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# Effects of a dietary synbiotic inclusion on bone health in broilers subjected to cyclic heat stress episodes

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**ABSTRACT** The objective of this study was to determine the effects of a dietary synbiotic inclusion on broiler bone health under daily cyclic heat stress. A total of 360 Ross 708 broilers were randomly assigned to 1 of 3 dietary treatments (8 replicates per treatment): a regular diet (control) and the regular diet mixed with a commercial synbiotic product at 0.5 (0.5X) or 1.0 (1.0X) g/kg. The synbiotic contains a prebiotic (fructooligosaccharides) and a probiotic mixture of 4 microbial strains (*Enterococcus faecium*, *Pediococcus acidilactici*, *Bifidobacterium animalis*, and *Lactobacillus reuteri*). Room temperature was gradually decreased from 34°C on d 1 by 0.5°C/d for the first 14 d; then a cyclic heat stress episode (32°C/9 h/d) was applied from d 15 to 42. Gait score assessment and the latency-to-lie test were conducted when broilers were

40 and 41 d of age, respectively. The tibia, femur, and humerus were collected for measuring bone parameters at 42 d of age. The data indicated that bone mineral density, bone mineral content, and bone area were higher and the level of gait score was lower in the 1.0X group ( $P = 0.05$ ) but not in the 0.5X group ( $P > 0.05$ ) compared to controls. The proportions of broilers showing signs of lameness were ranked 1.0X group (25%) < 0.5X group (45%) < control (54%). Compared to controls, broilers of 0.5X group stood longer ( $P = 0.03$ ) during the latency-to-lie test. In conclusion, under the present conditions the synbiotic profoundly improves multiple indices of leg health of broilers subjected to the cyclic heat episodes, resulting in an improvement in walking ability.

**Key words:** broiler, synbiotic, lameness, bone health, heat stress

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## INTRODUCTION

Leg disorder, such as lameness, is a serious welfare problem facing the poultry meat industry as lameness causes pain and impairs mobility (Bokkers and Koene, 2003; Reiter and Bessei, 2009). Lameness is also an economic issue that leads to huge productive loss annually due to poor growth, and high culling and mortality rates from starvation, dehydration, and carcass downgrading (Rath et al., 2000). Although multiple factors influence the incidence of leg disorders such as strain, age, sex, nutrition, housing, environment, and

management (Kestin et al., 1999; Sorensen et al., 2000; Toghyani et al., 2011; Schwan-Lardner et al., 2013), fast growth is a key factor affecting musculoskeletal health in commercial broiler flocks (Talaty et al., 2009; Toscano et al., 2013). Fast-growing broilers, for example, have both lower tibia mineral density and percentage of bone ash than slow-growing broilers (Shim et al., 2012), causing high bone porosity (Williams et al., 2004). Broilers with a poor gait score spend more time lying (86%) than non-lame (76%) ones (Weeks et al., 2000). The lack of activity further exacerbates lameness as that, similar in humans (Kjaer et al., 2015), mechanical loading is essential for maintaining normal bone formation as well as bone remodeling in chickens (Hester et al., 2013). For example, broilers kept in cages have lower leg bone mineralization resulted from hypoactivity compared to those reared in large enclosure barns where the chickens walk freely (Aguado et al., 2015).

Heat stress reduces BW gain of broilers as it reduces feed intake and nutrient resorption by impairing intestinal function (Belay and Teeter, 1993). Heat stress also causes bone loss in broilers (Hosseini-Vashan et al., 2016), laying hens (Koelkebeck et al., 1993),

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and turkeys (Jankowski et al., 2015), resulting from reduced calcium consumption and absorption. Although lameness is not triggered by repeated heat stress episodes (33°C, 3 d/wk from 4 to 6 wk of age), the subclinical incidence of tibial head necrosis is substantially greater in heat-stressed broilers at both 28 and 35 d of age (Wideman and Pevzner, 2012). As one of the reasons causing bone damage, elevated temperature stimulates the activity of the hypothalamic–pituitary–adrenal axis, increasing the levels of circulating corticosterone (Xu et al., 2018). Excess glucocorticoids negatively affect bone mass through inhibiting osteoblastogenesis, increasing osteoblast (bone formation cells) and osteocyte apoptosis, and promoting osteoclast (bone resorption cells) survival (Jia et al., 2006; Rauch et al., 2010; Henneicke et al., 2014). In addition, high body temperature increases reactive oxygen substances in mitochondria leading to oxidative stress (Huang et al., 2015), contributing to skeletal damage.

Numerous recent studies have revealed that the gut microbiota shape the development of the central nervous system including the hypothalamic–pituitary–adrenal axis and related functions (de Weerth, 2017). These findings provide a basis for therapeutic approaches to use prebiotics and probiotics to treat neuronal disorders, mental illness, and stress-caused damage through reducing cortisol level, inflammation, and oxidative damage (Felice and O'Mahony, 2017; Misra and Mohanty, 2017). Probiotic and prebiotic have also been reported to regulate bone health in humans and a variety of animals (Scholz-Ahrens et al., 2007; McCabe et al., 2015). Similarly, synbiotics, as a combination of probiotics and prebiotics, are able to improve femoral health in laying hens (Yan et al., 2018). However, the synbiotic function in broiler bone health, especially under high ambient temperature conditions, has not been examined. The objective of this study was to determine the effects of synbiotic on bone health in broilers subjected to cyclic heat stress episodes. We hypothesized that a dietary synbiotic would improve bone health and reduce lameness in broilers exposed to heat stress.

## MATERIALS AND METHODS

### *Birds, Management, and Diets*

A total of 360 1-d-old Ross 708 broiler chicks were obtained from a commercial hatchery (Miller Poultry, Orland, IN). Birds were weighed in groups of 15 chicks and placed into 1 of 24 floor pens (110 × 110 cm each), ensuring each pen had a similar average BW, within the same room at the Poultry Research Farm of Purdue University. The pens were randomly assigned to 1 of 3 dietary treatments of 8 replicates each for 42 d: a regular diet (Table 1) and the regular diet mixed with a multispecies synbiotic (PoultryStar® me, BIOMIN America, Inc., Overland Park, KS) at 0.5 g/kg (0.5X) or 1.0 g/kg of feed (1.0X). The composition of the synbiotic includes 4 probiotic strains (*Enterococcus faecium*,

**Table 1.** The diet ration formulation.

	Starter	Grower	Finisher
<b>Ingredient, %</b>			
Corn	52	52.3	62.8
Soybean meal, 48 % crude protein	40	39.1	29.7
Soybean 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 phosphate	1.75	1.48	1.17
Vitamin/mineral premix <sup>1</sup>	0.35	0.35	0.35
<b>Calculated analyses</b>			
Crude protein %	23.4	22.8	19.2
ME kcal/kg	3050	3151	3200
Ca %	0.95	0.85	0.75
Available P %	0.5	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

<sup>1</sup>Provided per kilogram 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 dihydroiodide, 2.10 mg; selenium from sodium selenite, 0.30 mg.

*Pediococcus acidilactici*, *Bifidobacterium animalis*, and *Lactobacillus reuteri*) and a prebiotic (fructooligosaccharides). The starter, grower, and finisher diets (Aviagen, 2014) were fed from d 1 to 14, 15 to 28, and 29 to 42, respectively. Room temperature was gradually decreased from 34°C on d 1 by 0.5°C/d for the first 14 d, and then a daily cyclic heat stress episode (32°C/9 h/d from 0800 to 1700 h) was applied from d 15 to 42. The lighting program was 24 L:0 D at d 1, 23L:1D at 30 lux at d 2 and 3, then 20L:4D at 10 lux until d 42. Feed and water were provided ad libitum throughout the trial. The experimental protocol was approved by the Purdue Animal Use and Care Committee (Number: 1111000262).

### *Data Collection*

At 40 d of age, 7 broilers per pen were randomly taken and individually evaluated for walking ability using a 3-point gait score system (0 = normal gait, 1 = gait with obvious impairment, and 2 = gait with severe impairment) as previously described (Webster et al., 2008). Briefly, each tested bird was allowed to walk a distance of 1 m in the floor pen where they were housed. The test was performed by one observer constantly. For statistical analysis, a mean gait score of each pen was calculated as the cumulated total scores divided by the bird number. The proportion of broilers per pen within a gait score (0, 1, or 2) was also calculated and expressed as a percentage.

At 41 d of age, 2 untested broilers per pen were randomly taken for the latency-to-lie test by using the

procedure described previously (Berg and Sanotra, 2003). Briefly, each tested bird was placed into a water tub filled with 3 cm deep of water at 28°C. The time which took the bird to sit down and touch the water was recorded. The test was stopped if a broiler still stood after 600 s and the observation of 600 s was recorded.

At 42 d of age, 2 broilers per pen (not used for gait score and latency-to-lie tests) were randomly taken, weighted, and sedated by using sodium pentobarbital (30 mg/kg of BW) injected intravenously via the brachial vein. Following cervical dislocation, the left wing, thigh, and drum were collected from each broiler, then frozen (−20°C) until analysis.

### Dual-energy X-ray Absorptiometry

The collected bone samples were thawed and scanned using a dual-energy X-ray absorptiometry equipment (Norland Medical Systems, Inc., Fort Atkinson, WI) with muscle, skin, and feathers intact to quantify areal bone mineral density (BMD), bone mineral content (BMC), and bone area of the whole humerus, femur, and tibia with fibula following the procedure previously reported (Hester et al., 2013).

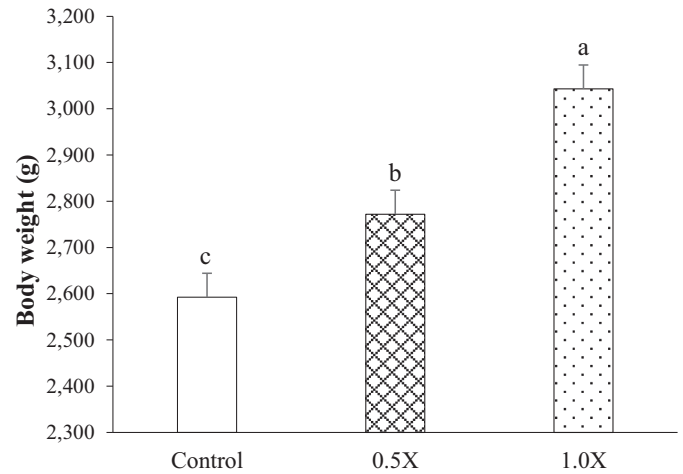
### Statistical Analysis

The experimental design was conducted in a randomized block design. Pen was considered as the experimental unit ( $n = 8$ ). The data were analyzed by using a 1-way ANOVA of the mixed model procedure of SAS 9.4 software (SAS Institute, Inc., Cary, NC). The fixed factor was the synbiotic treatment. The proportion of broilers within a gait score (0, 1, or 2) was analyzed by a nested design as treatment nested in gait score. The BW was used as a covariate for the measures of bone mineralization and bone area when necessary (Steel et al., 1997). Transformation of data was performed for normality when variances were not homogeneous (Steel et al., 1997). Statistical trends were similar for both transformed and untransformed data; therefore, the untransformed least square means and SEM were presented. The Tukey–Kramer test was used to analyze the differences among the means. Statistical significance was set at  $P < 0.05$ .

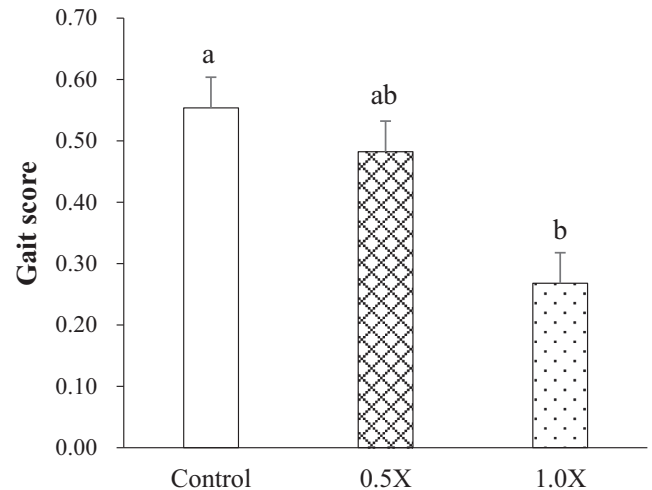
## RESULTS

Compared to controls, synbiotic fed broilers had a heavier BW, showing an increase with the dosage, i.e., 1.0X group > 0.5X group > controls ( $P = 0.01$ , Figure 1).

Gait score of the broilers was decreased with increased dosage in the order of controls > 0.5X group > 1.0X group (Figure 2); however, the significant difference was found between 1.0X group and control group only ( $P = 0.05$ ). In the current study, most of the broilers were categorized with a score of 0 (normal gait)



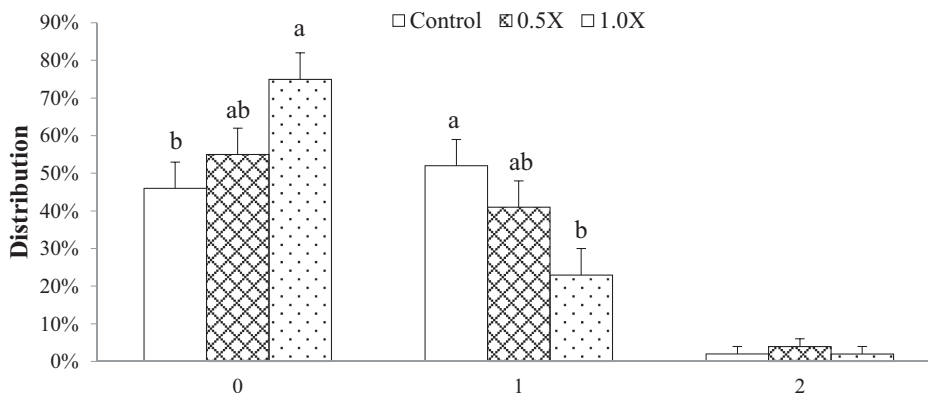
**Figure 1.** Effect of the dietary synbiotic inclusion on the BW of 42-day-old broilers subjected to daily cyclic heat stress episodes. Least square means lacking a common superscript differ ( $P < 0.05$ ). The synbiotic dosage was 0 (control), 0.5 (0.5X), or 1.0 (1.0X) g/kg of feed.  $N = 8$  replicates with the averaged data from 2 broilers each.



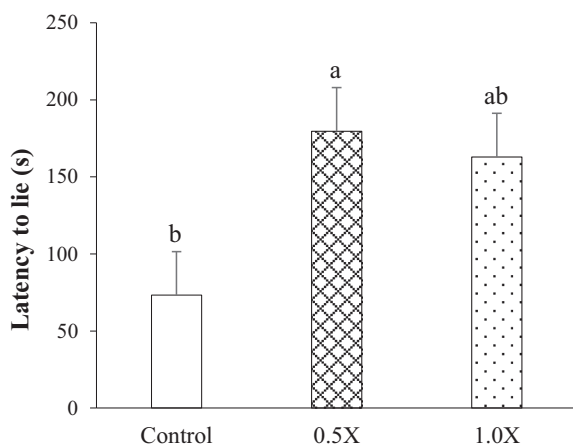
**Figure 2.** Effect of the dietary synbiotic inclusion on gait score in 40-day-old broilers subjected to daily cyclic heat stress episodes. Least square means lacking a common superscript differ ( $P < 0.05$ ). The synbiotic dosage was 0 (control), 0.5 (0.5X), or 1.0 (1.0X) g/kg of feed.  $N = 8$  replicates with the averaged data from 7 broilers each.

or 1 (obvious lameness), and only a small proportion (<2%) identified with a score of 2 (severe lameness) (Figure 3). There was a dosage effect of the synbiotic on the percentage of broiler in each gait score category. The proportion of broilers with a normal gait (score of 0) was increased with the synbiotic dosage: 46% (control) < 55% (0.5X group) < 75% (1.0X group) ( $P = 0.03$ , Figure 3), whereas the broilers with lameness (score 1) were decreased: 52% (controls) > 41% (0.5X group) > 23% (1.0X group) ( $P = 0.02$ , Figure 3). No differences were detected in broilers with obvious lameness (gait score 2) among treatments ( $P = 0.77$ ). Compared to the control group, the standing time during the latency to lie test was significantly increased in the 0.5 X group ( $P = 0.03$ ; Figure 4), and the 1.0 X group showed a tendency to increase ( $P = 0.08$ ).





**Figure 3.** The percentage of broilers within each gait score category by dietary treatments. Least square means lacking a common superscript differ ( $P < 0.05$ ). The synbiotic dosage was 0 (control), 0.5 (0.5X), or 1.0 (1.0X) g/kg of feed.  $N = 8$  replicates with the percentage data from 7 broilers per pen.



**Figure 4.** Effect of the dietary synbiotic inclusion on the latency-to-lie test of 41-day-old broilers subjected to daily cyclic heat stress episodes. Least square means lacking a common superscript differ ( $P < 0.05$ ). The synbiotic dosage was 0 (control), 0.5 (0.5X), or 1.0 (1.0X) g/kg of feed.  $N = 8$  replicates with the averaged data from 2 broilers of each.

Compared to control group, the mineralization (BMD and BMC) and area of both tibia and femur were increased in 1.0X group only ( $P < 0.05$  and  $0.01$ ; Table 2). 1.0X broilers also had a higher BMD ( $P = 0.05$ ) and BMC ( $P = 0.003$ ) of humerus with a tendency of higher area ( $P = 0.09$ ). When BW was taken into account, the significance of tibia and femur was vanished, but not the humerus.

## DISCUSSION

The thermal condition used in the current study, daily cyclic heat episode ( $32^{\circ}\text{C}/9\text{ h/d}$ ), as an environmental stressor, causing heat stress has been evidenced in previous studies (Huang et al., 2015; Mahmoud et al., 2015; Lu et al., 2017). In one of our parallel studies, Mohammed et al. (2018) reported that stressed broilers had higher panting and wing spreading, and these heat stress-associated behaviors were reduced by the synbiotic supplementation. In the current study,

**Table 2.** Effect of the dietary synbiotic inclusion on bone mineralization and area of 42-day-old broilers subjected to daily cyclic heat stress episodes.

Item	Treatment <sup>1</sup>			SEM	$P$	Adjusted $P^2$
	Control	0.5X	1.0X			
<b>Tibia</b>						
BMD ( $\text{g}/\text{cm}^2$ )	0.209 <sup>b</sup>	0.216 <sup>a,b</sup>	0.226 <sup>a</sup>	0.004	0.03	0.97
BMC (g)	2.82 <sup>b</sup>	3.02 <sup>a,b</sup>	3.30 <sup>a</sup>	0.09	0.002	0.76
Area ( $\text{cm}^2$ )	13.46 <sup>b</sup>	13.98 <sup>a,b</sup>	14.62 <sup>a</sup>	0.25	0.008	0.59
<b>Femur</b>						
BMD ( $\text{g}/\text{cm}^2$ )	0.180 <sup>b</sup>	0.185 <sup>b</sup>	0.199 <sup>a</sup>	0.004	0.006	0.81
BMC (g)	1.80 <sup>b</sup>	1.92 <sup>b</sup>	2.21 <sup>a</sup>	0.08	0.002	0.81
Area ( $\text{cm}^2$ )	9.99 <sup>b</sup>	10.39 <sup>a,b</sup>	11.08 <sup>a</sup>	0.25	0.01	0.82
<b>Humerus</b>						
BMD ( $\text{g}/\text{cm}^2$ )	0.215 <sup>b</sup>	0.214 <sup>b</sup>	0.234 <sup>a</sup>	0.006	0.05	0.05
BMC (g)	1.60 <sup>b</sup>	1.62 <sup>b</sup>	1.87 <sup>a</sup>	0.05	0.0003	0.0003
Area ( $\text{cm}^2$ )	7.47	7.62	8.00	0.17	0.09	0.09

<sup>a,b</sup>Least square means within a row lacking a common superscript differ ( $P < 0.05$ ).

$N = 8$  replicates with the averaged data from 2 broilers each.

<sup>1</sup>The synbiotic dosage was 0 (control), 0.5 (0.5X), or 1.0 (1.0X) g/kg of feed.

<sup>2</sup>BW was used as a covariate when significant.

BMD: bone mineral density; BMC: bone mineral content.

the BW of the synbiotic fed broilers was remarkably improved as compared to controls followed heat stress regardless of synbiotic dosage. Similar results of BW change have been reported in one of our parallel studies previously (Mohammed et al., 2018). In that study, the average BW was 2412.50 g (control group), 2515.86 g (0.5X group), and 2615 (1.0X group). Similarly, improved performance has been reported in broilers fed a diet with prebiotic (Sohail et al., 2012) or with probiotics such as *Lactobacillus* strains under heat stress (Faseleh Jahromi et al., 2016; Shokryazdan et al., 2017). However, whether the prebiotic or probiotic of the synbiotic alone has the similar functions will be tested in the future studies.

The cyclic heat stress, as previously reported, imposed with the intent to aggravate leg health; all measured parameters of skeletal health in the current study,

including bone mineralization (BMD and BMC) of leg and wing bones, gait score, and latency to lie, showed benefits in broilers fed the diet with the synbiotic. Both gait score and the latency to lie have been used for examining leg strength in broilers (Rault et al., 2017; Sun et al., 2018). In the current study, the broilers fed the synbiotic had a better gait score (1.0X group) and latency-to-lie test result (0.5X group and a tendency for 1.0X group) under heat stress. The results were in line with the changes of mineralization in both femur and tibia of 1.0X hens. It is interesting to find out that the synbiotic-induced improvement of leg strength occurred without detrimentally affecting growth. These results are contrary to the previous findings that gait score is positively correlated with BW, the heavier broilers having the poorer walking ability (Sorensen et al., 2000; Brickett et al., 2007). Broilers of the 1.0X group, compared to the controls, had a heavier BW with a better gait score. These differences could be related to improved resorption of intestinal nutrients and minerals as that feed intake was increased in the synbiotic fed broilers, especially in the 1.0X group (Mohammed et al., 2018). Similarly, dietary supplement with prebiotics, probiotics, or synbiotics in humans has significant effects on bone mineral metabolism (Skrypnik and Suliburska, 2018) and treatment of various disorders including rheumatoid arthritis (Khanna et al., 2017). The current results indicate that the walking ability of broilers under heat stress can be improved by feeding the synbiotic.

Between the 2 dosages of the synbiotic used in the current study, the higher dose (1.0X) fed broilers had better results as compared to the lower dosage (0.5X) fed broilers except for the latency-to-lie test. The reaction of the 0.5X group was intermediate and in many instances the changes did not differ from the controls. The synbiotic dosage effects may be related to the changes of the diversity of the intestinal microbiota (or microbiome) in the treated broilers. One of our parallel studies had reported that there was an effect of synbiotic dosage on beneficial bacterial counts (log 10 cfu/g) in the cecum of heat stressed broilers at 42 d of age, such as *Lactobacilli* spp., 6.05 (control) < 6.45 (0.5X) < 6.99 (1.0X) and *Bifidobacterium* spp., 5.44 (control) < 5.86 (0.5X) < 6.14 (1.0X) (Mohammed et al., 2017). However, the significant difference ( $P < 0.05$ ) was found between the control and 1.0X groups only. Both *Lactobacilli* spp. and *Bifidobacterium* spp. have been used as probiotics for improving health status and welfare in various animal species including poultry under both thermoneutral and heat stress conditions (Song et al., 2014; Faseleh Jahromi et al., 2016; Shokryazdan et al., 2017).

Several studies indicate that consuming synbiotics lead to improved skeletal health in poultry. Wideman et al. (2012) reported that broilers reared on wire floor to induce the bone disease showed a reduction in lameness by feeding the same synbiotic (at 0.55 g/kg

of feed, beginning at 1 d of age). Similarly, broilers raised on wire floor experienced a delayed onset as well as a lower incidence of bacterial chondronecrosis with osteomyelitis after fed a commercial synbiotic product including mannan oligosaccharide beta-glucan yeast cell wall and *Bacillus subtilis* (Wideman et al., 2015). Additionally, feeding a single species probiotic, *E. faecium* (0.55 g/kg of feed), beginning at 1 d of age, to broilers reared on wire resulted in a low incidence of femoral head transitional degeneration and tibial head necrosis (Wideman et al., 2012). Other studies in poultry have also shown that probiotic supplementation improves bone mass in laying hens (Abdelqader et al., 2013) and broilers (Sadeghi, 2014). Collectively, the current study provides further evidence that the synbiotic, PoultryStar, may improve skeletal health in broilers under heat stress.

The mechanisms of the synbiotic effects on skeletal health were not examined in this study. In the current study, however, the improved mineralization of leg bones, namely tibia and femur, may be induced by larger body size, as the significance was eliminated when BW was used as a covariate. But, BW was not a significant contributor for the increased bone mass of the humerus. Talaty et al. (2009) also reported that BW was not a covariate for bone width in broilers. Similar to the mechanisms found in humans (Skrypnik and Suliburska, 2018) and rodents (Scholz-Ahrens et al., 2016), the synbiotic-associated bone mass accrual may be related to the improvement of intestinal nutrients and minerals resorption. Previous studies have evidenced that synbiotics improve intestinal integrity, resulting in increasing absorption and bioavailability of minerals such as calcium and phosphorus for bone mineralization (Asemi et al., 2017). Compared with the functions under thermoneutral conditions, synbiotics in broilers under high ambient temperatures may be even more effective in enhancing intestinal mineral and nutrient absorption. It has been reported that under heat stress the inclusion of probiotics, prebiotics, or their combination improved gut health in both broilers (Sohail et al., 2012; Song et al., 2014) and laying hens (Deng et al., 2012). Chronic heat stress in broilers causes intestinal injury, such as reduced villus height (Sohail et al., 2012) and the reactive oxygen species-associated oxidative damage (Huang et al., 2015), by which heat stress hampers intestinal absorption of nutrients and minerals, such as Ca.

Taken together, the current study demonstrated that the synbiotic dietary inclusion increased BW, bone mineralization, and improved walking ability in broilers exposed to a daily cyclic heat episode. The results suggest that dietary supplementation of the multispecies synbiotic may be an alternative management strategy for effectively improving broiler health and welfare as well as production performance during hot weather, especially in the tropical and subtropical regions.

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## ABBREVIATIONS

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The following abbreviations may be used without definition in *Poultry Science*. Plural abbreviations do not require "s". Chemical symbols and three-letter abbreviations for amino acids do not need definition. Units of measure, except those shown below, should be abbreviated as listed in the *CRC Handbook for Chemistry and Physics* (CRC Press, 2000 Corporate Blvd., Boca Raton, FL 33431) and do not need to be defined.

A	adenine	MHC	major histocompatibility complex
ADG	average daily gain	mRNA	messenger ribonucleic acid
ADFI	average daily feed intake	min	minute
AME	apparent metabolizable energy	mo	month
AME <sub>n</sub>	nitrogen-corrected apparent metabolizable energy	MS	mean square
ANOVA	analysis of variance	n	number of observations
B cell	bursal-derived, bursal-equivalent derived cell	N	normal
bp	base pairs	NAD	nicotinamide adenine dinucleotide
BSA	bovine serum albumin	NADH	reduced nicotinamide adenine dinucleotide
BW	body weight	NRC	National Research Council
C	cytosine	NS	not significant
cDNA	complementary DNA	PAGE	polyacrylamide gel electrophoresis
cfu	colony-forming units	PBS	phosphate-buffered saline
CI	confidence interval	PCR	polymerase chain reaction
CP	crude protein	QTL	quantitative trait loci
cpm	counts per minute	pfu	plaque-forming units
CV	coefficient of variation	r	correlation coefficient
d	day	r <sup>2</sup>	coefficient of determination, simple
df	degrees of freedom	R <sup>2</sup>	coefficient of determination, multiple
DM	dry matter	RFLP	restriction fragment length polymorphism
DNA	deoxyribonucleic acid	RH	relative humidity
EDTA	ethylenediaminetetraacetate	RIA	radioimmunoassay
ELISA	enzyme-linked immunosorbent antibody assay	RNA	ribonucleic acid
EST	expressed sequence tag	rpm	revolutions per minute
g	gram	s	second
g	gravity	SD	standard deviation
G	guanine	SDS	sodium dodecyl sulfate
GAT	glutamic acid-alanine-tyrosine	SE	standard error
G:F	gain-to-feed ratio	SEM	standard error of the mean
GLM	general linear model	SNP	single nucleotide polymorphism
h	hour	SRBC	sheep red blood cells
HEPES	N-2-hydroxyethyl piperazine-N'-ethane-sulfonic acid	T	thymine
HPLC	high-performance (high-pressure) liquid chromatography	TBA	thiobarbituric acid
ICU	international chick units	T cell	thymic-derived cell
Ig	immunoglobulin	TME	true metabolizable energy
IL	interleukin	TME <sub>n</sub>	nitrogen-corrected true metabolizable energy
IU	international units	Tris	tris(hydroxymethyl)aminomethane
kb	kilobase pairs	TSAA	total sulfur amino acids
kDa	kilodalton	U	uridine
L	liter*	USDA	United States Department of Agriculture
L:D	hours light:hours darkness in a photoperiod	UV	ultraviolet
m	meter	vol/vol	volume to volume
μ	micro	vs.	versus
M	molar	wt/vol	weight to volume
MAS	marker-assisted selection	wt/wt	weight to weight
ME	metabolizable energy	wk	week
ME <sub>n</sub>	nitrogen-corrected metabolizable energy	yr	year

\*Also capitalized with any combination, e.g., mL.

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