Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress

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ABSTRACT The present study was aimed at elucidating the effects of supplementing mannan-oligosaccharides (MOS) and probiotic mixture (PM) on growth performance, intestinal histology, and corticosterone concentrations in broilers kept under chronic heat stress (HS). Four hundred fifty 1-d-old chicks were divided into 5 treatment groups and fed a corn-soybean diet ad-libitum. The temperature control (CONT) group was held at the normal ambient temperature. Heat stress broilers were held at 35 ± 2°C from d 1 until the termination of the study at d 42. Heat stress groups consisted of HS-CONT fed the basal diet; HS-MOS fed the basal diet containing 0.5% MOS; HS-PM fed the basal diet containing 0.1% PM; and HS-SYN (synbiotic) fed 0.5% MOS and 0.1% PM in the basal diet. Broilers were examined at d 21 and 42 for BW gain, feed consumption, feed conversion ratio (FCR), serum corticosterone concentrations, and ileal microarchitecture. The results revealed that the CONT group had higher ($P < 0.01$) feed consumption, BW gain, and lower FCR on d 21 and 42, compared with the HS-CONT group. Among supplemented groups, the HS-MOS had higher ($P < 0.05$) BW gain and lower FCR compared with the HS-CONT group. On d 21 and 42, the HS-CONT group had higher ($P < 0.05$) serum corticosterone concentrations compared with the CONT and supplemented groups. The CONT group had higher ($P < 0.05$) villus height, width, surface area, and crypt depth compared with the HS-CONT group. On d 21, the HS-PM had higher ($P < 0.05$) villus width and surface area compared with HS-CONT group. On d 42, the HS-SYN had higher ($P < 0.05$) villus width and crypt depth compared with the HS-CONT group. These results showed that chronic HS reduces broiler production performance, intestinal microarchitecture, and increases adrenal hormone concentrations. Also, supplementation of the MOS prebiotic and the PM can partially lessen these changes.

Key words: chronic heat stress, prebiotic, probiotic, ileal morphology, corticosterone

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INTRODUCTION

Heat stress (**HS**), one of the most serious climate problems of tropical and subtropical regions of world, negatively affects the production performance of poultry and livestock. Concisely, HS is characterized by endocrine disorders, reduced metabolic rate, lipid peroxidation, decreased feed consumption, decreased BW gain, higher feed conversion ratio (**FCR**), immunosuppression, and intestinal microbial dysbiosis (May et al., 1986; Lan et al., 2004; Sansonetti, 2004; Sohail et al., 2010, 2011). The hypothalamic-pituitary-adrenal (**HPA**) axis is a complex neuroendocrine pathway that controls reactions to stress. Birds experience activation of the HPA axis in an effort to combat stresses brought on by elevated environmental temperature (Siegel, 1960). Activation of the HPA axis is associated with overproduction of cortisol, corticosterone, and adrenocorticotropin. Indeed, managing the HPA axis activity is important for broiler growth and performance, because immunity and infection response are preferentially affected by overactivation of the HPA axis (Siegel, 1960).

The avian gastrointestinal tract harbors a diverse and dynamic population of microorganisms, living in symbiotic relationship with their host. This mutualistic relationship is important for host nutrition, metabolism, and immunity (Sohail et al., 2010). The complex ecosystem works as a virtual organ system that aids in host homeostasis (Forsythe et al., 2010). In adults, the intestinal microbial ecology is highly stable; however,

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it may be influenced by either feed or stress (Lan et al., 2004; Rehman et al., 2007). A few studies have described a link between the intestinal microbiome, the HPA axis, and stress (Gareau et al., 2007; Rhee et al., 2009). It is hypothesized that the intestinal microbiome may stimulate sensory neurons and the production of cytokines (Kawahito et al., 1994). In return, these cytokines and certain unknown factors secreted from enterochromaffin cells may influence adrenal gland function by dropping circulating corticosterone concentrations.

Recent research has focused on the importance of probiotics and prebiotics as functional foods to influence intestinal microarchitecture, microbial profiles, and broiler performance (Fuller, 1989; Patterson and Burkholder, 2003). Probiotics are live microbial feed supplements, which beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989). Prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). More recently, these feed additives have been supplemented to broilers under stress with some significant findings (Gareau et al., 2007; Silva et al., 2010). It is hypothesized that such supplements influence the intestinal microbiome and improve intestinal absorption, which altogether improve performance (Fuller, 1989; Sohail et al., 2011).

Previously, several dietary approaches have been examined, using vitamins, minerals, and probiotics, for the alleviation of HS in poultry (Zulkifli et al., 2000; Puthpongsiriporn et al., 2001; Lan et al., 2004). Probiotic supplementation was proven beneficial for improving chicken performance and immunity under HS conditions (Zulkifli et al., 2000; Lan et al., 2004). However, an understanding of the effects of prebiotic supplementations under HS conditions in broilers is lacking, especially in terms of intestinal histology and the HPA axis. We hypothesized that daily feeding of probiotic or prebiotic may improve intestinal histological indices and the HPA axis through the restoration of intestinal microbial ecology. Therefore, the aim of the current study was to explore the relationship between HS, the HPA axis, and diet, with the idea that supplementing a probiotic or a prebiotic may support a healthy microbiota, which would help broilers to better defend against HS.

MATERIALS AND METHODS

Birds Housing and Feeding

Four hundred fifty 1-d-old-chicks (Ross-708) of mixed sex were used in this experiment. The broilers were randomly divided into 5 treatment groups (n = 90) with 3 replicates (n = 30) for each group. The broilers were placed in floor pens on fresh wood-shavings litter in environmentally controlled houses. The temperature on d 1 for HS groups was maintained at 35 ± 2°C and was kept constant until d 42. For the temperature control (**CONT**) group, ambient temperature on d 1 was set at 35 ± 2°C and was decreased 3°C per week until it reached 26 ± 2°C; thereafter, the temperature was kept constant for the remaining period of the study. All birds were fed a corn-soybean meal basal diet (Table 1) and offered water ad libitum. Light was provided 23 h per day with 1 h of darkness. Heat stressed birds were fed a basal diet (HS-CONT group) or the same basal diet supplemented with 0.5% mannan-oligosaccharide (**MOS**; HS-MOS group; SAF-Mannan; Lesaffre Feed Additive, Marquette-Lez-Lille, France), 0.1% probiotic mixture (**PM**; HS-PM group; Protexin; Probiotics International Ltd., Somerset, UK), or a combination of both (0.5% MOS and 0.1% PM) as synbiotic (HS-SYN group) for the entire study period. The PM comprised of *Lactobacillus plantarum*, *Lactobacillus delbrueckii* ssp. Bulgaricus, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, and *Streptococcus salivarius* ssp. Thermophilus and *Enterococcus faecium* were approximately 2.00×10^9 total viable colony-forming units per gram. The experimental diets were formulated without antimicrobials or coccidiostats to meet or exceed the NRC (1994). The health status of the broilers for any infection or disease was constantly monitored by 2 experienced veterinarians at the poultry experiment facility, USDA, College Station, Texas.

Growth Performance

Weekly BW gain and feed intake per pen were measured. Initial body weights (d 1) were subtracted from the final BW to get BW gain. Feed consumption was calculated by subtracting residual feed from the offered feed. Data for feed consumption and BW gain were used to calculate the FCR. The FCR was adjusted for mortality and calculated on a per pen basis. Growth performance data were determined on d 21 and 42. For intestinal tissue sampling, 5 birds from each group were randomly killed by CO_2 inhalation.

Serum Corticosterone

On d 21, 30 birds from each group (10 from each of the 3 replicates) were randomly selected for blood collection. About 3 mL of blood was taken via jugular puncture and kept at 4°C overnight. Serum was harvested by centrifugation at 1,500 × *g* for 20 min. On d 42, the same birds were sampled again, following the same procedures as on d 21. Serum corticosterone was measured using an ELISA kit (Corticosterone EIA kit; Cayman Chemical, Ann Arbor, MI), following the recommended protocol.

Ileal Histology

Ilea fixed in formalin [10% (vol/vol) phosphate-buffered (pH 7.0) formaldehyde; Ricca Chemical Company, Arlington, TX] were dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Five-micrometer

Table 1. Ingredients and calculated composition of the basal diet

Item	Starter (1–14 d)	Grower (14–35 d)	Finisher (35–42 d)
Ingredient (g)			
Corn	584.1	612.8	645.5
Soybean meal, 49% CP	344.4	299.4	272.3
Blended fat	28.0	47.99	42.8
Limestone	15.7	15.8	15.8
Biofos 16/21P[1]	15.6	14.1	12.8
Trace mineral premix[2]	0.5	0.5	0.5
Vitamin premix[3]	2.5	2.5	2.5
Sodium chloride	4.1	3.0	3.5
L-Lysine HCl	1.6	1.3	1.6
DL-Methionine	1.5	1.5	1.5
L-Tryptophan	0.3	0.3	0.6
Calculated composition			
CP (%)	21.90	20.00	19.10
ME (kcal/kg)	3.05	3.20	3.20
Fat (%)	5.35	7.4	6.95
Calcium (%)	0.95	0.95	0.88
Total phosphorus (%)	0.70	0.62	0.61
Available phosphorus (%)	0.45	0.41	0.38
Crude fiber (%)	2.63	2.52	2.48
Lysine digest (%)	1.34	1.15	1.07
Methionine digest (%)	0.59	0.54	0.54
Total SAA[4] (%)	0.95	0.88	0.86
Threonine digest (%)	0.81	0.74	0.70

[1]Biofos 16/21: phosphorus 21%; calcium 15–18%; fluorine 0.21% (Mosaic Corp. Nutrition, South Riverview, FL).

[2]Mineral premix (each kg contained): K, 0.85 g; Na, 0.18 g; Mg, 18 g; Zn, 84 mg; Fe, 111.2 mg; Cu, 11.3 mg; Mn, 87 mg; Se, 0.15 mg; I, 0.1 mg (Producers Cooperative Association, Bryan, TX).

[3]Vitamin premix (each kg contained): vitamin A 4,409,200 IU; vitamin D_3 1,543,220 IU; vitamin E 18,371 IU; vitamin B_{12} 6.6 mg; riboflavin 2,338 mg; niacin 18,371 mg; D-pantothenic acid 8,084 mg; choline 191,066 mg; menadione 589 mg; folic acid 699 mg; pyridoxine 2,866 mg; thiamine 1,175 mg; D-Biotin 220 mg (RocheVitamins, Parsippany, NJ).

[4]SAA = total sulfur amino acids.

thick sections were cut with a microtome and mounted on slides. Four discontinuous paraffin-embedded sections per broiler ileal sample were processed for evaluation. These sections were deparaffinized in xylene, dehydrated in ethanol, and stained with hematoxylin and eosin. The slides were scanned (Epson 3170; EPSON Inc., Long Beach, CA) and examined (ImageJ-1997; NIH, Bethesda, MD) for determining villus height, width, and crypt depth (Awad et al., 2008). Villus height and villus width data were used to calculate villus surface area [$2\pi \times (W/2) \times L$], where W = villus width and L = villus length (Sakamoto et al., 2000).

Statistical Analysis

The Kolmogorov-Smirnov test was used to test the normal distribution of the data before statistical analysis was performed. Results are expressed as means ± SE. Growth performance, corticosterone, and histological data were subjected to ANOVA using SPSS statistical software (SPSS 13.0; Chicago, IL). Statistical differences among means ($P < 0.05$) were identified using Duncan's multiple range test. Differences in percentage mortality were analyzed by Pearson's χ^2 test.

RESULTS

The effects of HS and dietary supplements on BW gain, feed intake, FCR, and mortality rate are presented in Table 2. The CONT group had higher ($P < 0.05$) BW gain, feed intake, and lower FCR compared with the HS-CONT group. The HS-CONT group had 18.3% less BW gain on d 21 and 49.6% less on d 42 as compared with the CONT group. The HS-CONT birds consumed 15.4 and 25.4% less feed compared with the CONT group on d 21 and 42 respectively The CONT group attained 785 g more weight at d 42 than the HS-CONT group. Among the HS groups, the HS-MOS had higher ($P < 0.05$) BWgain and lower FCR compared with the HS-CONT group. Mannan-oligosaccharides supplementation increased BW gain by 8.1% on d 21and 17.7% on d 42, compared with the HS-CONT group. The supplementation of PM and SYN did not increase ($P > 0.05$) BW gain, feed consumption, or decrease FCR as compared with the HS-CONT on d 21 and 42. The percentage mortality was nonsignificant ($P > 0.05$) among groups, on d 21. On d 42, there were statistical differences ($P < 0.05$) of percentage mortality among different groups. The HS-CONT group had higher ($P < 0.05$) mortality compared with the CONT group. Among supplemented groups, HS-PM had higher ($P < 0.05$) mortality compared with HS-MOS and HS-SYN groups. On d 21 and 42, the HS-CONT group had higher ($P < 0.05$) serum corticosterone concentrations compared with CONT and the HS-supplemented groups (Table 3).

The CONT group had higher ($P < 0.05$) villus height, width, surface area, and crypt depth, compared with

Table 2. Body weight gain, feed consumption, and feed conversion ratio in broilers reared under normal temperature or heat stress conditions

	Treatment group[1]				
Day	CONT	HS-CONT	HS-MOS	HS-PM	HS-SYN
BW gain (g)					
21	$825.8^{a} \pm 8.77$	$698.4^{c} \pm 6.33$	$754.6^{b} \pm 26.35$	$728.2^{bc} \pm 10.20$	$714.9^{bc} \pm 5.80$
42	$2{,}411.3^{a} \pm 30.66$	$1{,}626.3^{c} \pm 143.89$	$1{,}906.7^{b} \pm 38.10$	$1{,}726.0^{bc} \pm 90.84$	$1{,}744.3^{bc} \pm 47.01$
Feed consumption (g)					
21	$1{,}082.8^{a} \pm 17.11$	$908.6^{c} \pm 25.51$	$990.6^{bc} \pm 31.55$	$1022.1^{ab} \pm 19.72$	$984.2^{bc} \pm 31.92$
42	$3{,}214.5^{a} \pm 94.87$	$2{,}688.8^{b} \pm 73.99$	$2{,}658.3^{b} \pm 82.37$	$2{,}769.7^{b} \pm 107.13$	$2{,}618.7^{b} \pm 40.56$
Feed conversion ratio (g of feed/g of weight gain)					
21	$1.31^{a} \pm 0.04$	$1.30^{a} \pm 0.02$	$1.31^{a} \pm 0.04$	$1.40^{a} \pm 0.04$	$1.37^{a} \pm 0.08$
42	$1.33^{c} \pm 0.05$	$1.67^{a} \pm 0.10$	$1.39^{bc} \pm 0.04$	$1.60^{ab} \pm 0.04$	$1.50^{abc} \pm 0.05$
Mortality (%)					
21	4.44	1.11	1.11	2.22	2.22
42†	5.56	10.00	1.11	7.78	2.22

[a–c]Means ± SE within a row lacking a common superscript differ ($P < 0.05$); †percentage values differ ($P < 0.05$) at d 42.

[1]CONT = control; HS = heat stress; MOS = mannan-oligosaccharide; PM = probiotic mixture; SYN = synbiotic.

the HS-CONT group, on d 21 and 42. On d 21, the HS-PM group had higher ($P < 0.05$) villus width and surface area compare with the HS-CONT group. On d 21, the crypt depth was highest ($P < 0.05$) in the HS-SYN group, whereas, on d 42, the HS-SYN group had higher ($P < 0.05$) villus width and crypt depth compared with the HS-CONT group (Table 4).

DISCUSSION

The study was planned to examine the effects of prebiotic and probiotic supplementations on broiler performance when reared under chronic HS. Results revealed that birds exposed to chronic HS had poor growth performance and increased corticosterone concentrations. Prebiotic and probiotic treatments increased growth performance and decreased corticosterone concentrations compared with the HS-CONT group, although the differences were not always discernible. Silva et al. (2010) observed that subjecting broilers to HS significantly suppressed growth performance, whereas, feeding MOS prebiotic can improve BW gain and lower FCR. Similarly, supplementing *Saccharomyces cerevisiae* probiotic in HS broilers significantly improved growth performance. Deteriorated performance of HS broilers can be attributed to a greater expenditure of energy for physiological adaptation to the stress condition instead of for growth enhancement (Lei and Slinger, 1970). Alternatively, it is believed that less weight gain in the HS groups is due to a smaller appetite and lower feed intake, as it may be a defense mechanism to help reduce heat production.

The rise in BW gain in supplemented broilers is believed to be a cumulative effect of prebiotic and probiotic foods, which serve to promote beneficial bacteria, intestinal function, and disease resistance (Rehman et al., 2007; Awad et al., 2008; Yang et al., 2008; Sohail et al., 2010). Oligosaccharides improve appetite and feed consumption in broilers, which eventually increase BW gain and feed efficiency (Gao et al., 2008). Keeping these arguments in view, it is safe to assume that supplements improved nutrient absorption from the intestine and counterbalanced the negative effect of HS. In the current study, only MOS significantly improved BW gain of HS broilers, whereas PM or SYN failed to influence the production performance. Heat stress at 35°C throughout the experimental trial increased mortality in the HS-CONT and the HS-PM groups compared with other groups. The rise in mortality in the HS-CONT and the HS-PM groups may be attributed to a fall in immunity and resistance against diseases. In a previous study, we observed that HS increases *Clostridium perfringens* load in chicken intestine (unpublished data). However, in the current study, no clinical signs of any specific infection or disease were observed in the birds. Adrenal corticosterone, the main glucocor-

Table 3. Corticosterone concentrations (pg/mL) in broilers reared under normal temperature or heat stress conditions

	Treatment group[1]				
Day	CONT	HS-CONT	HS-MOS	HS-PM	HS-SYN
21	$90.7^{b} \pm 22.56$	$877.4^{a} \pm 185.70$	$233.3^{b} \pm 75.50$	$93.4^{b} \pm 17.79$	$250.7^{b} \pm 86.52$
42	$51.8^{b} \pm 10.07$	$278.0^{a} \pm 87.48$	$81.5^{b} \pm 58.41$	$167.4^{ab} \pm 45.81$	$125.8^{ab} \pm 27.14$

[a,b]Means ± SE within a row lacking a common superscript differ ($P < 0.05$).

[1]CONT = control; HS = heat stress; MOS = mannan-oligosaccharide; PM = probiotic mixture; SYN = synbiotic.

Table 4. Ileal villus height (mm), villus width (mm), crypt depth (mm), and villus surface area (mm^2) of broilers reared under normal temperature or heat stress conditions

	Treatment group[1]				
Parameter	CONT	HS-CONT	HS-MOS	HS-PM	HS-SYN
d 21					
Villus height	$0.68^{a} \pm 0.01$	$0.59^{b} \pm 0.00$	$0.53^{c} \pm 0.00$	$0.56^{c} \pm 0.00$	$0.55^{c} \pm 0.00$
Villus width	$0.30^{b} \pm 0.01$	$0.22^{c} \pm 0.00$	$0.22^{c} \pm 0.00$	$0.56^{a} \pm 0.00$	$0.24^{c} \pm 0.00$
Crypt depth	$0.17^{b} \pm 0.00$	$0.13^{c} \pm 0.00$	$0.16^{b} \pm 0.00$	$0.16^{b} \pm 0.00$	$0.24^{a} \pm 0.00$
Villus surface area	$0.60^{b} \pm 0.01$	$0.41^{c} \pm 0.01$	$0.36^{c} \pm 0.01$	$1.0^{a} \pm 0.02$	$0.43^{c} \pm 0.01$
d 42					
Villus height	$1.08^{a} \pm 0.01$	$0.88^{b} \pm 0.02$	$0.87^{b} \pm 0.01$	$0.89^{b} \pm 0.02$	$1.04^{a} \pm 0.01$
Villus width	$0.30^{a} \pm 0.00$	$0.23^{c} \pm 0.00$	$0.27^{b} \pm 0.00$	$0.21^{c} \pm 0.00$	$0.25^{bc} \pm 0.00$
Crypt depth	$0.27^{a} \pm 0.10$	$0.19^{c} \pm 0.06$	$0.20^{c} \pm 0.06$	$0.20^{c} \pm 0.07$	$0.23^{b} \pm 0.06$
Villus surface area	$1.02^{a} \pm 0.03$	$0.64^{c} \pm 0.03$	$0.72^{bc} \pm 0.03$	$0.63^{c} \pm 0.02$	$0.81^{b} \pm 0.03$

[a–c]Means ± SE within a row lacking a common superscript differ ($P < 0.05$).

[1]CONT = control; HS = heat stress; MOS = mannan-oligosaccharide; PM = probiotic mixture; SYN = synbiotic.

ticoid of poultry, antagonizes immune response against infection (Tankson et al., 2001), and we observed an increase in its concentrations in the HS-CONT group.

Elevation of serum corticosterone level is considered as an indicator of HS (Quinteiro-Filho et al., 2010). The HPA axis is a complex neuroendocrine pathway that controls reactions to stress. The rise in the serum corticosterone concentrations is considered as an indication of overactivation of the HPA axis. In the present study, we observed that keeping birds under chronic HS increased the serum corticosterone concentrations compared with the CONT group and supplementations helped to normalize the serum corticosterone. Certainly, the overexcitement of the HPA axis is deleterious, given that it impairs growth and compromises body immune system (Zulkifli et al., 2000; Sohail et al., 2010; Haldar et al., 2011). In the current study, the supplemented groups had lower corticosterone concentrations compared with the HS-CONT group. As these supplements are believed to influence the gut health and microbiome, it can be postulated that a healthy and balanced microbial community may have helped normalize adrenal gland activity.

Maintenance of normal microarchitecture in the small intestine is very important for proper growth and development. In this study, we observed that HS decreased villus height, width, crypt depth, and villus surface area. Quite a few studies have reported that stress hampered the development of intestinal morphology and function (Mitchell and Carlisle, 1992). Stressors such as fasting or corticosterone injections have noxious effects on the intestinal microarchitecture, resulting in reduction in absorptive surface area (Yamauchi et al., 1995, 1996; Hu and Guo, 2008). However, not much is known about the effect of HS on intestinal morphology, aside from the Burkholder et al. (2008) report, describing a decrease in crypt depth in HS broilers compared with the CONT group.

Increase in villus height and width provide a greater surface area for nutrient digestion and absorption pursuant to increased mucosal enzymes, absorption, and nutrients transport system (Amat et al., 1996). In the present study, application of PM and SYN improved the ileal villus width, surface area, and crypt depth. Several other studies have reported beneficial effects of probiotics on intestinal microarchitecture (Awad et al., 2008; Rahimi et al., 2009).The fermentation profile of probiotic bacteria consists of several short-chain fatty acids that are believed to exert trophic effects on the intestinal microarchitecture (Wong et al., 2006). Therefore, the improvement in histology of intestine could possibly be the total effect of probiotics fed to HS broilers. Based on these findings, it can be concluded that the dietary supplementation of MOS and PM can partially help in reducing detrimental effects of chronic HS in broilers.

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