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Breast Meat Quality and Protein Functionality of Broilers with Different Probiotic Levels and Cyclic Heat Challenge Exposure

Hyun-Wook Kim[1], Traci Cramer[1], Osamudiamen O. E. Ogbeifun[1], Jin-Kyu Seo[1,2], Feifei Yan[3], Heng-Wei Cheng[4], and Yuan H. Brad Kim[1]*

[1]Meat Science and Muscle Biology Laboratory, Department of Animal Sciences, Purdue University, West Lafayette, IN 47907, USA
[2]Division of Applied Life Science (BK21 Plus), Gyeongsang National University, Jinju 52828, South Korea
[3]Department of Animal Sciences, Purdue University, West Lafayette, IN 47907, USA
[4]Livestock Behavior Research Unit, USDA-Agricultural Research Service, West Lafayette, IN 47907, USA
*Corresponding author: bradkim@purdue.edu (Yuan H. Brad Kim)

Abstract: This study was performed to evaluate the effect of probiotic feeding level on meat quality and protein functionality of breast muscle from chickens exposed to cyclic heat challenge. A total of 180 one-d-old male chicks were randomly allocated in 36 floor pens. From Day 15, the birds were exposed to 32°C for 10 h daily until the end of the experiment (Day 46). Three dietary treatments containing different levels of probiotic (a mixture of 4 lactic acid bacteria, 5.0×10^9 cfu/g) were prepared; regular diet without probiotic (control), regular diet with 0.5 g of probiotic/kg feed (probiotic 0.5) and regular diet with 1.0 g of probiotic/kg feed (probiotic 1). Both breast muscles (*M. pectoralis major*) were collected at 24 h postmortem, and the same side breast muscle was assigned to each experiment 1 (meat quality analysis, $n = 6$) and 2 (protein functionality analysis, $n = 3$). Probiotic feeding level did not affect initial pH and temperature declines ($P > 0.05$) of breast muscle until 6 h postmortem. However, the breast muscles from probiotic 1 group (5.92) showed a significantly higher ultimate pH than those from control (5.78) or probiotic 0.5 (5.82) groups at 24 h postmortem. No differences in chemical composition (moisture, protein, fat, ash, and phospholipids), water-holding capacity (cooking loss and display weight loss), shear force, and lipid oxidation stability were found in breast muscles from chickens exposed to cyclic heat challenge, regardless of probiotic levels ($P > 0.05$). An increase in probiotic level increased total protein solubility ($P = 0.0004$) and emulsion activity index of sarcoplasmic protein ($P = 0.0032$) of ground chicken breast. The results from the current study suggest that the supplementation of this commercial probiotic product could partially improve protein functionality of breast muscles from chickens exposed to cyclic heat challenge, in a dose-dependent manner within the applied level.

Keywords: chicken breast, heat stress, meat quality, probiotic, protein functionality
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Introduction

Heat stress due to an increase in ambient temperature lowers the productivity in the broiler industry, which could lead to substantial economic losses at $51.8 million annually (St-Pierre et al., 2003). Under high ambient temperature or hot seasonal condition, behavioral and physiological changes occur in the chicken body with needed thermoregulation. A major response of heat-stressed chickens is a reduction in feed consumption, which is followed by decreased growth per-

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formance (Azad et al., 2010a). With several metabolic changes, excessive generation of oxygen reactive species (ROS) due to heat stress causes oxidative damages to not only several organs, but also skeletal muscles in chicken (Lin et al., 2006; Mujahid et al., 2007). This, in turn, negatively affects protein functionality and oxidation stabilities of chicken skeletal muscles (Zhang et al., 2012). Thus, it has been well recognized that heat stress can cause undesirable chicken meat quality on color, water-holding capacity (WHC), oxidation stability, tenderness, and/or pale, soft, and exudative breast muscle (Fouad et al., 2016). While such negative impacts of heat stress were mostly found in acute heat-stressed chickens, it has been also reported that chronic heat exposure including cyclic heat stress could reduce the breast muscle size of chicken and its meat quality (Azad et al., 2010b; Zhang et al., 2012).

Microbial probiotic has been practically used as a functional supplement, instead of antibiotic, thereby improving not only growth performance, but also health of chickens (Khan and Naz, 2013). For these reasons, application of commercial probiotic products has been increasingly adopted in the poultry industry (Park and Kim, 2014). Recent studies have reported that probiotic supplementation could be an effective feeding strategy to reduce detrimental impacts of heat stress on growth performance and health of broiler chickens (Song et al., 2014; Jahromi et al., 2016). In addition, some previous studies found the beneficial impacts of probiotic feeding on broiler meat quality, such as WHC, oxidation stability, and tenderness (Aksu et al., 2005; Ali, 2010; Bai et al., 2016, 2017; Kim et al., 2017; Zhou et al., 2010). Our recent research also revealed that probiotic feeding could alleviate oxidative damage of breast muscle of chickens that were previously exposed to cyclic heat stress (Cramer et al., 2016). As a few studies also postulated that the efficacy of probiotic feeding on meat quality attributes could be affected by dosage-level (Aksu et al., 2005; Ali, 2010; Bai et al., 2016, 2017; Zhou et al., 2010), it would be reasonable to hypothesize that the beneficial impact of probiotic feeding on meat quality of heat-stressed broilers would be proportional to the amount of probiotic dosage level. Therefore, the objective of this study was to determine the impacts of different levels of probiotic feeding on meat quality and functional properties of breast muscles from chickens exposed under cyclic heat challenge.

Materials and Methods

The husbandry and the following procedures were approved by the Purdue Animal Use and Care Committee (PACUC Number 1111000262).

Animal management

One hundred and eighty 1-d-old male chicks (Ross 708 broiler) were purchased from a commercial hatchery (Miller Poultry, Orland, IN), group-weighed, and randomly allocated in 36 floor pens (5 birds per pen, 243 × 51 cm^2) in a single room at the Poultry Research Facility of Purdue University. The ambient temperature in the room gradually decreased from 35°C (Day 1) to 21°C (Day 14), for cyclic heat challenge, and increased from Day 15 to 32°C for 10 h daily until the end of the experiment (Day 46; Mahmoud et al., 2015). The lighting program was constantly maintained at 30 lx for 23L:1D (23 light:1 dark for a 24 h day) Day 1 up to Day 3 and then 10 lx for 20L:4D (20 light:4 dark) until Day 46. Twelve pens were randomly assigned to each of the 3 different dietary treatments (12 pens per treatment): regular diet without probiotic (control), regular diet with 0.5 g of probiotic/kg feed (probiotic 0.5), and regular diet with 1.0 g of probiotic/kg feed (probiotic 1). Regular diet was formulated according the recommendation for nutrients by the Aviagen (2014), and the ration formulation of regular diet is shown in Table 1. A commercial product used as a probiotic supplement, PoultryStar, is a probiotic mixture

Table 1. The ration formulation

Ingredient, %	Starter	Grower	Finisher
Corn	52.02	52.26	62.80
Soybean meal,48% CP	40.00	39.09	29.72
Soy oil	3.59	4.97	4.11
Sodium chloride	0.51	0.46	0.43
DL Methionine	0.3	0.24	0.23
$_{L}$–Lysine HCl	0.13	–	0.07
Threonine	0.06	–	–
Limestone	1.29	1.15	1.12
Monocalcium phos	1.75	1.48	1.17
Vitamin/mineral premix[1]	0.35	0.35	0.35
Calculated analyses			
Crude protein %	23.43	22.81	19.17
Poultry ME kcal/kg	3,050.00	3,150.76	3,200.00
Calcium %	0.95	0.85	0.75
Available phosphorus %	0.50	0.44	0.36
Methionine %	0.66	0.59	0.53
Methionine + Cystine %	1.04	0.97	0.86
Lysine %	1.42	1.29	1.09
Threonine %	0.97	0.89	0.74
Na %	0.22	0.20	0.19

[1]Provided per kg of diet: vitamin A, 13,233 IU; vitamin D3, 6,636 IU; vitamin E, 44.1 IU; vitamin K, 4.5 mg; thiamine, 2.21 mg; riboflavin, 6.6 mg; pantothenic acid, 24.3 mg; niacin, 88.2 mg; pyridoxine, 3.31 mg; folic acid, 1.10 mg; biotin, 0.33 mg; vitamin B12, 24.8 μg; choline, 669.8 mg; iron from ferrous sulfate, 50.1 mg; copper from copper sulfate, 7.7 mg; manganese from manganese oxide, 125.1 mg; zinc from zinc oxide, 125.1 mg; iodine from ethylene diamine dihydroidide, 2.10 mg; selenium from sodium selenite, 0.30 mg.

of 4 lactic acid bacteria (5.0×10^9 cfu/g, *Enterococcus faecium*, *Pediococcus acidilactici*, *Bifidobacterium animalis*, and *Lactobacillus reuteri*), which was provided by Biomin America Inc. (San Antonio, TX). Birds were fed a starter diet (from Day 1 to Day 14), a grower diet (from Day 15 to Day 28), and a finisher diet (from Day 29 to Day 46). Feed and water were provided during growth ad libitum.

Slaughter and sample collection

On Day 46, two birds were randomly selected from 6 pens per each treatment (12 birds for each dietary treatment) and transported to the Meat Laboratory at Purdue University within 20 min by a truck. The birds were electrically stunned, decapitated, bled for 120 sec, scalded, and placed in a rotary drum plucker. The featherless chicken carcasses were eviscerated manually, individually hung on a carcass hanging trolley, air-chilled in a 2°C chilling room for 24 h. At 24 h postmortem, both breast muscles (*M. pectoralis major*) were collected from each carcass, and the same side breast muscle was assigned to each experiment (experiment 1 and 2). In experiment 1 (for meat quality analysis), right side breast muscles were horizontally cut into 2 portions and assigned to 2 different postmortem storage days (Day 1 and Day 5). The breast portion was placed on a Styrofoam tray, overwrapped with commercial oxygen-permeable polyvinyl chloride (PVC) film (film thickness of 0.5 mil, Reynolds Food Service Packaging, Richmond, VA), and displayed in a 2°C chilling room under white fluorescent light (approximately 1,450 lx, color temperature = 3,500 K) for additional storage of 5 d. In experiment 2 (for protein functionality analysis), a total of 12 left side breast muscles were randomly assigned to 3 different batches (4 breast muscles/treatment/batch), vacuum-packaged, and stored in a –80°C freezer. The frozen samples were thawed in a 2°C chilling room for 24 h and ground using a meat grinder equipped with a 3/8 inch plate, and assays for protein functionality were conducted.

Experiment 1 (meat quality analysis of intact breast muscle)

pH and temperature declines. The pH decline of breast muscle was measured in duplicate using an insertion-type portable meat pH (HI 99163, Hanna Instruments Inc., Woonsocket, RI), and the temperature decline was monitored using the same machine. All measurements were performed at 0.25, 1, 2, 3, 4, 5, 6, and 24 h postmortem.

Proximate composition. Moisture (934.01; oven drying method), protein (990.03; combustion method), fat (920.39; ether extract method), and ash (942.05; muffle furnace technique) contents of breast muscle were determined in triplicate according to the AOAC method (AOAC, 2006).

Phospholipid content. Lipid faction was extracted from breast muscle using a chloroform/methanol solvent (2:1 ratio; Soyer et al., 2010). The extracted lipid was dissolved in chloroform (0.25 mg/mL), mixed with 1 mL of thiocyanate reagent (27 g of ferric chloride and 30 g of ammonium thiocyanate in 1 L of distilled water, DW), and vortex-mixed for 30 sec. The mixture was centrifuged at 750 × *g* for 10 min (20°C), and the absorbance of lower chloroform phase was read at 488 nm. The phospholipid content was calculated using standard curve of phosphtidylcholine in chloroform (5 to 50 µg/ml) and expressed as g phosphatidylcholine equivalents per 100 g fat (g phosphatidylcholine eq/100 g fat).

Cooking loss. A piece of meat (approximately 140 g) was taken from the same location in 1 d chicken breast muscles, which was placed in a commercial plastic bag (film thickness of 4 mil, Clarity, Bunzl Processor Division, North Kansas City, MO) and cooked in a 80°C water bath. The core temperature of samples was individually monitored by inserting a thermocouple (T-type, Omega Engineering, Stamford, CT) linked to a digital temperature logger (OctTemp2000, MadgeTech, Inc., Warner, NH). The cooked samples were cooled to room temperature for 3 h and re-weighed. Cooking loss was calculated as a percentage of the weight differences between raw and cooked samples (Kim et al., 2016).

Display weight loss. Display weight loss of breast muscle was calculated as a percentage of the weight differences between Day 1 and Day 5 (Kim et al., 2016).

Shear force. Four strips ($2.5 \times 1.0 \times 1.0$ cm^3) were obtained from the paralleled muscular fibers in the middle of each cooked breast sample. The shear force value of strips was determined using a Warner-Bratzler shear attachment on a texture analyzer (TA-XT Plus, Stable Micro System Ltd., Surrey, UK). Test speed was 2 mm/s, and the collected data were averaged (Kim et al., 2016).

Color measurement. Color of breast muscle was determined on Day 1 and Day 5 of display storage, using a Hunter MiniScan EZ colorimeter (Hunter, Reston, VA) equipped with a 25 mm (diameter) aperture. The illuminant was D_{65} source and the observer was standard 10°. On skin side surface, 5 random locations were taken to record Commission Internationale de I'Eclairage (CIE) L*, a*, and b* value, and the collected data were averaged. Hue angle was calculated as; hue angle = $\tan^{-1}$(b*/a*; American Meat Science Association, 2012).

Lipid oxidation (2-thiobarbituric acid reactive substances). Lipid oxidation of breast muscles was determined in triplicate according to the 2-thiobarbituric acid reactive substances (TBARS) method of Buege and Aust (1978) described by Kim et al. (2016).

Experiment 2 (protein functionality analysis of ground breast muscle)

Water-holding capacity. Salt-induced water uptake, cooking loss and final yield were determined in triplicate according to the method of Bowker and Zhuang (2016) with minor modification. Briefly, 15 g of ground breast (W1) were placed in a centrifuge tube (50 mL), mixed with 22.5 mL of cold 0.6 M NaCl solution, and centrifuged at 3,000 × *g* for 15 min (4°C). The supernatant was removed, and the swollen pellet (W2) was re-weighed. Salt-induced water uptake (%) was calculated as follow; [(W2 – W1) / W1 × 100]. The centrifuge tube containing swollen pellet was heated in a water bath (80°C) for 20 min, and then, the cooked pellet (W3) was weighed again. Cooking loss (%) was calculated as; [(W2 – W3) / W2 × 100], and final yield (%) was calculated as; [(W3 / W1) × 100].

Protein solubility. The solubilities of total and sarcoplasmic proteins were measured in triplicate according to the method of Bowker and Zhuang (2016). The concentration of solubilized proteins was determined using the Bio-Rad DC protein assay (Bio-Rad Laboratories, Hercules, CA). The protein solubility was expressed as mg soluble protein per g meat, and the solubility of myofibrillar protein was calculated as the difference in solubilities between total and sarcoplasmic proteins.

Emulsion activity index (EAI). The emulsion activity index (EAI) was determined in quadruplicate according to the method of Chan, Omana, and Betti (2011) described by Bowker and Zhuang (2016).

Statistical analysis

The experimental design was a randomized complete block design, in which a block was bird pen and processing batch in experiment 1 ($n = 6$) and 2 ($n = 3$), respectively. The model included probiotic feeding level effect and display storage effect (for color and TBARS), as main factors. All data were analyzed using the PROC MIXED procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC), to evaluate the significance of the main effects. Least squares means for all traits were separated (F test, $P < 0.05$) by using Fisher's protected least significant differences generated by the PDIFF option.

Results

Experiment 1 (meat quality of intact chicken breast)

The initial pH and temperature declines of breast muscles from chickens fed different levels of probiotic mixture under cyclic heat challenge are shown in Fig. 1. The pH of breast muscles rapidly decreased from 6.92 at 15 min postmortem to 5.89 at 6 h postmortem ($P < 0.0001$). The probiotic feeding levels did not affect the extent of pH decline up to 6 h postmortem ($P > 0.05$), then the pH of breast muscles from control or probiotic 0.5 groups slightly decreased. As a result, the breast muscle from probiotic 1 group had a higher ultimate pH (at 24 h postmortem; 5.92)

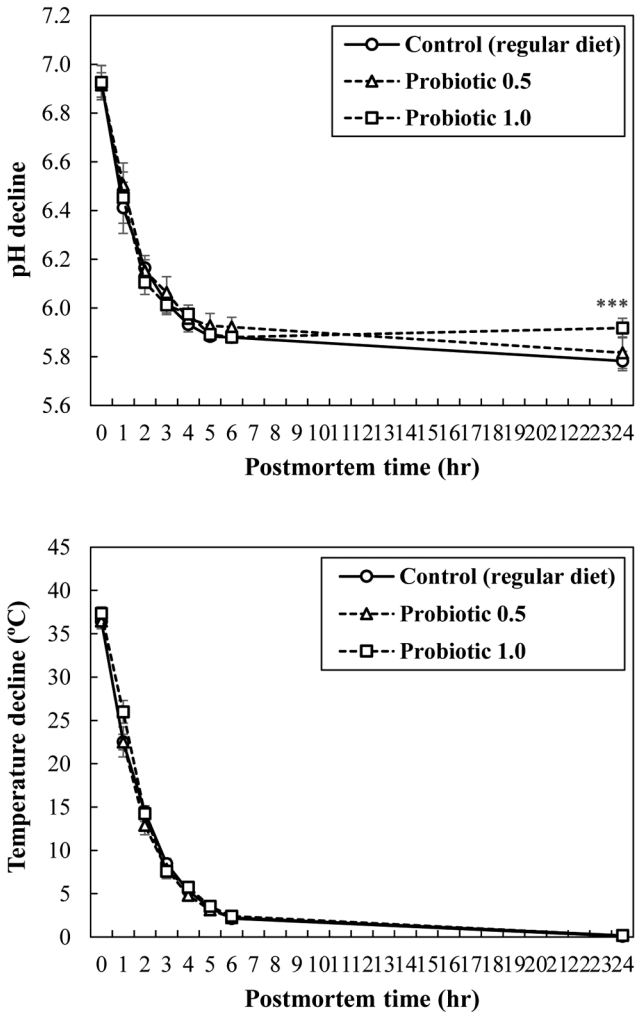

Figure 1. Initial pH and temperature declines of intact breast muscles from chickens fed different levels of probiotic mixture under cyclic heat challenge. ($n = 6$). Treatments: control, chickens fed only regular diet; probiotic 0.5, chickens fed regular diet plus 0.5 g probiotic/kg; probiotic 1.0, chickens fed regular diet plus 1.0 g probiotic/kg. ***, $P < 0.0001$.

than those from control (5.78) or probiotic 0.5 (5.82) groups ($P < 0.0001$). No significant difference in temperature decline of chicken breast muscles during 24 h postmortem was observed, regardless of probiotic feeding levels ($P > 0.05$; Fig. 1). Similarly, Wang et al. (2016) reported that heat stress did not affect initial temperature decline (from 15 min to 24 h postmortem) of breast muscles at commercial slaughter condition.

Proximate composition of breast muscles from chickens exposed to cyclic heat challenge was unaffected by probiotic feeding levels ($P > 0.05$; Table 2), in which moisture, protein, fat and ash contents were 75.5 to 75.9 g/100 g, 23.3 to 23.4 g/100 g, 1.5 to 2.1 g/100 g, and 1.2 g/100 g, respectively. As well as, probiotic feeding level did not alter the phospholipids (3.5 to 4.1 g/100 g fat) in breast muscles from chickens exposed to cyclic heat challenge ($P > 0.05$).

The effect of probiotic feeding levels on WHC and shear force of breast muscle from chickens exposed to cyclic heat challenge is presented at Table 3. No differences in cooking loss (18.2 to 18.7%) and display weight loss (2.9 to 3.3%) were found in chicken breast muscles, regardless of probiotic feeding levels ($P > 0.05$). In addition, breast muscles showed similar shear force (14.4 to 16.7 *N*; $P > 0.05$). These findings indicate that probiotic supplementation would have no influence on WHC and shear force of breast muscle from chickens exposed to cyclic heat challenge.

A change in color characteristics of breast muscles during 5 d of simulated retail display is shown in Table 4. No interactions between probiotic feeding levels and display storage time on CIE L* (lightness), CIE a* (redness), and hue angle (discoloration) breast muscles from chickens exposed to cyclic heat challenge were found ($P > 0.05$), except for CIE b* (yellowness). The feeding levels of probiotic did not influence any changes of color parameters in intact breast muscle during 5 d of display storage ($P > 0.05$). When display period increased, a decrease in lightness and redness, but an increase in yellowness was observed ($P < 0.0001$). Hue angle of chicken breast muscles, as an indicator of discoloration, slightly increased from 67.9 at Day 0 to 70.5 at Day 5 ($P = 0.0007$).

The effect of probiotic feeding levels on lipid oxidation (2-thiobarbituric acid reactive substances, TBARS)

Table 2. Proximate composition and phospholipids content of breast muscles from chickens fed different levels of probiotic mixture under cyclic heat challenge ($n = 6$)

Treatments[1]	Moisture, g/100 g	Protein, g/100 g	Fat, g/100 g	Ash, g/100 g	Phospholipids, g/100 g fat
Control (regular diet)	75.6	23.4	2.1	1.2	4.1
Probiotic 0.5	75.5	23.3	1.5	1.2	3.9
Probiotic 1.0	75.9	23.4	1.5	1.2	3.5
SEM[2]	0.164	0.153	0.179	0.011	0.318
P-value	0.51	0.96	0.33	0.13	0.75

[1]Treatments: control, chickens fed only regular diet; probiotic 0.5, chickens fed regular diet plus 0.5 g probiotic/kg; probiotic 1.0, chickens fed regular diet plus 1.0 g probiotic/kg.

[2]SEM: standard error of the mean.

Table 3. Water-holding capacity (WHC) and shear force of breast muscles from chickens fed different levels of probiotic mixture under cyclic heat challenge ($n = 6$)

	WHC, %		Shear
Treatments[1]	Cooking loss	Display weight loss	force, *N*
Control (regular diet)	18.4	3.2	14.8
Probiotic 0.5	18.2	2.9	16.7
Probiotic 1.0	18.7	3.3	14.4
SEM[2]	0.633	0.149	0.733
P-value	0.96	0.49	0.41

[1]Treatments: control, chickens fed only regular diet; probiotic 0.5, chickens fed regular diet plus 0.5 g probiotic/kg; probiotic 1.0, chickens fed regular diet plus 1.0 g probiotic/kg.

[2]SEM: standard error of the mean.

Table 4. Changes in color characteristics of breast muscles from chickens fed different levels of probiotic mixture under cyclic heat challenge ($n = 6$)

Main effects	CIE L* (lightness)	CIE a* (redness)	CIE b* (yellowness)	Hue angle (discoloration)
Probiotic feeding levels (P)				
Control (regular diet)[1]	61.4	6.6	17.0	69.4
Probiotic 0.5	62.0	6.5	17.3	69.3
Probiotic 1.0	61.5	6.5	17.3	68.6
SEM[2]	0.578	0.261	0.404	0.380
Display storage period (D)				
Day 0	62.5^{a}	6.9	17.0^{c}	67.9^{b}
Day 1	61.1^{b}	7.1	17.5^{b}	67.8^{bc}
Day 2	62.2^{a}	6.3	16.3^{d}	68.8^{b}
Day 3	62.6^{a}	6.1	16.3^{d}	69.4^{ab}
Day 4	60.6^{b}	6.6	18.2^{a}	70.2^{a}
Day 5	60.9^{b}	6.3	18.0^{a}	70.5^{a}
SEM	0.396	0.178	0.263	0.537
P-value				
P	0.79	0.88	0.79	0.2554
D	< 0.0001	< 0.0001	< 0.0001	0.0007
Interaction (P × D)	0.70	0.47	0.004	0.9902

[a–d]Different superscripts within each column indicate significant differences ($P < 0.05$).

[1]Treatments: control, chickens fed only regular diet; probiotic 0.5, chickens fed regular diet plus 0.5 g probiotic/kg; probiotic 1.0, chickens fed regular diet plus 1.0 g probiotic/kg.

[2]SEM: standard error of the mean.

of breast muscles from chickens under cyclic heat challenge is shown in Fig. 2. No significant interaction between probiotic feeding levels and display storage on TBARS value of breast muscle was found. At both Day 0 (24 h postmortem) and 5, although not significant, breast muscles from probiotic 0.5 or 1.0 groups had a numerically lower TBARS value than those from control group ($P > 0.05$). The TBARS value of chicken breast muscles increased from 0.03 mg MDA/kg muscle at Day 0 to 0.07 mg MDA/kg muscle at Day 5 ($P < 0.05$). In summary, our results show that an increase in probiotic feeding level increased ultimate pH of breast muscle from chickens under cyclic heat challenge, but little to no impacts on chemical composition, WHC, tenderness, color characteristics, and lipid oxidation stability of intact breast muscles were found.

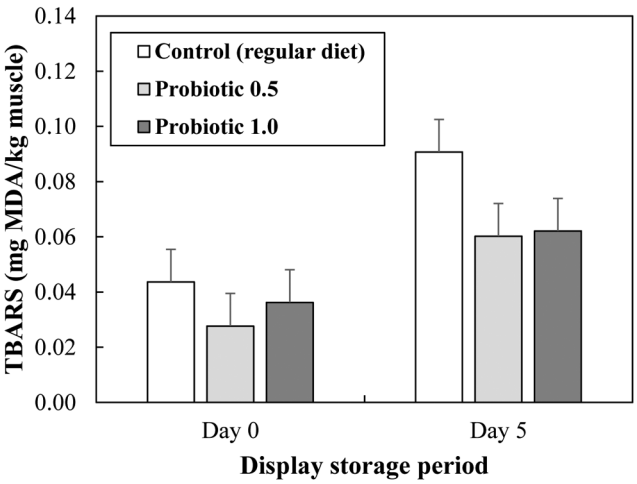

Figure 2. Lipid oxidation stability (2-thiobarbituric acid reactive substances, TBARS value) of intact breast muscles from chickens fed different levels of probiotic under chronic heat challenge. ($n = 6$). Control, chickens fed only regular diet; probiotic 0.5, chickens fed regular diet plus 0.5 g probiotic/kg; probiotic 1.0, chickens fed regular diet plus 1.0 g probiotic/kg.

Experiment 2 (protein functionality of ground broiler breast)

In experiment 2, protein functionality of ground breast muscle from chickens fed different probiotic levels under cyclic heat challenge was evaluated (Table 5). The increase in probiotic feeding level had no influence on salt-induced water uptake of ground breast from chicken exposed to cyclic heat challenge ($P > 0.05$), whereas ground breast from probiotic 0.5 or 1.0 groups had significantly higher cooking loss than that from control group. However, there was no significant difference in total yield of ground chicken breast.

Total protein solubility of breast muscles from chicken under cyclic heat challenge increased with probiotic feeding ($P = 0.0004$). Supporting this observation, the solubilities of sarcoplasmic and myofibrillar proteins were numerically increased with increasing probiotic feeding levels ($P > 0.05$).

Emulsion activity index (EAI) was measured to determine an ability of muscle proteins to stabilize hydrophilic-hydrophobic interaction as an emulsifier in meat emulsion system (Bowker and Zhuang, 2016). Probiotic supplementation to chickens exposed to cyclic heat challenge increased 2 times EAI of sarcoplasmic proteins from breast muscle ($P = 0.0032$), whereas EAI of myofibrillar proteins was unaffected by probiotic feedings level ($P > 0.05$). Consequently, our findings indicate that probiotic feeding levels could improve protein functionality of ground breast from chickens exposed to cyclic heat challenge, such as total protein solubility and emulsifying capacity of sarcoplasmic protein.

Table 5. Protein functionality of ground breast from chickens fed different levels of probiotic mixture under cyclic heat stress ($n = 3$)

	WHC, %			Protein solubility, mg/g muscle			Emulsion activity index, EAI	
Treatments[1]	Salt- induced water uptake	Cooking loss	Total yield	Total	Sarcoplasmic	Myofibrillar	Sarcoplasmic	Myofibrillar
Control (regular diet)	5.9	12.2[b]	88.8	132.4[c]	60.3	72.1	0.31[b]	1.03
Probiotic 0.5	6.8	17.1[a]	89.0	138.4[b]	60.3	78.1	0.60[a]	1.05
Probiotic 1.0	7.2	17.0[a]	89.4	150.0[a]	67.4	82.7	0.61[a]	1.07
SEM[2]	0.527	0.860	0.761	2.756	1.853	2.588	0.053	0.016
P-value	0.66	0.0014	0.96	0.0004	0.22	0.28	0.0032	0.59

[a–c]Different superscripts within each column indicate significant differences ($P < 0.05$).

[1]Treatments: control, chickens fed only regular diet; probiotic 0.5, chickens fed regular diet plus 0.5 g probiotic/kg; probiotic 1.0, chickens fed regular diet plus 1.0 g probiotic/kg.

[2]SEM: standard error of the mean.

Discussion

Under chronic heat stress condition, 3 major physiological changes potentially leading to meat quality decline may occur in living chicken; 1) decreases in protein synthesis and turnover (Temim et al., 2000), 2) increases in ante/postmortem glycolytic metabolisms (Zhang et al., 2012), and 3) excessive generation of ROS (Azad et al., 2010a). Consequently, it has been well documented that chronic heat stress could produce chicken breast muscle having low protein content and cause undesirable chicken meat quality on color, WHC, tenderness, and oxidation stability (Temim et al., 2000; Lu et al., 2007; Azad et al., 2010b; Zhang et al., 2012). In this current study, our results show that probiotic feeding level might not influence chemical composition, color, WHC, tenderness, and lipid oxidation of intact breast muscle from chickens exposed to cyclic heat challenge. However, 1 g/kg of probiotic feeding could change ultimate pH and some protein functionality (total protein sublimity and EAI of sarcoplasmic protein) of ground breast from chickens exposed to cyclic heat challenge.

The pH of meat is greatly associated with meat quality attributes (color, protein functionality, and tenderness) as well as quality variation of chicken breast muscle (Fletcher, 1999; Qiao et al., 2001). Zhang et al. (2012) found that breast muscle form chickens exposed to cyclic (5.78) or constant (5.72) heat stress exhibited lower ultimate pH than that from chickens raised at thermoneutral condition (5.88), as a result of increased lactate accumulation and the rate of postmortem glycolysis due to high activity of glycolytic enzymes (pyruvate kinase and lactic dehydrogenase). The reported pH value of breast muscle from chickens under cyclic heat challenge was similar to the pH of breast muscle from control group in this current study (Fig. 1). In addition, it was suggested that the decreased ultimate pH could be associated with poor quality characteristic of breast muscle from chronic heat-stressed broilers, particularly on color, WHC, and tenderness (Zhang et al., 2012). Some previous studies have found that microbial probiotic supplementation could increase ultimate pH of chicken breast muscle (Pelicano et al., 2003; Aksu et al., 2005; Zheng et al., 2015). Aksu et al. (2005) reported that dietary supplementation of 0.2% *Saccharomyces cerevisiae* increased pH of chicken breast muscle, from 6.24 to 6.31. A recent study conducted by Zheng et al. (2015) found that breast muscle (6.11) from chickens fed *Enterococcus faecium* had higher ultimate pH value than that from control without probiotic feeding (5.77), in which downregulation of glycolytic enzymes such as β-enolase and pyruvate kinase muscle isozyme was suggested as a possible cause for the high pH in breast muscle from chicken fed the probiotic. Therefore, the observed high ultimate pH of breast muscle from probiotic groups in the current study might be related to the down-regulating effect of probiotic supplementation on glycolytic enzymes that could alleviate an increase in glycolytic metabolism induced by high ambient temperature. This postulation would need to be confirmed by further investigation.

The overproduction of ROS under heat stress could cause oxidative damages to several organs including skeletal muscle tissue, which may lead to the decline of broiler meat quality (Wang et al., 2009; Azad et al., 2010a,b). According to Wang et al. (2009), lipid and protein oxidation induced by acute heat stress, together with decreased ultimate pH, could reduce protein functionalities of chicken breast muscle (e.g., WHC, protein solubility, and gel formation ability). Some previous studies have reported an antioxidant effect of probiotic feeding on lipid oxidation of chicken breast muscle (Aksu et al., 2005; Zhang et al., 2005; Ali, 2010; Aristides et al., 2012; Kim et al., 2017). Thus, although the amelioration of oxidation stability through probiotic supplementation could be expected to bring positive impacts on meat quality attributes of heat-stressed broiler, in this current study, the supplement effect of different probiotic levels on lipid oxidation stability of breast muscle was not obvious under cyclic heat challenge (Fig. 2).

In terms of protein functionality, our results indicate that probiotic supplementation could improve protein solubility and emulsifying capacity (of sarcoplasmic protein) of breast muscle from chickens exposed to cyclic heat challenge (Table 5). Protein solubility of chicken breast muscle, which is a primary factor affecting other protein functionalities, is dependent on pH between 5.5 and 7.0 (Xiong and Brekke, 1991). Qiao et al. (2001) reported that highly positive correlation between pH and emulsifying capacity ($r = 0.9572$, $P < 0.0001$) in ground chicken breast muscle. In this regard, the increased total protein solubility and EAI of sarcoplasmic protein in breast muscle from probiotic groups might be likely due to increased pH of breast muscle. However, emulsifying capacity of myofibrillar protein was not affected by probiotic feeding levels under cyclic heat challenge (Table 5). In addition, Chan et al. (2011) found that pH of poultry breast muscle mostly affected emulsifying capacity of sarcoplasmic protein, but not that of myofibrillar protein. This could elucidate no direct effects of probiotic supplementation on WHC of either intact or ground breast muscles from chickens exposed to cyclic heat challenge, in that myofibrillar proteins are largely associated with muscle pro-

tein functionalities, rather than sarcoplasmic proteins (Xiong and Brekke, 1989; Wang et al., 2009).

Conclusions

The results of this study indicate that an increase in probiotic feeding increased ultimate pH of breast muscles from chickens exposed to cyclic heat challenge, but had little to no impacts on meat quality attributes of intact chicken breast muscle. Increased total protein solubility and emulsifying capacity of sarcoplasmic protein of ground chicken breast from chickens exposed to cyclic heat challenge was observed with increasing probiotic feeding levels, which might be related to the increased ultimate pH value. Further studies on the determination of technological properties of emulsified meat products formulated with breast muscles from chickens exposed to heat stress and/or fed probiotic would be warranted to confirm beneficial effects of probiotic supplementation to ameliorate processing characteristics of breast muscles from heat-stressed chickens.

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