Research Note: Responses of growth performance, immune traits, and small intestinal morphology to dietary supplementation of chromium propionate in heat-stressed broilers

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ABSTRACT This study was conducted to investigate the dose responses of growth performance, immune traits, and small intestinal morphology to dietary supplementation of chromium propionate (**CrPro**) in heat-stressed broilers. A total of 252 1-day-old Cobb 500 male broilers were randomly assigned to 6 treatments with 7 replicate cages of 6 birds per cage. The dietary treatments consisted of a basal diet supplemented with 0, 0.2, 0.4, 0.8, 1.6, and 3.2 mg/kg Cr in the form of CrPro. The birds had ad libitum access to feed and tap water for an experimental period of 42 D. For induction of heat stress, the house temperature was set at 35°C ± 2°C from 22 to 42 D of age. No differences were detected among treatments in growth performance during the experimental period ($P > 0.05$). Serum IgA concentrations were not affected by treatment ($P > 0.05$). However, a quadratic response was detected for serum IgG ($P < 0.01$) and IgM ($P < 0.01$) concentration as dietary Cr supplementation was increased. The highest response of IgG and IgM in serum was observed for broilers fed a diet supplemented with 0.2 mg of Cr/kg. Dietary supplementation of Cr had no impacts on villus height, crypt depth, or the ratio of villus height to crypt depth in the jejunum and ileum. A quadratic response of villus height and the ratio of villus height to crypt depth and a linear response of crypt depth to increased dietary Cr supplementation were observed in the duodenum ($P < 0.01$). The results indicate that CrPro supplementation could modify the intestinal morphology of the duodenum and influence serum IgG and IgM concentrations in heat-stressed broiler chickens. Based on the results of this experiment, the 0.2-mg Cr/kg diet from CrPro increases immune response and intestinal health in heat-stressed broilers.

Key words: chromium, heat-stressed broiler, growth performance, immunity, intestinal morphology

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INTRODUCTION

Heat stress has been recognized as a major problem in the poultry industry worldwide because heat stress not only has detrimental effects on growth performance but also has a negative influence on carcass traits and immunity. In addition, heat stress has a negative impact on intestinal development, resulting in a decrease in nutrient utilization (Makanjuola and Adebiyi, 2012).

Dietary chromium (**Cr**) supplementation has been shown to increase growth performance (Huang et al., 2016) and immunological responses (Bahrami et al., 2012) in broiler chicks under heat-stressed conditions. Chromium propionate (**CrPro**), as a supplement of the broiler diet, recently was approved by the US Food and Drug Administration for supplementation to broiler diets at concentrations up to 0.20 mg Cr/kg (FDA, 2016). There is limited information on the dose response of CrPro on immune function and intestinal morphology. Therefore, we hypothesized that supplementation of CrPro may alleviate the negative impact of heat stress via enhancing intestinal health and immune function in stressed broilers. To test our hypothesis, the effect of increasing dietary levels of CrPro supplementation on growth performance, serum immunoglobulin, and small intestinal morphology was investigated in broilers under constant heat stress conditions.

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MATERIALS AND METHODS

Birds, Diets, and Experimental Design

All experimental procedures used in this experiment were reviewed and approved by the Animal Care and Use Committee of Chinese Southwest Minzu University. A total of 252 1-day-old Cobb 500 male broilers were stratified by weight and randomly assigned to 1 of the 6 treatments (7 replicate cages of 6 chicks per cage of each treatment). The treatment diets included a basal diet and the basal diets supplemented with 0.2, 0.4, 0.8, 1.6, and 3.2 mg/kg Cr from CrPro (Kemin Industries, Inc., Zhuhai, PR China). The broilers were housed in plastic-coated stainless-steel cages with fiberglass feeders in an electrically heated and thermostatically controlled room. Ambient temperature was set at 34°C during the first week of the experiment and then reduced by 3°C/wk to 26°C at the end of the third week (21 D). For induction of heat stress, the ambient temperature was set at 35°C ± 2°C from day 22 until the end of the trial at day 42 (Jahanian and Rasouli, 2015). The relative humidity was kept at 67%, and the lighting program consisted of 23 h light and 1 h of darkness during the experimental period.

The corn–soybean meal basal diet (Table 1) was formulated to meet or exceed all NRC nutrient requirements for male broilers (National Research Council, 1994). Nutrient composition of the starter and grower diets are presented in Table 1. Samples of experimental diets were collected weekly and composited by phase for Cr analysis (data not shown). The birds were allowed ad libitum access to the experimental diets and tap water containing no detectable Cr (<5.0 μg/L of water by analysis).

Sample Collection and Analysis

BW gain and feed intake were recorded on a cage basis on day 21 and 42. The mortality was recorded once observed to adjust the feed conversion ratio values. Two birds were selected from each replicate cage as per the average BW on day 42 after fasting for 12 h. Blood samples were collected via the wing vein and then centrifuged at 2,000 × *g* for 10 min at 25°C. The serum was obtained and stored at −20°C for immunoglobulin determination. After the blood samples were collected, the 2 birds were exsanguinated by cutting the jugular vein. The intestine was removed and divided into 3 segments of the duodenum (from the gizzard to the entry of the bile and pancreatic ducts), jejunum (from the entry of the ducts to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the ileocecal junction). Approximately 3 cm of the middle portion of the duodenum, jejunum, and ileum was collected. Then, the specimens were gently flushed with PBS to remove the gut contents and fixed in 4% paraformaldehyde solution at 4°C for histologic analysis.

Laboratory Analysis

Feed samples were dried for 48 h at 55°C in a forced air oven and then prepared for Cr analysis. The concentrations of Cr in diets were determined by inductively coupled plasma emission spectroscopy (Thermo Fisher Scientific, Waltham, MA) after wet digestions with HNO_3 and $HClO_4$. Water samples were analyzed for Cr without ashing. The concentrations of Ca and CP in the feed ingredient or diet samples were determined as described by the Association of Official Analytical Chemists (1990; 927.02, 988.05). Serum Ig concentrations (IgA, IgG, and IgM) were determined using double-antibody sandwich commercial ELISA kits (Bethyl Laboratories, Montgomery, TX) following the instructions.

After being fixed for about 24 h, the intestinal specimens were dehydrated using ethanol and embedded using paraffin. Then, the specimens were cut to 4 to 5 μm thick and stained with hematoxylin and eosin. The histologic sections were examined with an Axioplan 2 optical microscope (Carl Zeiss Jena GmbH, Oberkochen, Germany) equipped with a refrigerated QImaging Retiga-4000R digital camera (QImaging, Surrey, Canada) coupled with a charge-coupled device detector. The image was analyzed with Motic Images Advanced 3.2. The villus height and crypt depth of 10 well-oriented villi were measured per section, and the ratio of villus height to crypt depth was calculated.

Statistical Analysis

Data analysis was performed by ANOVA using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with cage (n = 7) as the experimental unit. Treatment effects were separated using least squares means with the PDIFF option. Orthogonal comparisons were applied for linear and quadratic responses with consideration to the unequally spaced treatment levels. Significance was declared at $P \leq 0.05$, and tendencies were discussed at $0.05 < P < 0.10$.

RESULTS AND DISCUSSION

ADG, feed intake, and feed conversion ratio were not affected by supplemental Cr in either the starter or grower period (data not shown). The effect of Cr supplementation on growth performance observed in heat-stressed broiler chickens was inconsistent. Some studies showed that dietary Cr supplementation could alleviate the adverse effects on the performance in heat-stressed broilers (Huang et al., 2016; Sahin et al., 2017). However, Habibian et al. (2013) reported that body mass, feed intake, and feed conversion rate were not affected by dietary Cr supplementation in heat-stressed broilers. A previous study in our laboratory found that supplementation of 0.4 and 2.0 mg/kg Cr from CrPro, $CrCl_3$, or Cr picolinate increased ADG in heat-stressed broilers (Huang et al., 2016). Female broilers were used in our previous study, and male broilers were used in the present study. This suggests that the response of female broilers to dietary Cr supplementation might be different from male broilers. Indeed, an interaction between dietary Cr supplementation and gender of broilers was detected recently in broilers (Spears et al., 2019).

Table 1. Composition of basal diets for broilers.

Item[1]/% (unless stated otherwise)	Starter (1–21 D)	Grower (22–42 D)
Ingredient		
Ground yellow maize	56.37	63.12
Soybean meal (44.16% CP)	32.05	25.90
Soybean oil	3.00	3.50
Corn gluten meal	4.50	4.00
Calcium hydrogen phosphate	1.91	1.50
Ground limestone	1.10	0.85
Salt	0.30	0.30
L-Lysine HCl	0.07	0.16
DL-Methionine	0.18	0.07
Premixs[2]	0.20	0.20
Maize starch + Cr	0.32	0.40
Nutrient composition (calculated)		
ME (MJ/kg)	12.59	12.97
CP[3]	22.21	19.52
Calcium[3]	1.10	0.90
Lysine	1.20	1.05
Methionine	0.54	0.39
Methionine + cysteine	0.90	0.73
Available phosphorus	0.45	0.38
Chromium analyzed[3] (mg/kg)	1.12	1.09

[1]Ingredient and nutrition composition was recorded as fed-basis.
[2]Provided as following per kilogram diet: vitamin A 8,400 IU, vitamin D_3 3,600 IU, vitamin E 13 IU, vitamin K 1.6 mg, thiamine 5.5 mg, vitamin B_2 6.8 mg, vitamin B_6 1.0 mg, vitamin B_{12} 0.01 mg, biotin 0.08 mg, folic acid 0.80 mg, pantothenic acid 10.2 mg, niacin 28.6 mg, choline (choline chloride) 1,000 mg, Cu (from $CuSO_4 \cdot 5H_2O$), 8 mg, Zn (from $ZnSO_4 \cdot H_2O$), 40 mg, Fe (from $FeSO_4 \cdot 7H_2O$), 80 mg, I (from $Ca(IO_3)_2$), 0.35 mg, Se (from Na_2SeO_3), 0.15 mg, Mn (from $MnSO_4 \cdot H_2O$), 80 mg.
[3]Analyzed values. Each value based on triplicate determinations.

The mechanism behind this needs to be investigated and clarified in future studies.

Chromium supplementation increased IgG and IgM in plasma or IgG in serum in heat-stressed broilers (Bahrami et al., 2012), suggesting that the dietary Cr could modify immunoglobulins in the circulation system. In the present study, serum IgG and IgM responded quadratically to increasing dietary Cr (Table 2). Broilers supplemented with 0.2 mg Cr/kg had greater IgG and IgM concentrations than controls. Increasing supplemental Cr from 0.2 to 0.4 or 0.8 mg Cr/kg decreased serum IgG and IgM concentrations. Serum IgG and IgM were similar in broilers supplemented with 0.8 and those fed 1.6 or 3.2 mg Cr/kg. This implies that dietary supplementation of 0.2 mg Cr/kg from CrPro is sufficient to maximize serum IgG and IgM concentrations in heat-stressed broilers. This is consistent with the maximum supplemental concentration (0.2 mg Cr/kg) of CrPro allowed by the US Food and Drug Administration (FDA, 2016). Serum concentrations of IgA were not affected by treatment (Table 2). The effect of Cr on immune functions could be owing to a decrease in the secretion of corticosteroids and potentiation in the action of insulin (Jahanian and Rasouli, 2015).

No differences were observed in villus height, crypt depth and the ratio of villus height to crypt depth of the

Table 2. Effects of chromium propionate supplementation on serum Ig concentration and duodenum morphology of male broilers under heat-stressed conditions.[1]

		Serum			Duodenum		
Treatments	Cr supplementation (mg/kg)	IgA (μg/mL)	IgG[2] (mg/mL)	IgM[2] (μg/mL)	Villus height (μm)	Crypt depth (μm)	Villus height/crypt depth
1	0	212.5	1.92^{b}	209.4^{b}	1,507^{b}	161.0^{b}	9.42^{c}
2	0.2	215.1	2.16^{a}	239.0^{a}	2,090^{a}	166.6^{b}	12.56^{a}
3	0.4	227.2	1.50^{c}	213.4^{b}	2,057^{a}	164.6^{b}	12.41^{a,b}
4	0.8	231.4	1.32^{c}	190.7^{b}	2,044^{a}	162.4^{b}	12.60^{a}
5	1.6	225.1	1.38^{c}	196.2^{b}	2,145^{a}	182.9^{a}	11.74^{a,b}
6	3.2	229.3	1.37^{c}	192.9^{b}	1,977^{a}	180.6^{a}	10.96^{b,c}
P value		0.197	<0.0001	0.001	0.0002	0.0001	0.0023
SEM		8.20	0.08	9.70	87	3.60	0.54
Linear		0.106	0.078	0.157	0.0762	<0.0001	0.6755
Quadratic		0.156	<0.0001	<0.0001	0.0005	0.1149	0.0063

[a–c]Means within a column without a common superscript letter are different ($P < 0.05$).
[1]Each value represents the mean of 7 cages with 2 broilers/cage of individual treatment.
[2]Orthogonal comparisons applied for linear and quadratic responses was conducted for the first 4 treatments.

jejunum and ileum (data not shown). However, the villus height of duodenum and villus height-to-crypt depth ratio increased quadratically ($P < 0.01$) as the dietary chromium dosage was increased (Table 2). Compared with control, birds fed diet with 0.2 mg Cr/kg had a higher ($P < 0.05$) villus height. Increasing supplemental Cr greater than 0.2 mg/kg did not further increase villus height. Crypt depth of the duodenum was affected linearly ($P < 0.01$) by dietary Cr. The greatest crypt depth was observed in broilers supplemented with 1.6 and 3.2 mg Cr/kg. Limited research has investigated the effect of dietary Cr on intestinal morphology during heat stress. Makanjuola and Adebiyi (2012) observed that the villus height was increased, and the crypt depth was reduced in the ileum of heat-stressed broilers supplemented with 0.15 and 0.25 mg Cr/kg from $CrCl_3$. The performance of broilers is directly related to their nutrition and the morphological function in the integrity of the digestive system (Marchini et al., 2011). Moreover, the structural integrity of the intestinal mucosal epithelium has a close connection with villus height and crypt depth, which are important indicators of intestinal digestion and absorption function. Heat stress has been shown to negatively affect gut morphology in broilers. Quinteiro-Filho et al. (2012) showed that mild acute multifocal lymphoplasmacytic enteritis in the duodenum, jejunum, and ileum was induced in heat-stressed broiler chickens. Marchini et al. (2011) reported that less crypt depth, mucous area, and villus height of the duodenum and lower intestinal length were noticed in heat-stressed chicks at 42 D of age. The present study suggests that supplementation of 0.2 mg Cr/kg diet from CrPro may partially alleviate the negative effect of heat stress on duodenal morphology.

In conclusion, dietary supplementation of 0.2 mg CrPro/kg increased serum IgG and IgM concentrations and improved the intestinal morphology of duodenum in broiler chicks under heat-stressed conditions. Results indicated that supplementation of 0.2 mg Cr/kg from CrPro would be beneficial for heat-stressed broilers to increase immune response and intestinal health.

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