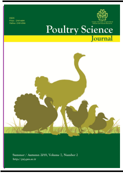

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Breast Muscle Characteristics of Avian Pathogenic *Escherichia Coli* Infected Broilers Fed with Antibiotics or Probiotic

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Abstract
The study was conducted to evaluate the impact of feeding multistrain probiotic on breast muscle characteristics of avian pathogenic *Escherichia coli* (APEC) infected broilers. Three hundred and thirty six Lohmann MB-202 day-old-chicks were divided to four treatment groups, including CNTRL (chicks receiving basal diet and not infected), APEC (chicks receiving basal diet and infected with APEC), APEC-AGP (chicks taking in basal diet containing 0.04% zinc bacitracin and infected with APEC) and APEC-PROB (chicks taking in basal diet containing 0.5% probiotic *Bacillus* and infected with APEC). At day 35, birds were randomly taken and slaughtered, and from which the breast muscles were collected for the determination of breast meat characteristics. The breast meat of CNTRL had lower ($P < 0.05$) pH values than APEC-AGP and APEC-PROB birds. The breast muscles from CNTRL exhibited higher ($P < 0.05$) water holding capacity (WHC) and water content than that from infected groups. Crude fat was higher ($P < 0.05$) in APEC-AGP and APEC-PROB than that of CNTRL and APEC meats. Crude ash was higher ($P < 0.05$) in APEC-PROB meat than that in other meats. The L* (lightness) values were higher ($P < 0.05$) in meats from APEC and APEC-AGP than in CNTRL and APEC-PROB meats. The a* (redness) values were higher ($P < 0.05$) in meat of CNTRL than in APEC-AGP and APEC-PROB. The samples from APEC-AGP had the highest ($P < 0.05$) values of b* (yellowness). Palmitic, stearic and linoleic acids were higher ($P < 0.05$) in meat from CNTRL and APEC compared to that from APEC-AGP and APEC-PROB. Oleic acid was higher ($P < 0.05$) in CNTRL than in APEC-AGP and APEC-PROB meats. CNTRL meat had higher ($P < 0.05$) antioxidant activity than APEC and APEC-AGP meats. The 21, 42.7, 40, 51 and 53 kDa bands were intense in all meat samples from APEC group, but less intense in some samples from CNTRL, APEC-AGP and APEC-PROB groups. In conclusion, APEC infection posed a negative effect on broiler meat characteristics. Probiotic seemed to counteract infection and thereby alleviate the detrimental effect of APEC infection on meat traits.

Introduction

During the last few decades the demand for broiler meat has been increasing substantially in Indonesia. The relatively low price and good nutritional properties make broiler meat the main choice of animal protein source for the Indonesian consumers. Currently, nutritional qualities are becoming a major consideration for the consumers of broiler meats. To respond such condition, it is therefore essential to produce broiler meats with high nutritional qualities. It has widely been known that some factors may affect the characteristics and qualities of broiler meat, such as genotype, sex, age, feed, environmental

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condition, stocking density, diseases and stress (Kralik *et al.*, 2017; Mir *et al.*, 2017).

Escherichia coli infection causing colibacillosis has been attributed to retarded growth rate and increased morbidity and mortality in poultry farm leading to economic loss (Mbanga and Nyararai, 2015). Besides causing production problems, infections may negatively affect the characteristics and qualities of broiler meat. Rajput *et al.* (2014) reported that challenge with *Eimeria maxima* reduced redness and water holding capacity (WHC) in broiler meat. Likewise, Wang *et al.* (2017) noticed that infection with *Clostridium perfringens* changed lipid and fatty acid profiles of broiler meat. In *E. coli*-infected birds, Istiqomah *et al.* (2013) found an increased pH in broiler meat, suggesting that *E. coli* infection decreased muscle glycogen deposition resulting in high meat pH post-mortem. Overall, the changes in lipid, protein and glucose absorption and metabolisms as a response to infections may be responsible for the changes in the broiler meat characteristics (Istiqomah *et al.*, 2013; Wang *et al.*, 2017).

In-feed antibiotics had traditionally been used to deal with pathogenic bacteria in broiler production. Considering the occurrence of antibiotic resistance both in birds and humans, the application of in-feed antibiotics is, however, no longer allowed in broiler production worldwide. The retraction of such additive from broiler feeds have been associated with a retarded growth rate and an outbreak of enteric problems such as colibacillosis (Sugiharto, 2016). For this reason, the substitutes for in-feed antibiotics are therefore of great importance for the sustainable broiler industry (Perdinan *et al.*, 2019). Besides promoting growth rate and controlling infectious agents, probiotic bacteria have been exhibited to alleviate the deleterious changes in the nutritional composition of broiler meats because of infections. In the work of Wang *et al.* (2017), it was clear that probiotic *Lactobacillus johnsonii* was capable of preventing the muscle from the adverse changes due to *C. perfringens* infections. Also, Zhou *et al.* (2016) showed that *Bacillus licheniformis*-based probiotic improved the expression of gene for biosynthesis of fatty-acid and β-oxidation of necrotic enteritis-infected chicks, and therefore probiotics may alleviate the negative effect of infections on meat quality.

In this current study, we incorporated multistrain probiotic in feeds as the substitute for commercial in-feed antibiotics for broiler chickens. The probiotics have previously been demonstrated capable of improving the digestive system and physiological traits as well as increasing the intestinal populations of useful bacteria in broilers (Isroli *et al.*, 2017; Sugiharto *et al.*, 2018a,b). However, the impact of multistrain probiotic on broiler meat-quality traits has never been studied. The current study was carried to assess the impact of feeding probiotic on breast muscle characteristics of avian pathogenic *Escherichia coli* (APEC) infected broilers.

Materials and methods

Ethical approval

The broiler chickens were raised according to the standard rearing of poultry mentioned in law of the Republic of Indonesia No. 18, year 2009 concerning animal farming, health and welfare.

Study design, broiler management and analyses

A total of 336 day-old-broiler chickens (unsexed Lohmann MB-202; body weight= 45.2 ± 0.37 g; means ± standard deviation) were raised in the current experiment. The birds were randomly divided to four groups of seven replicates (12 birds in each). The groups were CNTRL (chicks receiving basal diet and not infected), APEC (broiler chicken receiving basal diet and infected with APEC), APEC-AGP (broilerchicken taking in basal diet containing 0.04% zinc bacitracin and challenged with APEC) and APEC-PROB (broiler chicken taking in basal diet containing 0.5% probiotic and challenged with APEC). Antibiotic zinc bacitracin and probiotics were added ("on top") at the expense of the basal diet. Throughout the experiment (days 0 to 35), the birds were reared in an open-sided chicken house using rice husk as a litter stuff. The birds were permitted to have free access to diets and drinking water. The diets were formulated in mash form (Table 1) to fulfill the Indonesian National Standards for Feed of Broiler (SNI, 2006). The ration contained no enzymes, coccidiostat and other additive. The probiotic employed in the current work was the same as that of previously used by Isroli *et al.* (2017) and Sugiharto *et al.* (2018a,b). The product contained 12.10 log cfu/g of multi-strains *Bacillus*, i.e., *Bacillus cereus* (strain SIIA_Pb_E3), *B. licheniformis* (strain FJAT-29133), *B. megaterium (strain F4-2-27) and Bacillus* spp. *11CM31Y12.* The probiotic was also supplemented with 0.100 mg vit A, 0.018 mg vit D_3, 0.100 mg vit E, and some minerals including 1,200 mg Ca, 750 mg P, 0.006 mg Co, 0.08 mg Mg, 0.045 mg Cu, 0.180 mg S, 0.015 mg Se, 0.010 mg Zn, 0.030 mg I, 0.060 mg KCl, 0.060 mg Fe and 0.100 mg Mn.

Vaccinations with Newcastle disease (ND) and avian influenza (AI) vaccines were conducted on broiler chicken through intramuscular injections immediately after hatching. Referring to Istiqomah *et al.* (2013), the birds in APEC, APEC-AGP and APEC-PROB groups were inoculated with 0.5 mL of a bacterial culture containing 10^8 cfu/mL APEC at days 21, 23, 25 and 27. At the same times, the birds in CNTRL group received buffered saline water.

Table 1. Ingredients and chemical composition of basal diet

Items	Composition (%, unless otherwise stated)
Yellow corn (CP = 8.5%)	45.5
SBM (CP= 46%)	17.0
Wheat flour	10.0
Bread flour	5.00
Rice bran	4.45
CPO	3.50
CGM	3.60
DDGS	3.00
MBM	2.80
Chicken feather meal	2.00
Bone meal	1.50
L-Lysine	0.55
DL-Methionine	0.37
L-Threonine	0.08
Salt	0.15
Mineral-vitamin mix[1]	0.50
Calculated composition:	
ME (kcal/kg)[2]	3,200
CP	22.0
Crude fat	5.00
Crude fibre	5.00
Ash	7.00
Lys	1.30
Met+Cys	0.60
Thr	0.20

[1]Mineral-vitamin mix contained (per kg of diet) of Ca 2.250 g, P 0.625 g, Fe 3.570 mg, Cu 0.640 mg, Mn 5.285 mg, Zn 0.003 mg, Co 0.001 mg, Se 0.013 mg, I 0.016 mg, vitamin A 375 IU, vitamin D 150 IU, vitamin E 0.080 mg
[2]ME was calculated according to formula (Bolton, 1967): 40.81 {0.87 [CP + 2.25 crude fat + nitrogen- free extract] + 2.5}
CP: crude protein, SBM: soybean meal, CPO: crude palm oil, CGM: corn gluten meal, DDGS: distiller dried grains with solubles, MBM: meat bone meal, ME: metabolizable energy

The preparation of the APEC challenge culture was conducted according to Sugiharto *et al.* (2012) with some changes. In brief, the stock culture of APEC ATCC 8739 (Microbiologics, MediMark Europe) was retrieved overnight on nutrient agar at 38°C. The isolate was then recultured on the same media and conditions. The bacteria were harvested using peptone water (10 mL), and subsequently the suspension was diluted in 250 mL peptone water to achieve the inoculum of 1×10^8 cfu/mL. The plate count method using MacConkey agar (Merck KGaA, Darmstadt, Germany) was carried to ascertain the number of APEC colonies in the challenge culture. The challenges were carried out through direct intra-tracheal instillation by means of a 1.0 mL modified syringe with a blunt-ended pipette tip according to Kaikabo *et al.* (2017). At the final of rearing period, the birds were fasted (from feed, not water) for 8 h and one bird from each replicate (seven birds per treatment group) was randomly taken and slaughtered. For the determination of meat traits, the breast muscle was collected and immediately frozen (-20ºC) until analysis. The breast muscles were thawed at room temperature prior to analysis. The pH values of breast muscle was determined with the digital pH meter (Eutech EcoTestr pH 1, Thermo Fisher, Singapore). The water holding capacity (WHC) of meats was measured according to press methods with filter paper (Grau and Hamm, 1953). The proximate composition of breast muscle was assayed based on the standard proximate analysis (AOAC, 1995). The colour of broiler breast meat was assessed using digital colour meter in Mac OS X (set to CIE Lab). The colour was presented as L* (lightness), a* (redness) and b* (yellowness) values. The fatty acid content of breast muscle was measured with gas chromatography. The retention time of each sample was compared with the standard retention time to identify each fatty acid. Fatty acids were quantified by normalizing and transforming of the area percentage to mg per 100 g of edible portion, by mean of a lipid conversion factor (Holland *et al.*, 1998). To measure the 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging activity, meat sample was initially extracted using ethanol. The filtrate was subsequently subjected to the DPPH free radical scavenging assay according to Wu *et al.* (2009). The absorbance was assessed at 517 nm. The appearance of myofibrillar protein from breast meat was examined using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) according to Al-Baarri *et al.* (2018) with the slight modification in the gel concentration. The gradient polyacrylamide gel concentration from 4 to 15% from Bio-Rad Laboratories was used to provide the obvious separation of bands.

The data collected from the present study were analyzed based on the General Linear Models (GLM) procedure in SAS (SAS Inst. Inc., Cary, NC, USA). Further analysis based on Duncan's multiple-range test was performed if the differences ($P < 0.05$) across the groups were found. Data regarding the myofibrillar protein profiles were not statistically analysed.

Results

Data on physical and chemical characteristics of breast muscles of broilers are listed in Table 2. The breast muscle of CNTRL had lower ($P < 0.05$) pH values than APEC-AGP and APEC-PROB birds. The breast meat of broilers in CNTRL group had higher ($P < 0.05$) WHC and moisture content than that from APEC infected groups. Crude fat was higher ($P < 0.05$) in breast meat of APEC-AGP and APEC-PROB as compared to that of CNTRL and APEC breast meat. Crude ash was substantially higher ($P < 0.05$) in breast meat of APEC-PROB than that in other birds. The values of L* (Lightness) were higher ($P < 0.05$) in breast meats from APEC and APEC-AGP than in that from CNTRL and APEC-PROB groups. The a* (Redness) values were higher ($P < 0.05$) in breast meat of CNTRL than in APEC-AGP and APEC-PROB. The breast meat from APEC-AGP had the highest ($P < 0.05$) values of b* (Yellowness).

Table 2. Physical and chemical characteristics of broiler breast muscle

Items	Treatment groups				*SEM*	*P* value
	CNTRL	APEC	APEC-AGP	APEC-PROB		
pH	5.97^{b}	6.13^{ab}	6.23^{a}	6.27^{a}	0.06	0.01
WHC (%)	44.6^{a}	40.9^{b}	39.8^{c}	39.8^{c}	0.33	<0.01
Moisture (%)	76.1^{a}	75.5^{b}	74.9^{c}	75.2^{bc}	0.18	<0.01
Crude protein (%)	22.3	22.4	21.9	21.6	0.30	0.23
Crude fat (%)	0.65^{b}	0.74^{b}	1.05^{a}	1.08^{a}	0.09	<0.01
Crude ash (%)	0.26^{b}	0.27^{b}	0.32^{b}	0.48^{a}	0.05	0.01
L* (Lightness)	35.3^{b}	39.2^{a}	39.8^{a}	34.8^{b}	1.00	<0.01
a* (Redness)	25.7^{a}	23.0^{ab}	20.1^{b}	20.3^{b}	1.08	<0.01
b* (Yellowness)	35.9^{b}	37.5^{ab}	39.1^{a}	32.8^{c}	0.95	<0.01

^{a,b,c}Values with different letters within the same row are significantly different ($P < 0.05$)
CNTRL: chicken receiving basal diet and not infected, APEC: chicken receiving basal diet and infected with APEC, APEC-AGP: chicken receiving basal diet supplemented with 0.04% zinc bacitracin and infected with APEC, APEC-PROB: chicken receiving basal diet supplemented with 0.5% probiotic *Bacillus* and infected with APEC, WHC: water holding capacity, SE: standard error of means

The fatty acid content of breast muscles of broilers are presented in Table 3. Palmitic, stearic and linoleic acids concentrations were higher ($P < 0.05$) in breast meat from CNTRL and APEC when compared with that from APEC-AGP and APEC-PROB broilers. The concentration of oleic acid was higher ($P < 0.05$) in breast meat of CNTRL than in that from APEC-AGP and APEC-PROB broilers.

Table 3. Fatty acid content of broiler breast muscle

Items (%)	Treatment groups				*SEM*	*P* –value
	CNTRL	APEC	APEC-AGP	APEC-PROB		
Palmitic acid	18.5^{a}	20.0^{a}	6.40^{b}	7.78^{b}	3.14	0.01
Stearic acid	4.93^{a}	5.00^{a}	1.69^{b}	1.92^{b}	1.05	0.02
Oleic	31.8^{a}	29.2^{ab}	9.98^{c}	11.7^{bc}	6.52	0.03
Linoleic	4.79^{a}	5.12^{a}	1.89^{b}	1.57^{b}	0.99	0.01

^{a,b,c}Values with different letters within the same row are significantly different ($P < 0.05$)
CNTRL: chicken receiving basal diet and not infected, APEC: chicken receiving basal diet and infected with APEC, APEC-AGP: chicken receiving basal diet supplemented with 0.04% zinc bacitracin and infected with APEC, APEC-PROB: chicken receiving basal diet supplemented with 0.5% probiotic *Bacillus* and infected with APEC, *SEM*: standard error of means.

Figure 1 shows the DPPH radical scavenging activity of breast meats. Breast meat from CNTRL had a higher ($P < 0.05$) DPPH radical neutralizing activity when compared particularly to that from APEC and APEC-AGP chicken. No difference ($P > 0.05$) on DPPH radical scavenging activity was observed between breast meat from CNTRL and APEC-PROB chicken.

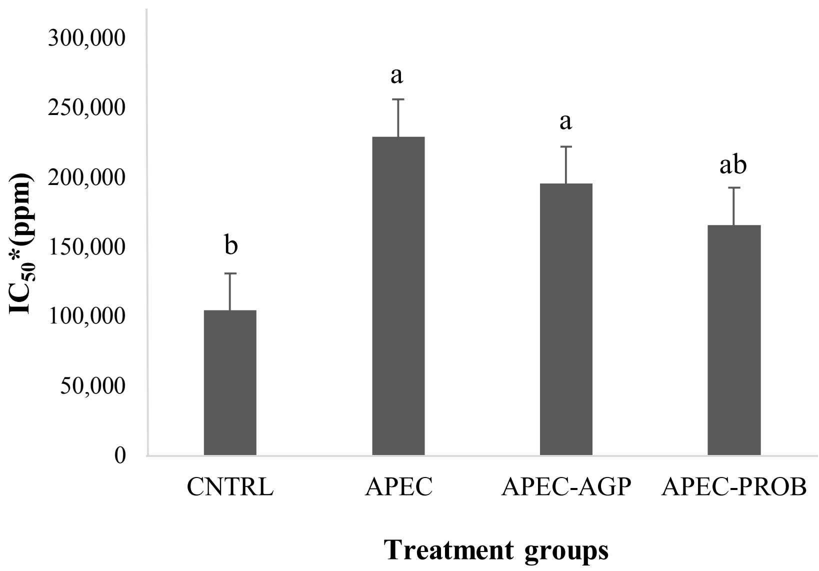

Figure 1. 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging activity of broiler breast muscle. IC_{50} is recognized as the potent concentration at which the DPPH radicals were removed by 50%. A lower IC_{50} value implies a higher of DPPH radical scavenging activity.

[a,b]Values with different letters within the same row are significantly different ($P < 0.05$). CNTRL: chicken receiving basal diet and not infected, APEC: chicken receiving basal diet and infected with APEC, APEC-AGP: chicken receiving basal diet supplemented with 0.04% zinc bacitracin and infected with APEC, APEC-PROB: chicken receiving basal diet supplemented with 0.5% probiotic *Bacillus* and infected with APEC

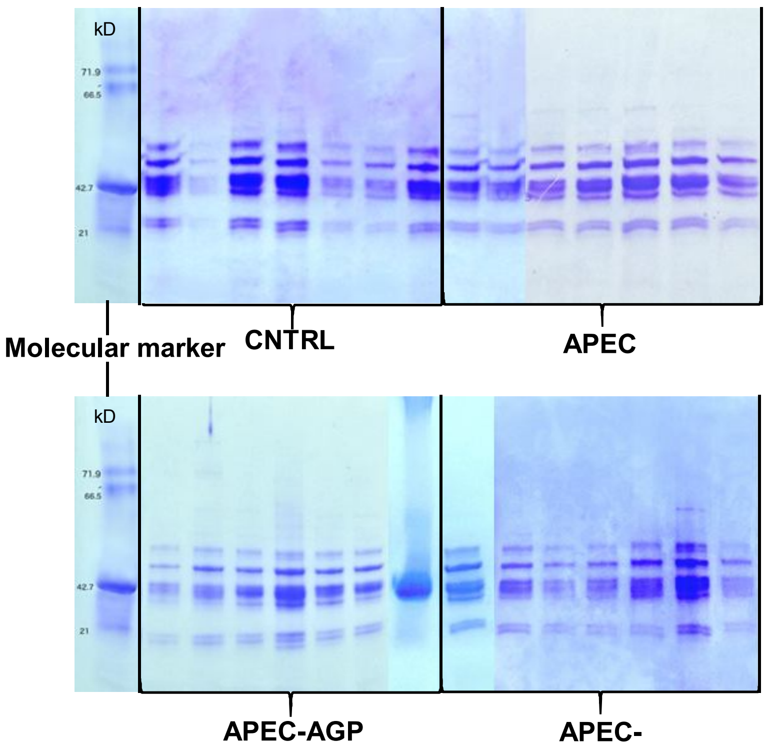

Figure 2. Myofibrillar protein profiles of broiler breast breast muscle on 1D-SDS–PAGE. CNTRL: chicken receiving basal diet and not infected, APEC: chicken receiving basal diet and infected with APEC, APEC-AGP: chicken receiving basal diet supplemented with 0.04% zinc bacitracin and infected with APEC, APEC-PROB: chicken receiving basal diet supplemented with 0.5% probiotic *Bacillus* and infected with APEC

The myofibrillar protein profiles of breast meat of broilers are shown in Figure 2. The 42.7 and 40 kDa bands appeared in all breast meats, but breast meat from APEC group seemed to have more intense 42.7 and 40 kDa bands in all samples. The 21 kDa band was intense in all samples of breast meats from APEC group. The 51 and 53 kDa bands were intense in all breast meat samples from APEC group, and less intense in some samples from CNTRL, APEC-AGP and APEC-PROB groups.

Discussion

In this study, APEC infection resulted in relatively higher pH values and lower WHC and moisture content of breast muscle. Similarly, Istiqomah *et al.* (2013) reported the increased pH values and decreased moisture content and WHC of breast meat with APEC infection. It seemed that the high pH values caused by APEC infection was associated with the depletion of muscle glycogen (Istiqomah *et al.*, 2013) and thus ultimately decreased WHC and water content in meat. Note that higher glycogen level in muscle is attributed to the higher moisture content as glycogen binds to water (Kerth, 2013). Studies have documented the capacity of antibiotic zinc bacitracin and probiotics (*Lactobacillus* spp. and *Aspergillus* probiotics) in elevating the WHC and moisture content of broiler meat (Contreras-Castillo *et al.*, 2008; Saleh, 2014). Conversely, the administration of either antibiotic zinc bacitracin or probiotic *Bacillus* could not alleviate the water loss from APEC infected broiler meat in the current work. The lack impacts of antibiotics oxytetracycline and neomycin as well as probiotic *Bacillus subtilis* (DSM 17299) on the water content and drip loss of broiler meats have also been recently reported by Abdulla *et al.* (2017). In this regard, different types of antibiotics, species of probiotic microorganisms and the particular condition during the chicken trial may result in divergent findings. In general, the values of WHC were relatively low across the breast muscle samples in the current study. The relatively high temperature during the study period (32±2ºC) may decrease the overall WHC values of broiler meats as recently documented by Xing *et al.* (2019). Indeed, the WHC values in the present study were actually higher than that of formerly revealed by Istiqomah *et al.* (2013), i.e., 30.11 to 36.24%.

It was apparent in this study that crude protein and crude fat did not change with APEC infection. Similarly, Istiqomah *et al.* (2013) found no changes in crude protein and fat content in broiler breast meat after APEC infection. Irrespective of APEC infection, administration of probiotic *Bacillus* resulted in increased crude fat content in broiler breast meat in the current study. This finding was in agreement with Endo and Nakano (1999) showing an increase in lipid content of broiler meat with probiotic (comprised of *Lactobacillus, Bacillus, Clostridium, Streptococcus, Saccharomyces* and *Candida species*) administration. The latter workers further suggested that the increased fat digestibility due to probiotic administration was responsible for the increased fat deposition in the broiler tissues. However, our result was different from Abdulla *et al.* (2017) reporting a decreased fat content in breast meat with administration of *B. subtilis* (DSM 17299). The differences in strains and species of probiotics used and experimental protocols applied may be responsible for the above discrepancy. It was observed in this study that meat fat content increased with the administration of zinc bacitracin. This was in contrast to Abdulla *et al.* (2017) documenting the reduced fat content in broiler breast meat after administrating antibiotics oxytetracycline and neomycin in diets. Former study documented that *E. coli* infection could reduce the content of ash in broiler meats (Istiqomah *et al.*, 2013). Different from the latter report, in this current work APEC infection did not change the ash content of broiler breast muscle since there was no difference in ash content between CNTRL and APEC meats. Regardless of APEC infection, probiotic administration resulted in higher crude ash content in broiler breast meats. Different data were documented by Abdulla *et al.* (2017), in which treatment with *B. subtilis* did not change the crude ash content of broiler meat. Indeed, some minerals were supplemented to probiotic *Bacillus* used in the present study. These minerals seemed therefore to contribute to the increased crude ash deposition in breast meats of APEC-PROB broilers.

Data in our current experiment showed that APEC infection increased the lightness values of broiler breast meats. It seemed that low muscle glycogen in the infected broilers resulted in higher ultimate pH and consequently increased the lightness values of breast meats. Different results were revealed by Wang *et al.* (2017) and Chodová *et al.* (2018) showing no changes in L* (lightness) values of broiler breast meats following the infections with *C. perfringens* and *Eimeria tenella*. In broiler chickens, Kannan *et al.* (1998) and Mir *et al.* (2017) noticed a clear relationship between corticosterone level and colour of meat, in which the increased circulating corticosterone was associated with the lighter of meat. In general, bacterial infection is associated with the increased stress and level of circulating corticosterone (Sharma *et al.*, 2017). Owing to this fact, the increased level of corticosterone due to APEC infection was most likely to be responsible for the lighter colour of breast meat from the APEC infected chicken. This stress hormone seemed to play a crucial role in the destruction of the intact muscular tissues leading to the less intense of meat colour (Kannan *et al.*, 1998). Interesting result was seen in the current study, in that probiotic treatment was capable of reducing the lightness values of broiler breast meat following APEC infection. In line with our data, Hossain *et al.* (2015) reported that probiotics containing *B. subtilis*, *Clostridium butyricum* and *Lactobacillus acidophilus* reduced the lightness of breast meat when compared with that from control and antibiotic (enramycin and avilamycin) treated broilers. Owing to the attribution of corticosterone to the paleness in broiler breast meats (Kannan *et al.*, 1998; Mir *et al.*, 2017), it is therefore most likely that probiotic reduce the circulating levels of corticosterone in APEC infected broilers (Sugiharto *et al.*, 2017) and thus ameliorating the muscular tissue destruction. There was a trend in this study that APEC infection reduced the redness values of broiler breast meats. As mentioned above, infection may increase the level of corticosterone, which plays an important role in the muscular tissues destruction especially myoglobin (gives red colour in meat) (Kannan *et al.*, 1998). In this study, the redness values were remarkably high across the meat samples. The definite explanation for the latter condition was not known, but freezing the raw meats prior to analysis was most likely to increase the redness values of broiler meats as reported by Lyon et al. (1976). It has been noted from the present study that APEC infection tended to increase the yellowness values of breast meats. The increased levels of stress hormone following infection seemed to be responsible for the latter condition. King and Chen Pas (1998) reported that treatment with adrenocorticotropic hormone resulted in increased yellowness values in broiler meats. Such treatment also produced broiler meats with lower WHC and lighter colour, which was also found in the breast meat from APEC infected broilers in the present study. With regard to the effect of probiotic *Bacillus*, the probiotic treatment was seen able to reduce the yellowness values of broiler breast meat in this study. In accordance with our data, Bai *et al.* (2017) showed a reduced yellowness values in breast meat from broiler chicken treated with probiotic *B. subtilis* fmbJ.

It was shown in the present project that infection with APEC did not change the fatty acid contents of broiler breast meats since there were no significant variations in fatty acid content between meats from CNTRL and APEC birds. This result differed from Wang *et al.* (2017) reporting an increased and decreased saturated and polyunsaturated fatty acids, respectively, in broiler meats following infection with *C. perfringens*. The different bacterial species used for infection and other experimental conditions may account for the above discrepancy findings. Irrespective of infection, our findings showed that administrations of zinc bacitracin and probiotic *Bacillus* decreased the contents of palmitic, stearic, oleic and linoleic acids in broiler breast meats. With regard particularly to probiotics, Saleh *et al.* (2012; 2013) reported significant decrease in the contents of palmitic and stearic acids in broiler meats with feeding probiotics *Aspergillus awamori* and *Saccharomyces cerevisiae*. Likewise, *Lactobacillus*-based probiotic decreased oleic acid (Kalavathy *et al.*, 2006) and probiotics *B. subtilis* KCTC 3239, *L. acidophilus* KCTC 3111, *Enterococcus faecium* KCTC 2022, and *S. cerevisiae* KCTC 7928 (used in combination with *Alisma canaliculatum*) reduced linoleic acid contents in the thigh meat of broiler (Hossain *et al.*, 2012). In the earlier studies, Kalavathy *et al.* (2006) and Hossain *et al.* (2012) revealed that probiotic administration may inhibit the synthesis or absorption of fatty acids leading to reduced fatty acid deposition in the muscle. It should however be noted that in another study, administration of probiotics (*Lactobacillus, Bacillus, Clostridium, Streptococcus, Saccharomyces* and *Candida*) did not affect the contents of palmitic, stearic, oleic and linoleic acids in broiler breast meats (Endo and Nakano, 1999). The absent effect of probiotics on palmitic, stearic, oleic and linoleic acids contents in breast meat has also been reported by Hossain *et al.* (2012). These variations of fatty acid content in broiler meats may be attributed to the different probiotic species and strains, meat samples (whether breast or thigh meat), diets and other experimental protocols and conditions. The inconsistent data related to fatty acid content in broiler meats was also observed with regard to the effect of in-feed antibiotics. It was noted in this study that treatment with zinc bacitracin reduced the contents of palmitic, stearic, oleic and linoleic acids in breast meat. In line with our finding, Ciftci *et al.* (2010) showed the decreased total saturated and polyunsaturated fatty acids in the thigh meat of broilers when feeding antibiotic avilamycin. Likewise, Hossain *et al.* (2012) noticed that dietary incorporation of chlortetracycline reduced the content of linoleic acid in the thigh meat of broilers. Conversely, the difference in fatty acid profile was not observed in breast meats between control and antibiotic treated broilers in the work of Ciftci *et al.* (2010) and Hossain *et al.* (2012).

It was shown in the present experiment that breast muscles from CNTRL showed a higher DPPH radical scavenging activity when compared particularly to that from APEC and APEC-AGP chicken. In line with our present finding, Wang *et al.* (2017) reported that breast and thigh meats from *C. perfringens* infected broilers had a lower catalase enzyme activity when compared with control. Note that catalase enzyme activity is positively related to the radical scavenging activity of the materials. Interestingly, no significant difference in DPPH radical scavenging activity was observed between breast meats from CNTRL and APEC-PROB birds. In accordance with our data, the administration of *L. johnsonii* was capable of preventing the decreased catalase enzyme activity in breast meat following *C. perfringens* infection (Wang *et al.*, 2017). Currently, the reason for which our probiotic preparation maintained the antioxidant activity in breast meat of APEC infected broilers is largely unknown, but the antioxidative activity of probiotic (Kadaikunnan *et al.*, 2015) seemed to protect the birds from oxidative stress that may decrease the antioxidant capacity of meat (Liao *et al.*, 2015).

It was noticed in this study that intense bands of 42.7 kDa (actin protein) and 40 kDa (tropomyosin) were observed in all meat samples from APEC birds. Conversely, less intense bands of 42.7 and 40 kDa were seen in some samples from other treatment groups. It has been suggested that actin may involve in the stimulation of host response to pathogens. In the case of viral infection, Öhman *et al.* (2009) reported that actin was greatly upregulated upon infection with influenza A virus. With regard to tropomyosin, O'Hara and Lin (2006) reported that parasite infection was associated with the accumulation of tropomyosin in the tissues. Owing to the above mentioned studies, it was therefore tempting to speculate that the more intense the 42.7 and 40 kDa bands may be attributed to APEC infection. Similar to control, some meat samples from APEC-AGP and APEC-PROB showed less intense of the 42.7 and 40 kDa bands. Perhaps, the potential of antibiotic and probiotic *Bacillus* in counteracting the APEC infection resulted in less infection and thus less actin and tropomyosin produced and deposited in the tissues. It was also shown in the present study that the 21 kDa band (troponin I) was intense in all samples from APEC group. It has been documented by Tanindi and Cemri (2011) that stress, trauma and inflammation may induce troponin isoform in the skeletal muscle. In this view, APEC infection that can induce stress and inflammation (Sharma *et al.*, 2017) may be attributed to troponin deposition in the breast muscle of broilers. In concomitant to control, some meat samples from APEC-AGP and APEC-PROB showed less intense of the 21 kDa band in the present study. The efficacy of antibiotic and probiotic in alleviating stress and inflammation in broiler chicken due to APEC infection was most likely to reduce the troponin I deposition in breast meats. It was shown from the 1D-SDS-PAGE that the 51 and 53 kDa bands (calsequestrin and desmin, respectively) were intense in all meat samples from APEC group, and less intense in some samples from CNTRL, APEC-AGP and APEC-PROB groups. Study documented that calcium-binding protein increased in the serum of humans due to *Plasmodium falciparum* malaria (Lee *et al.*, 2015). As a calcium-binding protein, the increased calsequestrin may therefore be attributed to the inflammatory condition as a result from infection. With respect to desmin, Wang *et al.* (2014) reported that *Schistosoma J*. Cercariae infection increased the expression of desmin protein in mice. Hence, the observed intense 53 kDa band in all meat samples in APEC group may indicate that all birds suffered from APEC infection. Interestingly, less intense of 53 kDa bands were seen on some samples from CNTRL, APEC-AGP and APEC-PROB. The latter condition may suggest that antibiotic and probiotic were capable of alleviating APEC infection in broiler chickens, and thus reducing desmin accumulation in the tissues.

Conclusion

It could be concluded that APEC infection posed a negative effect on broiler meat characteristics. Probiotic seemed to counteract infection and thereby ameliorate the detrimental effect of APEC infection on meat qualities.

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