Effect of a synbiotic supplement as an antibiotic alternative on broiler skeletal, physiological, and oxidative parameters under heat stress

J. Y. Hu,* A. A. Mohammed,*,† G. R. Murugesan,‡,§ and H. W. Cheng [#,1]

*Department of Animal Sciences, Purdue University, West Lafayette IN 47907, USA; †Department of Animal and Poultry Behavior and Management, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt; ‡BIOMIN America Inc., Overland Park, KS 66210, USA; §BIOMIN Holding GmbH, Getzersdorf, Austria; and #USDA-Agricultural Research Service, Livestock Behavior Research Unit, West Lafayette, IN 47907, USA

ABSTRACT The aim of this study was to examine if synbiotics can function as alternatives to antibiotics in broiler production under heat stress (**HS**). Day-old broiler chicks (528 birds) were randomly placed in floor pens within 2 identical temperature-controlled rooms (11 birds/pen and 24 pens/room). The pens of each room were evenly divided among 3 treatments (n = 8): basal diet (**CON**), the basal diet mixed with 50 ppm of bacitracin methylene disalicylate (**BMD**) or a synbiotic (50 ppm of PoultryStar me^{US}, **SYN**). From d 15, room 2 was under thermoneutral (**TN**) conditions (TN-CON, TN-BMD, and TN-SYN), while HS was applied to room 1 at 32°C for 9 hrs/d (0800 to 1700) (HS-CON, HS-BMD, and HS-SYN). Treatment effects on footpad dermatitis and gait score were measured on 5 birds/pen, and latency to lie (**LTL**) test was measured on 2 birds/pen at d 27 and d 41; and 1 broiler/pen was sampled on d 28 and d 42, respectively. Body, liver, and spleen weight were determined. Plasma levels of interleukins (**IL**), heat shock protein 70, immunoglobulin (**Ig**)Y, liver superoxide dismutase (**SOD**) and glutathione peroxidase (**GPx**) enzyme activities were examined. Heat stress suppressed BW and IgY concentrations on both d 28 and d 42, while suppressed plasma IL-6 concentrations, SOD activities, and LTL duration on d 28 only ($P < 0.05$). Among all treatments, SYN birds had the best foot and skeletal health scores on both d 27 and d 41 ($P < 0.05$). On d 42, SYN increased BW, and TN-SYN birds had higher relative spleen weight than both TN-BMD and TN-CON birds ($P < 0.05$). Antibiotic BMD increased BW ($P < 0.05$) but decreased SOD activities ($P < 0.05$) on d 42. These results indicate that the SYN supplementation decreases HS negative effect on broilers by improving BW, foot, and skeletal health, while BMD improves BW but also increases oxidative stress in broilers. The data suggest that synbiotic supplement may function as an alternative to antibiotics in broiler production during summer seasons, especially in the tropical and subtropical regions.

Key words: broiler, heat stress, synbiotic, antibiotic, welfare

INTRODUCTION

Heat stress (**HS**) is one of the top welfare issues affecting global poultry production. Chickens' thermoneutral zone is relatively narrow (16−25°C) and is vulnerable to HS due to the lack of sweat glands and feather coverage (Liu et al., 2020). Ambient temperature elevated above the upper limit of thermoneutral zone negatively affects production performance and skeletal health, compromises gut integrity, and related immune function, in severe cases, leading to morbidity and mortality (Lara and Rostagno, 2013). Excessive heat reduces calcium (**Ca**) absorption and decreases the metabolic conversion of vitamin D3 (cholecalciferol) to its biologically active form, 1,25-dihydroxyvitamine D3 (**1,25 (OH)$_2$D$_3$**) (Petruk and Korver, 2004), by which it reduces bone density (lowing Ca storage in bone), leading to poor skeletal health (Christakos et al., 2010). Femoral lesions have been found to be more severe in broilers under HS, which accelerates the development of lameness (Wideman and Pevzner, 2012). In addition, HS damages the integrity of intestinal tight junctions, increasing the permeability of pathogenic bacteria and related toxins from the gut to the systemic circulation (leaky gut), consequently elevating the incidence of bone diseases such as lameness, resulting in poor walking ability and footpad dermatitis (**FPD**) (Wideman, 2016).

Heat exposure suppresses both cell mediated and humoral immune responses, leading to alterations of cytokines and immunoglobulin syntheses. Under HS, there is an increase in the production of reactive oxygen species (**ROS**), causing oxidative stress and related inflammatory reactions in injured tissues (Montilla et al., 2014; Slimen et al., 2014). Excessive amounts of ROS enhance metabolic disorders of lipids, proteins, and nucleic acids, resulting in heat-induced oxidative damage by suppressing antioxidative enzymes, superoxide dismutase (**SOD**) and glutathione peroxidase (**GPx**), ultimately causing cell death (Slimen et al., 2014; Akbarian et al., 2016; Ghosh et al., 2018). The synthesis of heat shock proteins (**HSP**s) is associated with cytoprotective effects against HS, which prevents protein improper folding or damage and inhibits apoptosis of cells (Richter et al., 2010).

Subtherapeutic antibiotics, such as bacitracin methylene disalicylate (**BMD**), have been used as feed additives to prevent inflammation and infectious diseases and to promote growth performance in animal production since 1920s (Engberg et al., 2000; Castanon, 2007). One of the active models of antibiotics is to directly inhibit certain intestinal pathogens for reducing inflammation (Shin et al., 2020; Strzępa et al., 2017). However, use of antibiotics in animal husbandry has caused public concerns about drug residues in meat products and the development of antibiotic-resistant bacteria (Kirchhelle et al., 2020; Nadeem et al., 2020). Many countries have already taken actions to control antibiotics usage, and the use of antibiotics for growth promotion (**AGP**s) has been banned in European Union since 2006 (Millet and Maertens, 2011). In 2012, the United States Food and Drug Administration (**FDA**) issued a proposal that requires veterinarians' oversight for using antibiotics in livestock to limit preventive use of antibiotics (Kuehn, 2014). Eliminating antibiotics in poultry production with no reliable alternatives has raised considerable consequences, such as comprised production performance and increased incidence of infectious diseases and associated mortality (Cervantes, 2015). Restoration of disrupted gut microbiota and impacted intestinal homeostasis without medical intervention has become a critical issue in the poultry industry. Several alternatives of non-therapeutic antibiotics have been tested, where prebiotics and probiotics have been considered for replacing antibiotics as growth promoters and prevention of diseases in poultry productions (Redweik et al., 2020). A synbiotic is defined as "a mixture of probiotics and prebiotics that aids the host by improving the survival and activity of beneficial microorganisms in the gut" (Gyawali et al., 2019). The beneficial effects of supplementing synbiotics include improvement of growth performance, gut integrity, and immune function as well as inhibition of colonization of pathogens in broilers with and without stress challenges (Awad et al., 2009; Hassanpour et al., 2013; Min et al., 2016; Chen et al., 2018; Yan et al., 2019). Currently, there are limited studies comparing the effects of synbiotics and antibiotics on growth and skeletal health of broilers under hot ambient conditions. Particularly, climate change in the recent decades has resulted in more hot days with more frequent and unexpected heat waves. Therefore, the objective of this study was to determine if effects of synbiotics on production, skeletal health, immune response, and oxidative status are comparable with antibiotics in broilers subjected to HS. We hypothesize that synbiotics would perform at the levels similar to antibiotics in HS broilers due to their multiple beneficial effects on gut health and immune function.

MATERIALS AND METHODS

Birds and Treatment

Day-old Ross 708 male broiler chicks (528 birds) were obtained from a commercial hatchery (Townline Poultry Farm, Zeeland, MI). The chicks were weighed in a group of 11 birds each, then randomly assigned to floor pens (0.17 m^2/bird) with similar average BW within 2 identical temperature- and lighting-controlled rooms (24 pens/room). Birds were reared for 42 d. The pens of each room were divided among 3 dietary treatments (n = 8): basal diets (**CON**), the basal diets mixed with antibiotic (**BMD**. 50 ppm, bacitracin methylene disalicylate, BMD50, Zoetis, Kalamazoo, MI) or a synbiotic (**SYN**, 50 ppm, PoultryStar me^{US}, BIOMIN America Inc., Overland Park, KS; Table 1). The SYN contains a prebiotic, fructo-oligsaccharides, and a probiotic mixture of 4 microbial strains, *Bifidobacterium animalis, Pediococcus acidilactici, Enterococcus faecium,* and *Lactobacillus reuteri.* The level of the SYN was recommended by the company (BIOMIN America Inc., San Antonio, TX) and tested in our previous studies (Yan et al., 2019). The antibiotic dose was adapted from the previous studies conducted in broilers (Koltes et al., 2017; de Oliveira et al., 2019; Zoetis, 2020). One room was maintained under thermoneutral condition (**TN,** 24 ± 2°C); while HS was applied to another room (32°C for 9 h, 0800−1700) from d 15 to d 42. There were 6 treatments defined as: TN-CON, TN-BMD, TN-SYN, HS-CON, HS-BMD, and HS-SYN, respectively. The bird management was performed according to the guidelines of Ross broilers (Aviagen, 2018). Feed and water were provided with free access. The diets were mixed at the Purdue University feed mill (West Lafayette, IN) using a step-increase procedure (Mohammed et al., 2018). Room temperature and humidity were monitored by data loggers (HOBO, Onset Computer Corporation, Bourne, MA) fixed 30 cm above the litter surface. All procedures of animal care used in this experiment were approved by the Purdue Animal Use and Care Committee (PACUC Number: 1712001657).

Physiological Sampling

On d 28 and 42, 1 bird per pen was used for sample collection, respectively (8 birds/treatment/time point). To balance sampling effects on the measured parameters, the sampled birds of each treatment were taken by

Table 1. Basal diet ration formulation.

	Starter (1−14 d)	Grower (15−28 d)	Finisher (29−42 d)
Ingredient, %			
Corn ground	57.66	63.76	66.9
Soybean meal 47.5%	35.27	29.68	26.3
Soybean oil degummed	3.00	3.00	3.52
Calcium carbonate	1.41	1.38	1.49
Phosphate monocalcium	1.42	1.02	0.82
L-Lysine	0.11	0.1	0.02
Salt plain	0.48	0.46	0.48
L-Threonine 98%	0.06	0.04	0.00
DL-Methionine	0.24	0.21	0.12
Vitamin/mineral premix[1]	0.35	0.35	0.35
Calculated analyses			
Crude protein %	23.4	22.8	19.2
Poultry ME kcal/kg	3050	3151	3200
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 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 diaminedihydroidide, 2.10 mg; selenium from sodium selenite, 0.30 mg.

repeating the cycle of the TN-CON, TN-BMD, TN-SYN, HS-CON, HS-BMD, and HS-SYN until the end. Each sampled bird was weighed and sedated with sodium pentobarbital (30 mg of pentobarbital/kg of BW) injected into the brachial vein; then 10 mL blood was collected into an EDTA (Ethylenediaminetetraacetic acid) coated tube through cardiac puncture. The birds were euthanized via cervical dislocation immediately after blood collection. Whole liver and spleen were weighed and reported as both absolute and relative organ weight (absolute organ weight/body weight). Approximately 1 cm^2 sample of each liver were collected from the same location for oxidative status analysis. Blood samples were centrifuged at 700 × g for 20 min at 4°C for plasma collection. Plasma and liver tissue samples were stored at −80°C until further analysis.

Plasma Cytokines and Immunoglobulin Y

Plasma levels of interleukin (**IL**)-2, IL-6, IL-10, HSP 70 (Catalog#: MBS005165, MBS037319, MBS007312, MBS017726, MyBioSource, San Diego, CA), and immunoglobulin (**Ig**) Y (Catalog#: E33-104, Bethyl, Montgomery, TX) were measured using the commercially available ELISA kits following the individual manufacturer's protocols. The optical density of each sample was read at 450 nm (Epoch Microplate Spectrophotometer, BioTek, Winooski, VT). All samples were measured in duplicates with CV ≤15%.

Liver Superoxide Dismutase Enzyme and Glutathione Peroxidase Enzyme Activities

The liver SOD (ESOD-100, BioAssay Systems, Hayward, CA) and GPx (EGPX-100, BioAssay Systems)

enzyme activities were measured using commercially available kits following the corresponding manufacturer's protocols. The optical density of SOD and GPx was read at 440 nm and 340 nm, respectively, using a microplate spectrophotometer (BioTek). All samples were measured in duplicates with CV ≤15%.

Gait Score, Footpad Dermatitis, and Latency to Lie

Gait Score (**GS**), FPD, and LTL were tested on d 27 and 41, respectively. Gait score was determined from the marked 5 birds (labeled with leg bands) per pen (40 birds/treatment: 5 birds/pen × 8 pens/treatment) using a 3-point gait scoring system adopted from Webster et al. (2008): score 0, birds with no impairment of walking ability; score 1, birds with obvious impairment but still ambulatory; and score 2, birds with severe impairment and not able to walk. The numbers collected from the 5 birds were averaged for statistical analysis.

Footpad dermatitis was scored from the left foot of the same 5 birds per pen using a 0 to 2 scale, 0 indicates no blemish or discoloration of foot pad, 2 indicates severe lesion with black coloration, and 1 intermediate. The numbers collected from the 5 birds were averaged for statistical analysis (Kjaer et al., 2006).

The LTL test was performed by modified the protocol published previously (Webster et al., 2008). Briefly, birds were made to stand in a container (size 45 × 40 × 30 cm) with shallow warm water inside the testing room. The time it took for the bird to first attempt to sit down was recorded. The test was terminated after 600 s if the bird was still standing. Two birds per pen were randomly selected for the testing, and the

results were averaged for statistical analysis (16 birds/treatment). To avoid reuse of the birds in the following test and sample collection, the tested bird was marked for identification.

Statistical Analysis

A 3 × 2 factorial design was used in the study, and the main factors were diet (CON, BMD, and SYN) and room temperature (TN and HS). Each pen was considered as an experiment unit. Physiological data were subjected to two-way ANOVA using the MIXED method of SAS 9.4 software (SAS Institute Inc., Cary, NC). A one-way ANOVA was used to partition treatment difference under each environmental condition, Tukey-Kramer test was used to partition the difference among the treatments if there was significance. A two-way non-parametric analysis Aligned Ranks (**ART**) was used for LTL, FPD, and GS results. Transformation of data (Box-Cox or log transformation) was performed if the data were not normally distributed or not homoscedastic.

Statistical trend was similar for both transformed and untransformed data, thus untransformed data were reported. The data were presented as least square means (**LSMeans**) ± standard error of the mean (**SEM**). Statistical difference was reported when $P \leq 0.05$, and a trend was reported when $0.05 < P \leq 0.10$.

RESULTS

Production Performance and Physical Parameters

On d 28, HS suppressed BW ($P_{temperature} = 0.01$) and absolute liver weight ($P_{temperature} = 0.05$) without dietary and temperature interaction effect (Table 2). No overall dietary influence was found in liver weight, but under TN condition, CON birds tended to have lower relative liver weights than SYN ($P = 0.08$) and BMD ($P = 0.08$) supplemented birds. Among HS treatments, HS-SYN birds had greater relative spleen weight than HS-CON ($P = 0.01$) but not HS-BMD birds. Absolute

Table 2. Effect of the dietary synbiotic inclusion on body and organ weights of 28-day-old and 42-day-old broilers under thermoneutral and heat stress conditions.

	Absolute liver weight (g)	Relative liver[1]	Absolute spleen weight (g)	Relative spleen[1]	BW (kg)
28-d					
TN-CON	28.83	1.99[Y]	1.19	0.83	1.44
TN-BMD	32.20	2.30[X]	1.44	1.04	1.39
TN-SYN	29.76	2.08[X]	1.26	0.89	1.42
HS-CON	28.91	2.12	1.14	0.84[Y]	1.36
HS-BMD	27.58	2.11	1.29	0.99[XY]	1.32
HS-SYN	25.46	2.08	1.34	1.09[X]	1.22
Feed additives					
CON	28.87	2.06[B]	1.16	0.84[B]	1.40
BMD	29.89	2.21[A]	1.37	1.02[A]	1.35
SYN	27.61	2.08[B]	1.30	0.99[AB]	1.32
Temperature					
TN	30.26[a]	2.13	1.30	0.92	1.42[a]
HS	27.32[b]	2.10	1.26	0.98	1.30[b]
SEM	0.99	0.05	0.06	0.06	0.03
P-value					
Diet	0.41	0.08	0.18	0.10	0.50
Temperature	0.05	0.74	0.58	0.33	0.01
Diet × temperature	0.34	0.17	0.47	0.14	0.36
42-d					
TN-CON	41.58[Y]	1.54	2.24[XY]	0.84[y]	2.70[y]
TN-BMD	54.26[X]	1.90	2.11[Y]	0.74[y]	2.86[x]
TN-SYN	53.74[X]	1.78	2.81[X]	0.93[x]	3.00[x]
HS-CON	43.87	1.69	1.98	0.76	2.59[y]
HS-BMD	43.73	1.76	2.23	0.90	2.49[xy]
HS-SYN	46.85	1.70	2.19	0.79	2.75[x]
Feed additives					
CON	42.72[B]	1.61	2.11	0.80	2.65[a]
BMD	49.00[A]	1.83	2.17	0.82	2.67[a]
SYN	50.30[A]	1.74	2.50	0.86	2.67[a]
Temperature					
TN	49.86[a]	1.74	2.39[A]	0.84	2.85[a]
HS	44.82[B]	1.72	2.13[B]	0.82	2.61[b]
SEM	2.56	0.11	0.19	0.06	0.08
P-value					
Diet	0.10	0.17	0.17	0.70	0.04
Temperature	0.08	0.83	0.08	0.68	0.0003
Diet × temperature	0.17	0.37	0.10	0.02	0.20

[a,b]Least squares means within a column for the 3 dietary treatments under 2 temperature conditions lacking a common superscript differ ($P \leq 0.05$. n = 8 per treatment) and [AB]least square means represents a trend difference ($0.05 < P \leq 0.1$).

[x,y]Least squares means within a column for the 3 dietary treatments under 1 temperature condition (HS or TN) ($P \leq 0.05$) and [XY]least square means represents a trend different ($0.05 < P \leq 0.1$).

[1]Relative liver was calculated as absolute liver weight (g)/BW(kg) × 100; relative spleen weight was calculated as absolute spleen weight (g)/BW (kg) × 100.

liver and spleen weight were not different among the 6 groups (Table 2).

On d 42, SYN and BMD supplemented birds had increased BW ($P_{\text{diet}} = 0.04$) with a trend to have greater absolute liver weights ($P_{\text{diet}} = 0.10$) compared with CON birds, mostly due to the changes under TN condition. Heat stress suppressed BW ($P_{\text{temperature}} = 0.0003$) and had a trend to decrease absolute liver ($P_{\text{temperature}} = 0.08$) and spleen weights ($P_{\text{temperature}} = 0.08$). The beneficial effect of dietary supplements on reducing HS induced negative effects on production performance was only found in HS-SYN birds compared to HS-CON ($P = 0.04$). In addition, a diet by temperature interaction was determined in relative spleen weight ($P_{\text{diet*temperature}} = 0.02$) due to the effects of SYN under TN condition, in which TN-SYN birds had a higher relative spleen weight than both TN-BMD and TN-CON birds ($P < 0.05$).

Physiological and Oxidation Parameters

On d 28, birds exposed to HS had increased plasma IL-6 ($P_{\text{temperature}} = 0.05$) with decreased IgY ($P_{\text{temperature}} = 0.04$) concentrations than birds raised under TN condition (Table 3). There was a trend overall dietary effect on plasma IgY concentration ($P_{\text{temperature}} = 0.10$) that was driven primarily from birds raised under TN condition, where IgY concentrations were decreased in TN-CON birds compared to both TN-BMD ($P = 0.03$) and TN-SYN birds ($P = 0.03$), while no difference was found among diet treatments under HS. There was no diet ($P_{\text{diet}} > 0.05$) or temperature ($P_{\text{temperature}} > 0.05$) effect on IL-2 and IL-10 concentrations. Liver SOD enzyme activity tended to decrease with heat exposure ($P_{\text{temperature}} = 0.06$, Table 4) due to the effects mainly found in CON birds. The SOD enzyme activity in HS-CON birds was significantly reduced compared to TN-CON birds (1.02 vs. 0.65, $P = 0.01$); while there were no dietary supplement effects under both TN and HS ambient conditions ($P_{\text{diet}} > 0.05$). No dietary ($P_{\text{diet}} > 0.05$) or temperature ($T_{\text{emperature}} > 0.05$) effect was found on GPx and HSP70 concentrations.

On d 42, HS decreased the concentrations of plasma IgY ($P_{\text{temperature}} = 0.01$, Table 3), while no difference was found in measured cytokines ($P_{\text{temperature}} > 0.05$). In addition, there were no dietary effects on the measured

Table 3. Effect of the dietary synbiotic inclusion on plasma cytokines and immunoglobulin Y of 28-day-old and 42-day-old broilers under thermoneutral and heat stress conditions.

	IL-2[1] (pg/mL)	IL-6[1] (pg/mL)	IL-10[1] (pg/mL)	IgY[1] (mg/dL)
28-d				
TN-CON	145.18	34.35	81.22	10.20[Y]
TN-BMD	162.37	36.02	83.37	24.92[X]
TN-SYN	142.40	34.38	77.15	20.83[X]
HS-CON	153.49	39.26	80.60	9.30
HS-BMD	160.86	37.02	79.74	11.77
HS-SYN	183.21	36.74	84.11	10.18
Feed additives				
CON	149.34	36.81	80.91	9.75[B]
BMD	161.61	36.52	81.56	18.34[A]
SYN	162.80	35.56	80.63	15.51[A]
Temperature				
HS	132.31	37.68[a]	81.48	10.42[b]
TN	126.29	34.92[b]	80.58	18.65[a]
SEM	18.5	1.81	9.62	4.31
P-value				
Diet	0.74	0.81	0.99	0.10
Temperature	0.29	0.05	0.89	0.04
Diet × temperature	0.48	0.48	0.79	0.40
42-d				
TN-CON	130.02	45.03	85.20	78.26
TN-BMD	117.38	43.08	78.21	88.27
TN-SYN	131.46	41.38	85.81	73.05
HS-CON	130.20	41.56	84.36	58.82
HS-BMD	126.74	50.53	84.75	62.70
HS-SYN	139.98	44.07	70.67	53.46
Feed additives				
CON	135.00	43.29	84.78	68.54
BMD	129.10	46.81	81.48	75.48
SYN	123.79	42.73	78.24	63.26
Temperature				
HS	132.31	45.39	79.93	58.33[b]
TN	126.29	43.16	83.07	79.86[a]
SEM	18.57	3.65	11.01	9.06
P-value				
Diet	0.83	0.39	0.80	0.40
Temperature	0.70	0.51	0.75	0.01
Diet × temperature	0.89	0.42	0.67	0.93

[a,b]Least squares means within a column for the 2 temperature conditions lacking a common superscript differ ($P \leq 0.05$. n = 8 per treatment), and [AB]least square means represents a trend among treatments ($0.05 < P \leq 0.1$).
[X,Y]least square means represents a trend among treatments within 1 environmental condition ($0.05 < P \leq 0.1$).
[1]Abbreviations: IL-2, Interleukin 2; IL-6, interleukin 6; IL-10, interleukin 10; IgY, immunoglobulin Y.

Table 4. Effect of the dietary synbiotic inclusion on heat shock protein 70 (HSP70) and oxidative status indicators of 28-day-old and 42-day-old broilers under thermoneutral and heat stress conditions.

	HSP70 (pg/mL)[1]	GPX activity (U/L)[1]	SOD activity (U/mL)[1]
28-d			
TN-CON	748.75	613.55	1.02
TN-BMD	931.62	575.10	0.83
TN-SYN	723.21	509.25	0.82
HS-CON	743.54	620.09	0.65
HS-BMD	740.74	502.09	0.78
HS-SYN	777.18	591.87	0.73
Feed additives			
CON	746.14	616.82	0.83
BMD	836.18	538.59	0.80
SYN	750.19	550.56	0.78
Temperature			
HS	753.82	571.35	0.72^B
TN	801.19	565.97	0.89^A
SEM	109.65	50.53	0.09
P-value			
Diet	0.70	0.27	0.70
Temperature	0.58	0.91	0.06
Diet × temperature	0.49	0.43	0.30
42-d			
TN-CON	936.61	642.64	0.86^x
TN-BMD	766.10	575.49	0.58^y
TN-SYN	853.28	587.64	0.72^x
HS-CON	848.54^X	602.32	0.75^x
HS-BMD	853.68^{XY}	573.11	0.54^y
HS-SYN	708.58^Y	587.35	0.94^x
Feed additives			
CON	892.57^A	622.48	0.81^{ab}
BMD	809.89^{AB}	574.30	0.56^b
SYN	780.93^B	587.49	0.83^a
Temperature			
HS	803.60	587.59	0.77
TN	852.00	601.92	0.75
SEM	114.76	34.29	0.12
P-value			
Diet	0.08	0.49	0.04
Temperature	0.64	0.50	0.85
Diet × temperature	0.10	0.69	0.63

[a,b]Least squares means within a column for the 3 dietary treatments lacking a common superscript differ ($P \leq 0.05$. n = 8 per treatment), and [A,B]least square means represents a trend among treatments ($0.05 < P \leq 0.1$).

[x,y]Least squares means within a column for the 1 temperature condition (HS or TN) and 3 dietary treatments ($P \leq 0.05$) and [XY]least square means represents a trend among treatments within 1 environmental condition ($0.05 < P \leq 0.1$).

[1]Abbreviations: GPX, glutathione peroxidase; HSP70, heat shock protein 70; SOD, superoxide dismutase.

cytokines and IgY levels in broilers ($P_{diet} > 0.05$). BMD additive decreased SOD activities in broilers as compared to CON and SYN broilers under both TN and HS conditions ($P_{diet} = 0.04$, Table 4). SYN birds tended to have lower HSP70 than CON birds but not BMD birds ($P_{diet} = 0.08$), while the difference was mainly contributed from HS groups as HS-SYN birds tended to have lower HSP70 concentrations than HS-CON birds ($P = 0.06$, Table 4). GPx enzyme activity was not different among the 6 groups ($P > 0.05$, Table 4).

Latency to Lie, Footpad Dermatitis, and Gait Score

On d 27, main effects of both diet and temperature were significant for LTL test ($P_{diet} < 0.05$, $P_{temperature} = 0.05$) and effect of diet was significant for FPD and GS ($P_{diet} < 0.05$, Table 5).

Under TN condition, TN-SYN birds had lower levels of FPD and GS compared with TN-CON birds ($P < 0.05$). TN-SYN birds also had longer durations during

LTL tests than TN-BMD birds ($P < 0.05$), while TN-BMD birds had similar levels of LTL duration, FPD, and GS as those of TN-CON birds ($P > 0.05$). Under HS condition, HS-SYN birds had longer LTL durations and lower GS than both HS-BMD and HS-CON birds ($P < 0.05$). HS-SYN birds as well as HS-BMD birds had fewer incidences of FPD than HS-CON birds ($P < 0.05$).

On d 41, there was no temperature effect on the measured parameters ($P_{temperature} > 0.05$, Table 5), while dietary effects on leg health were persisted. There was an overall diet effect ($P_{diet} = 0.02$) on LTL duration between SYN and BMD treatments but not CON treatment due to TN-SYN birds had a greater LTL duration compared with TN-BMD birds ($P < 0.05$). FPD condition was also improved in SYN birds compared to both CON and BMD birds ($P_{diet} = 0.0001$). Under TN condition, SYN birds had improved FPD than both BMD and CON birds ($P < 0.05$). Under heat exposure, both HS-SYN and HS-BMD birds had better FPD condition than HS-CON birds ($P < 0.05$). Overall, GS level was the best in SYN birds and the worst in BMD birds ($P_{diet} < 0.0001$). Compared to TN-CON birds, GS levels were

Table 5. Effect of the dietary synbiotic inclusion on latency to lie, footpad dermatitis, and gait score of 27-day-old and 41-day-old broilers under thermoneutral and heat stress conditions.

Treatment	Latency to lie (min)	Footpad dermatitis (0−2 scale)	Gait score(0−2 scale)
27-d			
TN-CON	3.15[xy]	0.73[x]	0.68[x]
TN-BMD	2.96[y]	0.40[xy]	0.43[xy]
TN-SYN	4.26[x]	0.20[y]	0.28[y]
HS-CON	1.86[y]	0.73[x]	0.63[x]
HS-BMD	1.38[y]	0.40[y]	0.48[x]
HS-SYN	3.80[x]	0.20[y]	0.18[y]
Feed additives			
CON	2.51[b]	0.70[a]	0.65[a]
BMD	2.17[b]	0.41[b]	0.45[b]
SYN	4.03[a]	0.24[b]	0.23[c]
Temperature			
HS	2.34[b]	0.44	0.43
TN	3.46[a]	0.45	0.46
SEM	0.60	0.09	0.06
P-value			
Diet	0.003	0.0006	<0.0001
Temperature	0.05	0.78	0.48
Diet × temperature	0.69	0.69	0.42
41-d			
TN-CON	2.05[xy]	0.85[x]	0.55[y]
TN-BMD	1.35[y]	0.75[x]	0.80[x]
TN-SYN	3.47[x]	0.38[y]	0.30[z]
HS-CON	2.27	0.93[x]	0.55[y]
HS-BMD	2.05	0.68[y]	0.85[x]
HS-SYN	3.39	0.63[y]	0.40[y]
Feed additives			
CON	2.19[ab]	0.89[a]	0.55[b]
BMD	1.70[b]	0.71[a]	0.83[a]
SYN	3.43[a]	0.50[b]	0.35[c]
Temperature			
HS	2.56	0.74	0.60
TN	2.29	0.66	0.55
SEM	0.66	0.71	0.06
P-value			
Diet	0.02	0.0001	<0.0001
Temperature	0.63	0.15	0.26
Diet × temperature	0.85	0.08	0.65

[a,b]Least squares means within a column for the 2 temperature conditions and 3 dietary treatments lacking a common superscript differ ($P \leq 0.05$).

[x-z]Least squares means within a column for the 1 temperature condition (HS or TN) and 3 dietary treatments lacking a common superscript differ ($P \leq$ 0.05).The data of latency to lie were collected from 16 birds of 8 replicates per treatment; and the scores of footpad dermatitis and gait were collected from 40 birds of 8 replicates per treatment.

improved in TN-SYN birds ($P < 0.05$) but reduced in TN-BMD birds ($P < 0.05$). Under HS conditions, the GS level of HS-SYN birds was similar to that of HS-CON birds but was lower compared with HS-BMD birds ($P < 0.01$).

DISCUSSION

Heat stress is one of the detrimental factors that lead to impaired health and welfare in poultry species (Lara and Rostagno, 2013). In the current study, broilers exposed to HS had impaired growth performance compared to broilers raised under TN ambient conditions regardless of dietary treatments. Similar negative effects have been reported in multiple studies conducted in broilers under different HS severities (degree of temperature and its duration; Liu et al., 2020; Ruff et al., 2021). The outcomes from the current study indicate that the inclusions of SYN and BMD were able to improve broiler growth performance under the TN rearing conditions; while under the HS environments,

the beneficial effects were only seen in SYN fed birds during the 42-d trial due to its functions on the antioxidative system and regulating HSP70 synthesis and immunity.

One of the strategies for using BMD in broiler production is to preserve gut integrity and homeostasis by targeting primarily gram-positive bacteria through inhibiting synthesis of bacterial proteins in the intestinal tract (Singh et al., 2008). However, long-term use of antibiotics could lead to disrupted beneficial bacteria causing undesired shift in the gut commensal microbiome composition, which adversely affects host health (Konstantinidis et al., 2020). Synbiotic supplements can improve intestinal integrity by providing beneficial bacteria, which enables the gastrointestinal tract to absorb nutrients more efficiently and releases various biochemical factors with antimicrobial, antioxidant, and or immunomodulatory activities to prevent translocation of pathogens, and ultimately to improve animal growth performance (Huang et al., 2019; Shini et al., 2020). In the current study, however, BMD was only able to improve BW of broilers reared under TN conditions,

without heat exposure. Similar effect of dietary BMD was shown by Song et al. (2011), who reported that BMD does not counteract the negative effects of HS on growth performance in finishing pigs. The BMD effects could be related to increased villus height and crypt depth in the duodenum, resulting in a great surface for nutrient digestion and resorption, and through reducing harmful gut bacteria and related subclinical disorders. However, few published studies have documented the response of farm animals fed BMD in a HS environment. In addition, long-term use of BMD could cause imbalance of gut microbiota, clear out normal gut microflora, and promoting growth of resistant strains. Antimicrobial resistance has become a global threat to the one health approach (Aslam et al., 2018).

Broilers' foot health was improved with the SYN inclusion in the current study, which could be related to SYN improving litter quality. Previous studies have shown that the SYN prevents growth of *Campylocacter jejuni,* a common pathogen leading to bacterial foodborne infection in broilers (Ghareeb et al., 2012). *Campylobacter jejuni* caused diarrhea (enteritis) in broilers, which not only affects growth performance but also leads to damage of the feet and legs due to standing and lying on wet litter (Humphrey et al., 2014). Wet litter is a major factor causing FPD, a thickness and discoloration with or without lesions of the footpad (Shepherd and Fairchild, 2010). The severity of FPD is positively correlated with *Campylobacter* infection in broilers (Colles et al., 2008). In the present study, broilers supplemented with the SYN had the best foot condition on both d 27 and d 41, which could directly result from the dryer litter with provision of beneficial bacteria against pathogens. Although the quality of litter was not examined in this study, previous studies have shown that the intensity of FPD is correlated with excreta related litter moisture content (wet litter) (Toppel et al., 2019) which is affected by diet nutrition with various supplements (Swiatkiewicz et al., 2017).

Broilers fed with the SYN were able to stand longer during LTL test (a common indicator of leg strength), indicating that SYN fed birds had a stronger leg musculoskeletal system. It has been reported previously that broilers fed with the SYN product (Wideman et al., 2012) have a lower incidence of bacterial chondronecrosis with osteomyelitis lameness as well as lower incidence of femoral head transitional degeneration and tibial head necrosis due to prevention of gut bacterial translocation and hematogenous distribution. The direct link between prebiotic and probiotic supplements and bone health has been demonstrated in a variety of animal models (zebrafish, rodents, and chicken) and humans (Scholz-Ahrens et al., 2007; Tu et al., 2021). In this aspect, it could be argued that broilers fed with the SYN have improved skeletal health under both TN and HS conditions. The similar results have been reported in one of our previous studies, the SYN improves broiler bone mineral density, bone mineral content, GS, and LTL (Yan et al., 2019). Several other beneficial bacteria strains, such as *Bacillus subtilis, Saccharomyces*

cerevisiae, and *Lactobacillus acidophilus,* with the similar effects on skeletal health including the improvement of bone mineral density and breaking force have been reported previously (Saleh et al., 2012; Abdelqader et al., 2020; Li et al., 2020; Khan et al., 2019).

Heat stress activates the hypothalamus-pituitary-adrenal (**HPA**) axis, stimulating release of glucocorticoids which play an important role in regulation of the immune system (Bae et al., 2019; Bagath et al., 2019). Glucocorticoids regulate the synthesis and release of both proinflammatory and anti-inflammatory cytokines to modulate the healing process of stress-induced inflammatory damage (Siddiqui et al., 2020). Generally, T lymphocyte subtype Th1 is responsible for releasing IL-2, IL-8, IFN-γ, and TNF-α, while Th2 subtype functions in synthesizing and releasing IL-4, IL-5, IL-10, and IL-13, playing important roles in the development of humoral immune response (Romagnani, 1997). Among the immune regulators, IL- 6, as a proinflammatory cytokine, is produced mainly by macrophages in response to various stimulations (Tanaka et al., 2014). High IL-6 levels have been found in association with many diseases and related tissue damage (Tanaka et al., 2014). In the present study, plasma proinflammatory cytokine IL-6 was elevated in broilers exposed to HS on d 28, which may indicate HS-induced inflammation in broilers. In agreement with the current results, increased plasma concentrations and upregulated mRNA expressions of several proinflammatory cytokines including IL-6 as well as TNF-α, IL-2, IL-1b, and IL-8 have been reported previously in HS broilers (Alhenaky et al., 2017; Baxter et al., 2020; Humam et al., 2021).

Heat stress suppressed the concentrations of total plasma IgY, the major immunoglobulin subclass in broiler chickens, on both d 28 and d 42. This finding agrees with the data reported by Honda et al. (2015), who observed decreased plasma IgY after 19 d of heat exposure. On d 28, both SYN and BMD supplemented birds tended to have higher IgY than CON birds, suggesting an increased immune response in these birds. The reasons for this change could be due to the protective effect of BMD and SYN in against intestinal inflammation. Heat stress disturbs commensal bacteria and increases intestinal permeability allowing for translocation of pathogens, endotoxin, and biochemical factors, leading to systematic inflammation (De Boever et al., 2008; Alhenaky et al., 2017). Antibiotics target certain pathogens to reduce infection, however, chronic antibiotic administration can lead to commensal microbe depletion and microbiota composition imbalance, ultimately affecting host health (Ubeda and Pamer, 2012). This hypothesis has been evidenced by the reconstruction of disrupted intestinal flora after administration of commensal gut microbiota in germ free mice (Mazmanian et al., 2005). In addition, immunological effects of probiotics have been discovered in different hosts, including broilers (Azad et al., 2018; Tuo et al., 2018; Wang et al., 2018). Results of higher antibody titers against new castle disease (An et al., 2008;

Shahir et al., 2014) and infectious bursal disease (Rehman et al., 2020) have been determined in broilers fed prebiotic and/or probiotic supplements. In the current study, SYN supplemented birds had a higher relative spleen weight compared with both BMD and CON birds on d 42 under TN condition. The spleen is a major immune organ in birds, and weight changes have been used as an indicator of immunity in response to stress (Scanes, 2020). Greater relative spleen weight may indicate an enhanced immune response and lower stress reaction in broilers fed with the SYN supplements. These results may indicate that provision of beneficial bacteria via dietary supplementation of probiotics or synbiotics could be a harmless strategy for restoring commensal bacterial balance, protecting against pathogens, and stimulating the immune responses in broilers as well as other animals.

Heat stress causes a surge of ROS, which contributes to oxidative stress (Slimen et al., 2014). Oxidative stress occurs when overproduced ROS overcomes the capacity of antioxidant enzymes such as SOD activity (Slimen et al., 2014). The drop of SOD activity between TN-CON and HS-CON birds on d 28 evidenced that the HS induced oxidation, while SYN supplements were able to attenuate the negative effect. In addition, HS-BMD birds had reduced SOD enzyme activity compared with both HS-CON and HS-SYN birds. The decreased SOD enzyme activities in the BMD supplemented group indicated a higher oxidative status in the broilers. Antibiotic administration has been shown to increase intracellular oxidation by potentially increase ROS production due to gut bacteria death (Liu et al., 2016; Sannasimuthu et al., 2020). Oxidative stress negatively affects broiler performance as well as meat quality, causing profound economic loss (Altan et al., 2003). Moreover, oxidative stress is associated with expression of HSP70 (Grunwald et al., 2014). Our data showed plasma levels of HSP 70 tended to be lower in HS-SYN but not HS-BMD broilers compared to it of HS-CON birds on d 42. Heat shock proteins have cytoprotective activities, which aid protein assembling and folding as well as degradation of improperly folded proteins to prevent cell apoptosis (Jee, 2016; Ikwegbue et al., 2018). As a chaperone protein, HSP70 is synthesized rapidly after the animals being exposed to high environmental temperature (Feder and Hofmann, 1999). Increased circulating HSP70 and upregulated gene expression have been reported in HS broilers (Tang et al., 2018; Siddiqui et al., 2020). The lower HSP70 found in the SYN supplemented birds could be an indicator for attenuated HS response and oxidative stress by increasing the birds' adaptation to HS via the gut-immune and gut-brain axes.

In conclusion, our results showed that antibiotics BMD can promote broiler growth performance but may lose efficacy under HS and potentially lead to oxidative stress. The SYN supplements attenuate negative effect of HS by improving BW, foot health, leg strength, immunity, and antioxidant capability. The dietary supplementation of SYN may be a viable BMD alternative for broilers production, especially in the tropical and subtropical regions.

REFERENCES

Abdelqader, A., M. Abuajamieh, F. Hayajneh, and A. R. Al-Fataftah. 2020. Probiotic bacteria maintain normal growth mechanisms of heat stressed broiler chickens. J. Therm. Biol. 92:102654.

Akbarian, A., J. Michiels, J. Degroote, M. Majdeddin, A. Golian, and S. De Smet. 2016. Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. J. Anim. Sci. Biotechnol. 7:37.

Alhenaky, A., A. Abdelqader, M. Abuajamieh, and A. R. Al-Fataftah. 2017. The effect of heat stress on intestinal integrity and Salmonella invasion in broiler birds. J. Therm. Biol. 70:9–14.

Altan, Ö., A. Pabuçcuoğlu, A. Altan, S. Konyalioğlu, and H. Bayraktar. 2003. Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. Br. Poult. Sci. 44:545–550.

An, B. K., B. L. Cho, S. J. You, H. D. Paik, H. I. Chang, S. W. Kim, C. W. Yun, and C. W. Kang. 2008. Growth performance and antibody response of broiler chicks fed yeast derived β-glucan and single-strain probiotics. Asian-Australas. J. Anim. Sci. 21:1027–1032.

Aslam, B., W. Wang, M. I. Arshad, M. Khurshid, S. Muzammil, M. H. Rasool, M. A. Nisar, R. F. Alvi, M. A. Aslam, M. U. Qamar, M. Salamat, and Z. Baloch. 2018. Antibiotic resistance: a rundown of a global crisis. Infect. Drug Resist 11:1645–1658.

Aviagen, 2018. Ross broiler management handbook. https://en.avia gen.com/assets/Tech_Center/Ross_Broiler/Ross-BroilerHand book2018-EN.pdf. Accessed Jan. 2022.

Awad, W. A., K. Ghareeb, S. Abdel-Raheem, and J. Böhm. 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poult. Sci. 88:49–55.

Azad, M. A. K., M. Sarker, and D. Wan. 2018. Immunomodulatory effects of probiotics on cytokine profiles. Biomed. Res. Int. 2018:8063647.

Bae, Y. S., E. C. Shin, Y. S. Bae, and W. Van Eden. 2019. Editorial: Stress and immunity. Front. Immunol. 10:1–2.

Bagath, M., G. Krishnan, C. Devaraj, V. P. Rashamol, P. Pragna, A. M. Lees, and V. Sejian. 2019. The impact of heat stress on the immune system in dairy cattle: a review. Res. Vet. Sci. 126:94–102.

Baxter, M. F. A., E. S. Greene, M. T. Kidd, G. Tellez-Isaias, S. Orlowski, and S. Dridi. 2020. Water amino acid-chelated trace mineral supplementation decreases circulating and intestinal HSP70 and proinflammatory cytokine gene expression in heat-stressed broiler chickens. J. Anim. Sci. 98:1–13.

Castanon, J. I. R. 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poult. Sci. 86:2466–2471.

Cervantes, H. M. 2015. Antibiotic-free poultry production: is it sustainable? J. Appl. Poult. Res. 24:91–97.

Chen, Y., C. Wen, and Y. Zhou. 2018. Dietary synbiotic incorporation as an alternative to antibiotic improves growth performance, intestinal morphology, immunity and antioxidant capacity of broilers. J. Sci. Food Agric. 98:3343–3350.

Christakos, S., D. V. Ajibade, P. Dhawan, A. J. Fechner, and L. J. Mady. 2010. Vitamin D: metabolism. Endocrinol. Metab. Clin. North Am. 39:243–253.

Colles, F. M., T. A. Jones, N. D. McCarthy, S. K. Sheppard, A. J. Cody, K. E. Dingle, M. S. Dawkins, and M. C. J. Maiden. 2008. Campylobacter infection of broiler chickens in a free-range environment. Environ. Microbiol. 10:2042–2050.

De Boever, S., R. Beyaert, F. Vandemaele, K. Baert, L. Duchateau, B. Goddeeris, P. De Backer, and S. Croubels. 2008. The influence of age and repeated lipopolysaccharide administration on body temperature and the concentration of interleukin-6 and IgM antibodies against lipopolysaccharide in broiler chickens. Avian Pathol. 37:39–44.

De Oliveira, M. J. K., N. K. Sakomura, J. C. De Paula Dorigam, K. Doranalli, L. Soares, and G. D. S. Viana. 2019. Bacillus amyloliquefaciens CECT 5940 alone or in combination with antibiotic growth promoters improves performance in broilers under enteric pathogen challenge. Poult. Sci. 98:4391–4400.

Engberg, R. M., M. S. Hedemann, T. D. Leser, and B. B. Jensen. 2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. Poult. Sci. 79:1311–1319.

Feder, M. E., and G. E. Hofmann. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu. Rev. Physiol. 61:243–282.

Ghareeb, K., W. A. Awad, M. Mohnl, R. Porta, M. Biarnés, J. Böhm, and G. Schatzmayr. 2012. Evaluating the efficacy of an avian-specific probiotic to reduce the colonization of Campylobacter jejuni in broiler chickens. Poult. Sci. 91:1825–1832.

Ghosh, N., A. Das, S. Chaffee, S. Roy, and C. K. Sen. 2018. Reactive oxygen species, oxidative damage and cell death. In Immunity and inflammation in health and diseaseAcademic Press, 45–55.

Grunwald, M. S., A. S. Pires, A. Zanotto-Filho, J. Gasparotto, D. P. Gelain, D. R. Demartini, C. M. Schöler, P. I. H. de Bittencourt, and J. C. F. Moreira. 2014. The oxidation of HSP70 is associated with functional impairment and lack of stimulatory capacity. Cell Stress Chaperones. 19:913–925.

Gyawali, R., N. Nwamaioha, R. Fiagbor, T. Zimmerman, R. H. Newman, and S. A. Ibrahim. 2019. The Role of Prebiotics in Disease Prevention and Health Promotion. Academic Press, Cambridge, MA, 151–167.

Hassanpour, H., A. K. Z. Moghaddam, M. Khosravi, and M. Mayahi. 2013. Effects of synbiotic on the intestinal morphology and humoral immune response in broiler chickens. Livest. Sci. 153:116–122.

Honda, B. T. B., A. S. Calefi, C. Costola-De-Souza, W. M. Quinteiro-Filho, J. G. Da Silva Fonseca, V. F. De Paula, and J. Palermo-Neto. 2015. Effects of heat stress on peripheral T and B lymphocyte profiles and IgG and IgM serum levels in broiler chickens vaccinated for Newcastle disease virus. Poult. Sci. 94:2375–2381.

Huang, L., L. Luo, Y. Zhang, Z. Wang, and Z. Xia. 2019. Effects of the dietary probiotic, enterococcus faecium NCIMB11181, on the intestinal barrier and system immune status in Escherichia coli O78-challenged broiler chickens. Probiotics Antimicrob. Proteins. 11:946–956.

Humam, A. M., T. C. Loh, H. L. Foo, W. I. Izuddin, I. Zulkifli, A. A. Samsudin, and N. M. Mustapha. 2021. Supplementation of postbiotic RI11 improves antioxidant enzyme activity, upregulated gut barrier genes, and reduced cytokine, acute phase protein, and heat shock protein 70 gene expression levels in heat-stressed broilers. Poult. Sci. 100:100908.

Humphrey, S., G. Chaloner, K. Kemmett, N. Davidson, N. Williams, A. Kipar, T. Humphrey, and P. Wigley. 2014. Campylobacter jejuni is not merely a commensal in commercial broiler chickens and affects bird welfare. MBio 5:1–7.

Ikwegbue, P. C., P. Masamba, B. E. Oyinloye, and A. P. Kappo. 2018. Roles of heat shock proteins in apoptosis, oxidative stress, human inflammatory diseases, and cancer. Pharmaceuticals 11:1–18.

Jee, H. 2016. Size dependent classification of heat shock proteins: a mini-review. J. Exerc. Rehabil. 12:255–259.

Khan, A. Z., I. U. Khan, S. Khan, S. Afzal, M. Hamid, M. Tariq, I. U. Haq, N. Ullah, M. A. Khan, S. Bilal, K. Huwang, and R. Liu. 2019. Selenium-enriched probiotics improve hepatic protection by regulating pro-inflammatory cytokines and antioxidant capacity in broilers under heat stress conditions. J. Adv. Vet. Anim. Res. 6:355–361.

Kirchhelle, C., P. Atkinson, A. Broom, K. Chuengsatiansup, J. P. Ferreira, N. Fortané, I. Frost, C. Gradmann, S. Hinchliffe, S. J. Hoffman, J. Lezaun, K. Outterson, S. H. Podolsky, S. Raymond, A. P. Roberts, A. C. Singer, A. D. So, L. Sringernyuang, E. Tayler, S. Rogers Van Katwyk, and C. I. R. Chandler. 2020. Setting the standard: multidisciplinary hallmarks for structural, equitable and tracked antibiotic policy. BMJ Glob. Heal. 5:1–14.

Kjaer, J. B., G. Su, B. L. Nielsen, and P. Sørensen. 2006. Foot pad dermatitis and hock burn in broiler chickens and degree of inheritance. Poult. Sci. 85:1342–1348.

Koltes, D. A., H. D. Lester, M. Frost, D. Aldridge, K. D. Christensen, and C. G. Scanes. 2017. Effects of bacitracin methylene disalicylate and diet change on gastrointestinal integrity and endotoxin permeability in the duodenum of broiler chicken. BMC Res. Notes. 10:8–13.

Konstantinidis, T., C. Tsigalou, A. Karvelas, E. Stavropoulou, C. Voidarou, and E. Bezirtzoglou. 2020. Effects of antibiotics upon the gut microbiome: a review of the literature. Biomedicines. 8:1–15.

Kuehn, B. M. 2014. FDA moves to curb antibiotic use in livestock. JAMA - J. Am. Med. Assoc. 311:347–348.

Lara, L. J., and M. H. Rostagno. 2013. Impact of heat stress on poultry production. Animals. 3:356–369.

Li, C., Z. Niu, M. Zou, S. Liu, M. Wang, X. Gu, H. Lu, H. Tian, and R. Jha. 2020. Probiotics, prebiotics, and synbiotics regulate the intestinal microbiota differentially and restore the relative abundance of specific gut microorganisms. J. Dairy Sci. 103:5816–5829.

Liu, L., M. Ren, K. Ren, Y. Jin, and M. Yan. 2020. Heat stress impacts on broiler performance: a systematic review and meta-analysis. Poult. Sci. 99:6205–6211.

Liu, X., M. Marrakchi, M. Jahne, S. Rogers, and S. Andreescu. 2016. Real-time investigation of antibiotics-induced oxidative stress and superoxide release in bacteria using an electrochemical biosensor. Free Radic. Biol. Med. 91:25–33.

Mazmanian, S. K., H. L. Cui, A. O. Tzianabos, and D. L. Kasper. 2005. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell. 122:107–118.

Millet, S., and L. Maertens. 2011. The European ban on antibiotic growth promoters in animal feed: from challenges to opportunities. Vet. J. 187:143–144.

Min, Y. N., H. L. Yang, Y. X. Xu, and Y. P. Gao. 2016. Effects of dietary supplementation of synbiotics on growth performance, intestinal morphology, sIgA content and antioxidant capacities of broilers. J. Anim. Physiol. Anim. Nutr. (Berl). 100:1073–1080.

Mohammed, A. A., J. A. Jacobs, G. R. Murugesan, and H. W. Cheng. 2018. Effect of dietary syn-biotic supplement on behavioral patterns and growth performance of broiler chickens reared under heat stress. Poult. Sci. 97:1101–1108.

Montilla, S. I. R., T. P. Johnson, S. C. Pearce, D. Gardan-Salmon, N. K. Gabler, J. W. Ross, R. P. Rhoads, L. H. Baumgard, S. M. Lonergan, and J. T. Selsby. 2014. Heat stress causes oxidative stress but not inflammatory signaling in porcine skeletal muscle. Temperature. 1:42–50.

Nadeem, S. F., U. F. Gohar, S. F. Tahir, H. Mukhtar, S. Pornpukdeewattana, P. Nukthamna, A. M. Moula Ali, S. C. B. Bavisetty, and S. Massa. 2020. Antimicrobial resistance: more than 70 years of war between humans and bacteria. Crit. Rev. Microbiol. 46:578–599.

Petruk, A., and D. R. Korver. 2004. Broiler breeder egg production and quality are affected by timing of increased dietary Ca relative to photostimulation. Can. J. Anim. Sci. 85:411–420.

Redweik, G. A. J., J. Jochum, and M. Mellata. 2020. Live bacterial prophylactics in modern poultry. Front. Vet. Sci. 7:1–15.

Rehman, A., M. Arif, N. Sajjad, M. Q. Al-Ghadi, M. Alagawany, M. E. Abd El-Hack, A. R. Alhimaidi, S. S. Elnesr, B. O. Almutairi, R. A. Amran, E. O. S. Hussein, and A. A. Swelum. 2020. Dietary effect of probiotics and prebiotics on broiler performance, carcass, and immunity. Poult. Sci. 99:6946–6953.

Richter, K., M. Haslbeck, and J. Buchner. 2010. The heat shock response: life on the verge of death. Mol. Cell. 40:253–266.

Romagnani, S. 1997. The Th1/Th2 paradigm. Immunol. Today. 18:263–266.

Ruff, J., G. Tellez, A. J. Forga, R. Señas-Cuesta, C. N. Vuong, E. S. Greene, X. Hernandez-Velasco, Á. J. Uribe, B. C. Martínez, J. A. Angel-Isaza, S. Dridi, C. J. Maynard, C. M. Owens, B. M. Hargis, and G. Tellez-Isaias. 2021. Evaluation of three formulations of essential oils in broiler chickens under cyclic heat stress. Animals. 11:1–13.

Saleh, A. A., Y. Z. Eid, T. A. Ebeid, A. Ohtsuka, M. Yamamoto, and K. Hayashi. 2012. Feeding Aspergillus awamori reduces skeletal muscle protein breakdown and stimulates growth in broilers. Anim. Sci. J. 83:594–598.

Sannasimuthu, A., D. Sharma, B. A. Paray, M. K. Al-Sadoon, and J. Arockiaraj. 2020. Intracellular oxidative damage due to antibiotics on gut bacteria reduced by glutathione oxidoreductase-derived antioxidant molecule GM15. Arch. Microbiol. 202:1127–1133.

Scanes, C. G. 2020. Avian physiology: are birds simply feathered mammals? Front. Physiol. 11:1–11.

Scholz-Ahrens, K. E., P. Ade, B. Marten, P. Weber, W. Timm, Y. Açil, C. C. Glüer, and J. Schrezenmeir. 2007. Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. J. Nutr. 137:838S−46S.

Shahir, M. H., O. Afsarian, S. Ghasemi, and G. Tellez. 2014. Effects of dietary inclusion of probiotic or prebiotic on growth performance, organ weight, blood parameters and antibody titers against influenza and newcastle in broiler chickens. Int. J. Poult. Sci. 13:70–75.

Shepherd, E. M., and B. D. Fairchild. 2010. Footpad dermatitis in poultry. Poult. Sci. 89:2043–2051.

Shin, Y. H., S. Kang, W. S. Byun, C. W. Jeon, B. Chung, J. Y. Beom, S. Hong, J. Lee, J. Shin, Y. S. Kwak, S. K. Lee, K. B. Oh, Y. J. Yoon, and D. C. Oh. 2020. Absolute configuration and antibiotic activity of piceamycin. J. Nat. Prod. 83:277–285.

Shini, S., D. Zhang, R. C. Aland, X. Li, P. J. Dart, M. J. Callaghan, R. E. Speight, and W. L. Bryden. 2020. Probiotic Bacillus amyloliquefaciens H57 ameliorates subclinical necrotic enteritis in broiler chicks by maintaining intestinal mucosal integrity and improving feed efficiency. Poult. Sci. 99:4278–4293.

Siddiqui, S. H., D. Kang, J. Park, M. Khan, and K. Shim. 2020. Chronic heat stress regulates the relation between heat shock protein and immunity in broiler small intestine. Sci. Rep. 10:1–11.

Singh, M., S. S. Chauhan, and P. Kumar. 2008. Effect of supplementation of diets with BMD and Virginiamycin on the growth performance, carcass characteristics and bacterial population in broiler chickens. Vet. World. 1:141–143.

Slimen, I. B., T. Najar, A. Ghram, H. Dabbebi, M. Ben Mrad, and M. Abdrabbah. 2014. Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. A review. Int. J. Hyperth. 30:513–523.

Song, R., D. N. Foster, and G. C. Shurson. 2011. Effects of feeding diets containing bacitracin methylene disalicylate to heat-stressed finishing pigs. J. Anim. Sci. 89:1830–1843.

Strzępa, A., M. Majewska-Szczepanik, F. M. Lobo, L. Wen, and M. Szczepanik. 2017. Broad spectrum antibiotic enrofloxacin modulates contact sensitivity through gut microbiota in a murine model. J. Allergy Clin. Immunol. 140:121–133 e3.

Swiatkiewicz, S., A. Arczewska-Wlosek, and D. Jozefiak. 2017. The nutrition of poultry as a factor affecting litter quality and foot pad dermatitis - an updated review. J Anim Physiol Anim Nutr (Berl) 101:e14–e20.

Tanaka, T., M. Narazaki, and T. Kishimoto. 2014. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol 6: a016295.

Tanaka, T., M. Narazaki, and T. Kishimoto. 2014. IL-6 in inflammation, immunity, and disease. Cold Spring Harb. Perspect. Biol. 6: a016295.

Tang, S., B. Yin, J. Xu, and E. Bao. 2018. Rosemary reduces heat stress by inducing CRYAB and HSP70 expression in broiler chickens. Oxid. Med. Cell. Longev. 2018:7014126.

Toppel, K., F. Kaufmann, H. Schon, M. Gauly, and R. Andersson. 2019. Effect of pH-lowering litter amendment on animal-based welfare indicators and litter quality in a European commercial broiler husbandry. Poult. Sci. 98:1181–1189.

Tu, Y., R. Yang, X. Xu, and X. Zhou. 2021. The microbiota-gut-bone axis and bone health. J. Leukoc. Biol 110:525–537.

Tuo, Y., X. Song, Y. Song, W. Liu, Y. Tang, Y. Gao, S. Jiang, F. Qian, and G. Mu. 2018. Screening probiotics from Lactobacillus strains according to their abilities to inhibit pathogen adhesion and induction of pro-inflammatory cytokine IL-8. J. Dairy Sci. 101:4822–4829.

Ubeda, C., and E. G. Pamer. 2012. Antibiotics, microbiota, and immune defense. Trends Immunol. 33:459–466.

Wang, W. C., F. F. Yan, J. Y. Hu, O. A. Amen, and H. W. Cheng. 2018. Supplementation of Bacillus subtilis-based probiotic reduces heat stress-related behaviors and inflammatory response in broiler chickens. J. Anim. Sci. 96:1654–1666.

Webster, A. B., B. D. Fairchild, T. S. Cummings, and P. A. Stayer. 2008. Validation of a three-point gait-scoring system for field assessment of walking ability of commercial broilers. J. Appl. Poult. Res. 17:529–539.

Wideman, R. F. 2016. Bacterial chondronecrosis with osteomyelitis and lameness in broilers: a review. Poult. Sci. 95:325–344.

Wideman, R. F., and I. Pevzner. 2012. Dexamethasone triggers lameness associated with necrosis of the proximal tibial head and proximal femoral head in broilers. Poult. Sci. 91:2464–2474.

Wideman, R. F., K. R. Hamal, J. M. Stark, J. Blankenship, H. Lester, K. N. Mitchell, G. Lorenzoni, and I. Pevzner. 2012. A wire-flooring model1 for inducing lameness in broilers: evaluation of probiotics as a prophylactic treatment. Poult. Sci. 91:870–883.

Yan, F. F., A. A. Mohammed, G. R. Murugesan, and H. W. Cheng. 2019. Effects of a dietary synbiotic inclusion on bone health in broilers subjected to cyclic heat stress episodes. Poult. Sci. 98:1083–1089.