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Effects of Chromium Picolinate Supplementation on Growth Performance, Small Intestine Morphology and Antioxidant Status in Ducks Under Heat Stress Conditions

Efectos de la Suplementación con Picolinato de Cromo en el Rendimiento de Crecimiento, Morfología del Intestino Delgado y Estado Antioxidante en Patos Bajo Condiciones de Estrés Calórico

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SUMMARY: The experiment was conducted to evaluate the effects of dietary supplemental chromium (Cr) on growth performance, meat quality, intestinal morphology, mucosa Hsp70 mRNA expression and antioxidant status of ducks reared under heat stress conditions. All ducks were randomly divided into three treatment groups, respectively, control group (Control, 23 ± 2 °C), heat stress group (HS, 32 ±2 °C), Cr picolinate group (CrPic, 32 ± 2 °C, 0.2 mg Cr/kg). Feed and distilled-deionized water were available ad libitum for an experimental phase of 35 days. Samples were collected on the day 14, 21 and 35 to determine biological and hematological values. Results showed that heat stress or dietary supplemental Cr both didn't have distinct influence on growth performance ($P>0.05$), compared to controls. Ducks fed 0.2 mg Cr/kg diet had greater ultimate pH (pHu)($P<0.05$) than HS group. At day 14, the ratio of villus height to crypt depth (V/C) in CrPic group significantly increased ($P<0.05$) than that of HS group in jejunum. Heat stress remarkably increased Hsp70 mRNA expression in jejunum compared with controls ($P<0.05$). While the expression of Hsp70 mRNA in CrPic group was significantly decreased compared with HS ($P<0.05$). At day 21, the V/C of ileum in CrPic group significantly increased compared with HS group ($P<0.05$). Serum SOD levels in CrPic group were significantly higher than those in HS group ($P<0.05$). At day 35, Hsp70 mRNA expression and serum T-SOD levels in CrPic group significantly increased compared with controls ($P<0.05$). T-AOC in HS group significantly decreased compared with controls ($P<0.05$). Results indicate that dietary Cr supplementation doesn't influence ducks' growth performance, but has a positive effect on meat quality, small intestine morphology, also regulates Hsp70 mRNA expression under heat stress conditions, and enhances the antioxidant status.

KEY WORDS: Chromium; Heat stress; Duck; Small intestine; Hsp70.

INTRODUCTION

Heat stress is of major concern in all aspects of poultry production, especially in hot regions of the world, due to the negative effects on feed intake, body weight gain, mortality, hatchability, and other important traits governing the economic success of the poultry industry. More specifically, heat stress will cause decreases in the feed conversion and intake, weight gain, growth rates (Bartlett & Smith, 2003); decreases in blood lymphocytes and spleen weight (Trout & Mashaly, 1994); increases in expression of Hsp70 mRNA (Gaughan *et al*., 2013). It also has been indicated that heat stress negatively affects intestinal health including intestinal villi development (Pearce *et al*., 2013), the integrity of the intestinal epithelium (Morales *et al*., 2016), the intestinal mucosa immune function (Borsoi *et al*., 2015), and the balance of the digestive tract flora (Burkholder *et al*., 2008; Quinteiro Filho *et al*., 2012). High ambient temperature has been implicated in disrupting the body redox balance through increasing reactive oxygen species (ROS) production (Del Vesco *et al*., 2014) or reducing antioxidant defenses (Di Trana *et al*., 2006), causing oxidative damage. In addition, increasing demand for food coupled with threats of global warming will further accentuate heat stress-related problems (Renaudeau *et al*., 2012).

The primary role of Cr in metabolism is attributed to a regulatory role in insulin action. Cr is a component of an

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oligopeptide low molecular weight Cr-binding substance, chromodulin, functioning as a part of the insulin signaling auto-amplification mechanism (Schwarz & Mertz, 1959; Mertz, 1967, 1969). Furthermore, Cr, by decreasing high levels of insulin and preventing auto-oxidation of glucose, may act as an indirect antioxidant (Roussel *et al*., 2007). It has been recognized that Cr is an essential trace element for human and the National Academy of Science (NRC 2002) has established a daily adequate intake (AI) of 35 and 25 mg/day of trivalent Cr for adult male and females, respectively. What's more, previous studies involving broilers fed supplemental Cr have shown a positive effect on growth performance, carcass traits, and antioxidant capacity when under heat stress conditions (Toghyani *et al*., 2012; Huang *et al*., 2016). Immunological function has also been enhanced by trivalent Cr, and its effects seem to be more pronounced during times of stress (Wang *et al*., 2007; Lien *et al*., 2014). Thus, dietary supplemental Cr is a rational strategy to overcome the adverse consequences in poultry production at high environmental temperature. Nevertheless, an appropriate recommendation on the Cr requirement of poultry has yet not been made (NRC, 1994) and the research studies carried out over the past decades were mostly based on mammal or broilers. Poultry species are less heat-tolerant than mammalian species due to a limited heat dissipation capacity (Yahav *et al*., 2004). And compared with broilers, ducks is more sensitive to HS. Therefore, whether Cr has a positive effect on heat-stressed ducks or not is remain unknown. Taking these factors into consideration, we hypothesized that heat-stressed ducks might have impairments in many aspects of health, and dietary addition of Cr could ameliorate these impairments induced by heat stress. Therefore, the objective of this study was to investigate the effects of organic Cr on the growth performance, meat quality, intestinal morphology, Hsp70 mRNA expression and antioxidant status of ducks under heat stress conditions.

MATERIAL AND METHOD

Ducks, Diets, and Managements. All experimental procedures described herein were approved by the Huazhong Agricultural University for Nationalities Animal Care and Use Committee. A total of 180 1-day-old ducks (Cherry Valley) were used for this study, whose initial body weight was 60 ± 5 g. The ducks were randomly assigned into three treatment groups, each treatment consisted of five replicate cages that contained 12 ducks per cage. The ducks in all groups were given the basal diet including maize-soybean meal diet. The corn-soybean meal basal diets (Table I) were formulated to meet or exceed the nutrient requirements of ducks National Research Council (NRC, 1994).

Table I. Ingredients and nutrient analyses of the basal diets.

Items	Starter diet _0-14 days_	Grower diet _15-35 days)
Ingredients_%_		
Corn	57.94	63.45
Wheat bran	5	8
Soybean meal	27	17
Rapeseed meal	6	8
limestone	1.2	1.2
Dicalcium phosphate	1.1	0.8
Vitamin and mineral premix[1]	1.0	1.0
Salt	0.4	0.35
Synthetic lysine	0.2	0.1
Synthetic methionine	0.16	0.1
Nutrient		
ME_Mcal/kg_	2.89	2.93
Crude protein	19.5	16.9
Calcium[2]	0.83	0.75
Available phosphorus	0.39	0.34
Total phosphorus	0.64	0.58
Calcium and phosphorus ratio	1.3:1	1.3:1

1.Vitamin and mineral premixes supplied per kilogram diet: vitamin A 13000IU; vitamin D3 6600IU; vitamin E 83mg; vitamin B2 10mg; vitamin K3 2mg; pantothenic acid 8mg; niacin 42mg; folic acid 1.6mg; biotin 0.05mg; Fe 80mg; Mn 80mg; Cu 5mg; Zn 70mg; I 3mg; Co 1mg; Se 0.2mg
2. The protein, calcium and total phosphorus content of the feedstuffs were analysis values. Other nutrient levels were theoretical values

Prior to the experiment, the house was disinfected with white wash and fumigated with formalin gas. Feeders and drinkers were properly disinfected with 5 % $KMnO_4$ solution and dried under direct sunlight. The brooder temperature was maintained at 35±0.5 °C and gradually decreased to 25°C during the first week, using a 24 h artificial light regime throughout the experimental period. After which, the control group were maintained at room temperature (21°C to 25°C), fed with the basal diet. Heat Stress group fed with the basal diet under an ambient temperature of 30-34 °C; CrPic group were fed with basal diet mixed with 200 mg/kg CrPic under an ambient temperature of 30-34 °C. All ducks had ad libitum access to feed and water during the 35-day study.

Sample Collection. On day 14 of the experiment, one duck was randomly selected in each repeat, according to the average BW, within the cage following a 12-h fast, weighed individually, and humanely harvested according to the welfare of animal slaughter regulations of China. Carcass weight measurements were collected

after defeathering. Blood was collected from the jugular vein in tubes and tubes (EDTA, anticoagulant) respectively, kept on ice. Two-centimeter intestinal segments of the distal duodenum, jejunum, and ileum (3 cm proximal to Meckel's diverticulum) were removed and fixed in 4 % (w/v) paraformaldehyde for analysis of intestinal morphology. Appropriate size of jejunum was removed, immediately frozen in liquid nitrogen and then stored at -80 °C until analysis for RNA.

Growth performance. Daily feed intake was accurately recorded and calculated every day. Weighing fasting ducks in the morning after 12 hours of feed deprivation at 7, 14, 21, 28, and 35 days of the experiment to record body weight. And feed efficiency per pen was calculated as body mass gain per unit feed intake. According to feed intake and daily gain to calculate average daily gain (ADG), average daily feed intake (ADFI), feed/gain (F/G).

Meat quality. The left pectoralis major was used to assess pH and drip loss. The pH of breast muscle was determined at 15 min named initial pH (pHi) and 24h named ultimate pH (pHu) postmortem by a universal Sartorius pH meter. Each sample was measured 3 times, and the mean value was recorded as the final result. To determine drip loss, approx. 10 g left pectoralis major was obtained and weighed. Then each meat sample was packed in a zip-sealed plastic bag and stored at 4. After 24 h, surface moisture was absorbed with filter paper, and the muscle was reweighed. The difference in weights of samples before and after storage was expressed as percent drip loss.

Intestinal Morphology. The tissue samples were dehydrated and embedded in paraffin wax by standard techniques, sectioned (3-4 mm). Each sample was subsequently stained (hematoxylin and eosin) according a routine protocol, and examined under a microscope using an ocular micrometer. Villus height was measured from the tip of the villi to the villous-crypt junction, whereas crypt depth was measured from this junction to the base of the crypt (Nabuurs *et al*., 1993). The villus height and crypt depth of 10 well-oriented villi were measured per section. The average value for each tissue was obtained from 5 sections. Morphology data were analyzed using the GLM procedure in SAS.

Expression of Hsp70 mRNA in Jejunum. The jejunum intestine lumen was washed with 4 % saline. Then, they were preserved in 2 mL centrifugal tube and well-marked, quickly set in liquid nitrogen frozen preservation at -70 °C to be measured for RT-PCR. Total RNA was extracted with TRIzol reagent according to the manufacturer's instructions (Invitrogen, USA). The concentrations and purity of isolated RNA were determined with a spectrophotometry (Smart Spec plus, BIO-RAD, USA). First-strand complementary DNA (cDNA) was synthesized using oligo (dT)18 primers and SuperScript II reverse transcriptase according to the manufacturer's instructions (Invitrogen, China). Synthesized cDNA was diluted five times with sterile water and stored at -80 °C before use. Real-time quantitative PCR (qPCR) was examined with SYBR Premix Ex Taq TM (Takara, China) and detected with the ABI PRISM 7500 qPCR system (Applied Biosystems) following the manufacturer's guidelines. The Hsp70 primer pair was deduced from a partial sequence for ducks (NCBI, No.EU678246), shown in Table II. The mRNA relative levels were calculated according to the 2-Ct method, accounting for gene-specific efficiencies and were normalized to the mean expression of the housekeeping gene b-actin.

Serum Antioxidant Enzyme and MDA. The levels of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), total antioxidant capacity (T-AOC), and malondialdehyde (MDA) in serum were determined by commercial kits (Jiancheng Biochemical Reagent Co., Nanjing, China) according to the manufacturer's instructions.

Statistical Analysis. All data were analyzed by one-way analysis of variance (ANOVA) according to the general linear model procedures (GLM) of SAS 9.0 (SAS Institute Inc., Cary, NC) as a randomized block design. The model included terms for treatment and block. Each pen served as the experimental unit for growth performance, and individual duck was considered as the experimental unit for other indexes. All measurement results were expressed as mean±standard deviation (Mean±SD).The differences of means between treatments were compared by Duncan's multiple range test and were considered statistically significant at $P<0.05$.

Table II. Gene-specific primers for HSP70 and b-actin used in the qPCR.

Gene	Primer sequence (5_–3_)	Primer length (bp)
Hsp70	Up: CGGTGGAGGATGATAAACTG	20
	Down: CTCTTTCTGCTTGTGCTCGT	20
_-actin	Up: GACATTGACATCAGGAAGGAC	18
	Down: CTTGATCTTCATTGTGCTGG	20

RESULTS

Growth performance. Table III lists the effects of heat stress and Cr supplementation on growth performance of ducks. Feed intake, weight, daily gain and feed conversion ratio at 21 and 35 days of age were not significantly affected (P>0.05) by heat stress or supplementation with Cr in duck's diet, compared with controls.

Meat quality. The effects of heat stress and Cr supplementation on meat quality of ducks were displayed in Table IV. Three groups didn't have significant variation in both drip loss and initial pH (P>0.05). Compared with the control group, the HS group had lower ultimate pH (P=0.056). Moreover, in contrast with the HS group, the CrPic group had increased ultimate pH significantly (P=0.023).

Morphological Measurement of Small Intestine Mucosa. Small Intestine morphology of ducks is presented in Table V, Table VI and Table VII. The effects of heat stress and Cr supplementation on small intestine at day 14 are shown in Table V. At day 14, no differences (P>0.05) were observed in villus height, crypt depth and V/C ratio of duodenum and ileum in three treatment groups. What's more, three treatment groups both had no significant difference in jejunum's villus height (P>0.05). The HS group had deeper

Table III. Effects of chromium supplementation on growth performance of ducks (n=25).

Day	Item	Control	HS	CrPic
1-21d	Feed intake(g)	1410.48±70.09	1357.87±72.11	1363.07±43.58
	21 day weight(g)	651.56±13.78	656.69±31.01	656.92±14.20
	Daily gain(g)	31.18±0.57	31.42±1.52	31.49±0.69
	Feed conversion ratio(g/g)	2.23±0.09	2.16±0.06	2.17±0.09
22-35d	Feed intake(g)	2408.93±136.35	2243.64±124.37	2266.80±149.78
	21 day weight(g)	1440.26±91.21	1370.09±98.04	1356.49±82.34
	Daily gain(g)	51.14±7.01	49.88±4.67	49.30±4.48
	Feed conversion ratio(g/g)	32.0±0.25	3.27±0.27	3.29±0.15

a,b Means within a column with different letters differ significantly (P<0.05).

Table IV. Effects of chromium supplementation on meat quality in ducks (n=25).

Item	Control	HS	CrPic
Drip loss(%)	4.31±0.81	4.14±1.32	4.66±1.64
Initial pH	6.01±0.07	5.98±0.21	6.03±0.17
Ultimate pH	6.32±0.01^{ab}	6.12±0.18^{b}	6.37±0.16^{a}

a,b Means within a column with different letters differ significantly (P<0.05).

Table V. Effects of organic chromium on morphology of intestine of ducks at day 14 (n=25).

Item	villus height (_m)	crypt depth (_m)	V/C[1]
Duodenum			
Control	303.60±26.18	68.80±4.57	4.47±0.24
HS	284.90±26.53	68.87±9.72	4.23±0.57
CrPic	307.74±28.30	69.91±7.83	4.29±0.43
Jejunum			
Control	309.28±24.59	66.95±7.08^{b}	4.68±0.31^{ab}
HS	304.69±24.62	69.21±1.76^{a}	4.23±0.12^{b}
CrPic	342.55±14.41	69.05±5.95^{ab}	5.02±0.24^{a}
Ileum			
Control	298.96±36.38	69.90±9.19	4.39±0.33
HS	298.05±38.57	76.30±12.44	3.99±0.21
CrPic	297.37±40.33	74.69±3.06	4.00±0.48

a,b Means within a column with different letters differ significantly (P<0.05).
1V/C = villus height: crypt depth.

(P<0.05) crypt depth than controls. The CrPic group tended to decrease crypt depth with no significance (P=0.33), but remarkable increased (P<0.05) the V/C ratio, compared with HS group. The effects of organic chromium on the morphology of intestine of ducks at day 21 are presented in Table VI. At day 21, HS would significantly decreased villus height in ileum compared with controls (P<0.05). The CrPic group tended to improve villus height under HS condition with no significance (P=0.18). And the V/C ratio in CrPic group was significantly higher than that in HS group (P<0.05). At day 35, there were no significant differences (P>0.05) were observed in villus height, crypt depth and V/C ratio of duodenum and ileum between three treatment groups. In jejunum, HS had decreased V/C ratio significantly compared with controls. Dietary supplemental CrPic tended to increase V/C ratio in contrast with HS group, however, the difference was not significant (P=0.20).

Table VI. Effects of organic chromium on morphology of intestine of ducks at day 21 (n=25).

Item	Villus height (_m)	crypt depth (_m)	V/C[1]
Duodenum			
Control	378.59±32.40	94.45±10.92	4.08±0.29
HS	428.90±25.39	92.10±5.84	4.50±0.39
CrPic	408.78±27.07	92.38±11.10	4.47±0.28
Jejunum			
Control	397.87±41.74	71.62±2.99	5.60±0.51
HS	382.08±55.93	73.35±8.96	5.22±0.28
CrPic	411.78±67.39	73.26±7.22	5.65±0.49
Ileum			
Control	330.91±26.02^{a}	66.09±2.06	4.57±0.35^{ab}
HS	286.02±16.61^{b}	63.54±5.56	4.17±0.43^{b}
CrPic	298.42±23.86^{ab}	62.34±4.96	4.90±0.28^{a}

a,b Means within a column with different letters differ significantly (P<0.05).
1V/C = villus height: crypt depth.

Table VII. Effects of organic chromium on morphology of intestine of ducks at day 35 (n=25).

Item	villus height (_m)	crypt depth (_m)	V/C[1]
Duodenum			
Control	455.99±39.05	86.95±11.55	5.38±0.57
HS	442.64±68.90	85.32±7.64	5.21±0.64
CrPic	447.55±59.27	81.92±9.73	5.50±0.18
Jejunum			
Control	493.82±81.49	83.84±4.45	5.74±0.17^{a}
HS	436.74±61.48	84.69±16.29	5.31±0.82^{b}
CrPic	477.15±22.06	84.90±2.30	5.69±0.24^{ab}
Ileum			
Control	383.44±48.24	78.86±8.97	4.91±0.40
HS	335.62±23.47	80.62±9.31	4.22±0.39
CrPic	342.15±41.23	68.53±2.12	5.07±0.66

a,b Means within a column with different letters differ significantly (P<0.05).
1V/C = villus height: crypt depth.

Table VIII. Effects of supplemental chromium on HSP70 mRNA expression in jejunum (n=25).

Item	Date		
	14d	21d	35d
Control	1.05±0.93^{b}	1.82±0.26	2.28±1.38^{b}
HS	2.98±1.37^{a}	1.76±1.50	3.22±1.67^{a}
CrPic	0.66±0.46^{b}	1.44±1.35	3.44±0.50^{a}

a,b Means within a column with different letters differ significantly (P<0.05).

Hsp70 mRNA Expression in Jejunum Mucosa. The effects of heat stress and Cr supplementation on jejunum mucosa Hsp70 mRNA transcript expression are shown in Table VIII. The results revealed that at day 14, the Hsp70 mRNA expression was significantly (P<0.05) higher in HS group as compared to the control group, while significantly decreased (P<0.05) in CrPic group compared with HS group. At day 21, there were no significant differs between three treatments. At day 35, the expression of jejunum mucosa Hsp70 mRNA significantly increased (P<0.05) in both HS and CrPic groups contrast with controls.

Serum Antioxidant Parameters in ducks. The effects of dietary supplementation of Cr on serum antioxidant status are provided in Table IX. At 14th day, Serum T-AOC activities decreased significantly in CrPic group compared with control group. And no differences were observed in concentration of T-SOD and GSH-Px in those three treatment groups. In addition, ducks reared under HS had greater (P<0.05) serum MDA level than controls. And dietary supplementation of Cr could significantly decrease serum MDA level (P<0.05) compared with HS group.

At 21th day, serum T-AOC, MDA and GSH-Px levels were both not affected by HS or CrPic, compared to controls. HS would significantly decrease (P<0.05) Serum T-SOD levels compared to control group. And Serum T-SOD levels in CrPic group were significantly higher than those in HS group (P<0.05).

At 35th day, the levels of T-AOC remarkably decreased (P<0.05) in HS group, compared with the control group. Adding CrPic tended to increase Serum T-AOC levels, but didn't show significant differences (P=0.38) compared to HS group. CrPic would remarkably improve (P<0.05) Serum T-SOD levels compared to controls, while didn't increase Serum SOD levels obviously in contrast to HS group (P=0.15). Besides, three treatment groups both had no significant difference in Serum MDA and GSH-Px levels (P>0.05).

Table IX. Effects of chromium supplementation on serum antioxidant status in ducks (n=25).

Day	Item	Control	HS	CrPic
14d	T-AOC(U/mL)	25.95±5.00^{a}	21.29±5.72^{ab}	17.39±3.54^{b}
	T-SOD (U/mL)	80.14±17.76	75.33±16.47	74.74±9.46
	GSH-Px(U/mL)	359.29±64.26	325.12±48.89	324.14±27.68
	MDA(nmol/mL)	6.09±1.62^{b}	10.75±1.37^{a}	4.86±0.47^{b}
21d	T-AOC(U/mL)	16.33±4.77	19.49±1.04	16.10±1.97
	T-SOD(U/mL)	87.35±13.34^{a}	66.21±11.68^{b}	82.83±12.97^{a}
	GSH-Px(U/mL)	378.66±62.06	399.45±61.98	423.53±8.69
	MDA(nmol/mL)	11.11±5.93	6.67±2.85	8.02±2.49
35d	T-AOC(U/mL)	23.56±2.03^{a}	18.49±6.50^{b}	20.17±2.33^{ab}
	T-SOD(U/mL)	67.64±11.59^{b}	75.64±13.58^{ab}	87.89±6.55^{a}
	GSH-Px(U/mL)	389.60±51.53	393.98±18.57	362.79±60.75
	MDA(nmol/mL)	17.75±11.22	13.23±4.29	9.22±5.05

DISCUSSION

High ambient temperature will adversely affect performance and physiological traits of poultry. The reduction in feed intake and increase in excretion of minerals under HS are accompanied by decreased performance and a depressed health status and antioxidant system. Heat stress decreases feed intake, daily gain and feed conversion rate in poultry (Mashaly *et al*., 2004). And there are researches indicated that HS would reduce growth rate and feed conversion ratio of broiler chicken by reducing the feed intake (Quinteiro *et al*., 2012). However, according to our experiment, HS didn't have significant influence on growth performance of the ducks during the study period. Also based on Kim (2009) research, high ambient temperature didn't affect the feed conversion ratio of pigs. HS also significantly decreases Japanese quails' feed intake and body weight, whereas remarkable increases feed conversion ratio (Sahin *et al*., 2010). Hester *et al*. (1981) also demonstrated that ducks exposed to a constant temperature of 29.4 °Cshowed an increase in relative weight. These results are not consistent, the degree of heat stress may partially explain these discrepancies and the differences between experimental animals' species, breed or age. In this study, the effect is not obvious; this may have some connections with the amount of the organic Cr.

Similar observations have been demonstrated in previous studies examining the influence of Cr under heat stress conditions (Samanta *et al*., 2008; Toghyani *et al*., 2012). Several researches on broilers revealed improvement in weight gain and feed conversion with Cr supplementation under conditions of induced stress (Huang *et al*.). Cr has been proved as an essential element in the form of trivalent, Cr (III), and hexavalent, Cr (VI). During the past few years, the Cr function has been revealed on a molecular level (Racek, 2003; Clodfelder *et al*., 2004). Cr is widely applied in the form of organic compounds: yeast extract or Cr picolinate (Racek). After absorption in the gastrointestinal tract, Cr is most likely transported into cells and bounds to the plasma protein transferring (Vincent, 2000). Cr is involved in carbohydrate, lipid and protein metabolism through activating certain enzymes in poultry (Torki *et al*., 2014). As a strong and effective antioxidant, Cr also alleviates productive and metabolic decline in poultry exposed to HS (Sahin *et al*., 2002; Dogukan *et al*., 2009).

Chronic heat stress has been reported to increase the production of lactate in muscle, which in turn increased the rate of pH decline and subsequently decreased the quality of breast meat in broilers (Zhang *et al*., 2012). Turkeys reared continuously at a high temperature for 5 weeks, had lower breast muscle's pHi and pHu, and more drip loss (Armstrong *et al*.). This result agreed with the findings obtained in heat-stressed Ross chicks by Aksit (2006) and showed that a high ambient temperature would cause a lower pH, higher lightness, redness values and redness: yellowness in meat. Organic Cr can participate in carbohydrates, protein, and fat metabolism, enhance the function of insulin, so that an increase in muscle glycogen storage decomposition reduces, and inhibits the rapid decline of the post-mortem muscle pH. Cr supplementation of 500 μg/kg Cr has an improvement in the pH value of the breast muscles and leg muscles. In the current experiment, adding organic Cr under constant high temperature conditions has no effect on initial pH and drip loss of the ducks, but it would increase ultimate pH significantly ($P<0.05$).

A normal morphology and integrity of the small intestine is important to prevent bacteria translocation from

the intestinal tract to the body. What's more, the structural integrity of the intestinal mucosal epithelium has a close relationship, especially villous height, width, thickness and area, which are the important indicators of intestinal digestion and absorption function (Samal, Sun *et al*. 1994; Fukuhara *et al*. 2005; Lien *et al*.). It was revealed that chickens exposed to acute heat stress (30 °C/24 h) presented a reduction of the ileum's crypt depths but no significant differences in the villus height and villus: crypt ratio (Burkholder *et al*.), this showed that heat stress via changing the intestinal morphology and structure increased the organism risk of illness. Compared with birds kept at 21 °C, birds kept at 34 °C had decreased villus height, ratio of villus height to crypt depth and fewer intraepithelial lymphocytes (Deng *et al*., 2012), suggested that thermal stress damaged the normal intestinal health. In our study, at day 35, ducks in HS group had deeper crypt depth than those in CrPic group in ileum.

Cr supplementation has a great benefit to intestine, indicating that the supplements of Cr are able to alleviate the negative effects of the heat stress (Sahin *et al*., 2010; Mirzaei *et al*., 2011).

During our experiments, we tested the morphology of intestine at different time, respectively, day 14, 21, and 35, and the results varied in different intestinal segments. For example, at day 14, ducks supplied with CrPic had significantly increased the V/C ratio compared with the HS group in jejunum. At day 21, the CrPic group had higher V/C ratio of ileum than that in HS group. And at day 35, dietary supplemental CrPic tended to increase V/C ratio of jejunum in contrast with HS group, however, the result was not very significant. Based on our experiments, the results were not consistent with the previous studies. This may due to the difference between experimental animals or the concentration of Cr.

Heat shock proteins (HSP), also known as stress proteins, are expressed in different cell types and involved in many promoter pathways (Lindquist & Craig, 1988). HSP 70 is the most conserved Hsp in birds and mammals (Craig and Gross, 1991) and it serves as a cellular thermometer that regulates the expression of all heat shock proteins protecting the organism against the upshifting in body temperature (Gabriel *et al*., 1996). What's more, Hsp70 is famous for its ability to prevent apoptosis and oxidation and its function as molecular chaperones and anti-stress protection (Xing *et al*., 2015). Previous studies have indicated that the contents of Hsp70 increase in heart, liver, kidney (Endong *et al*., 2004) and brain tissues (Al-Aqil & Zulkifli, 2009) of broilers under heat stress. What's more, Dogukan *et al*. observed that Cr histidinate (CrHis) significantly decreased lipid peroxidation levels and Hsp expression in the kidneys of experimentally induced diabetic rats. This study supported the efficacy of CrHis to expression of Hsp70. However, the studies for Cr supplementation on Hsp70 mRNA were few in ducks. In our study, at day 14, the Hsp70 mRNA expression was significantly higher in HS group as compared to the control group, while significantly decreased in CrPic group compared with HS group. These results indicated the role of Cr for Hsp70 under heat stress conditions. Besides, it still needs further study on the specific mechanism that either Cr reduces the expression of Hsp70 mRNA by increasing the body's anti-stress ability or Cr improve the anti-stress ability of the body by increase the expression of Hsp70.

Environmental stress including high ambient temperature causes oxidative stress in vivo leading to an imbalance in oxidant/antioxidant system in poultry (Sahin *et al*., 2002). HS can disturb redox homeostasis by enhancing production of radical (Del Vesco *et al*., 2014) and causing oxidative damage of lipids, proteins and DNA (Schrauwen *et al*., 2010). Furthermore, animals stressed under improper environmental conditions are found to have higher lipid peroxidation levels in plasma and tissues due to increased production of free radicals (Naziroglu *et al*., 2000). High concentration of trivalent Cr has been demonstrated to cause oxidative damage, including DNA damage and lipid peroxidation by stimulating the formation of reactive oxygen species such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals(Tian *et al*., 2014). Additionally, some studies showed that additional low levels of Cr up to 400 mg/kg decreased oxidative stress in rats (Jain *et al*., 2007). Furthermore, it has been reported that, in high temperature condition, the diet adding 0.6-1.0 mg/kg chrome could strengthen body oxidation prevention function, restraint ROS, protect kidney tissue structure integrity, support body normal physiologic function. According to previous studies, Cr supplementation decreased the LP (MDA concentration) and increased the activities of antioxidant enzymes (GSH-Px, GSH Rx, and RBCC), suggesting that Cr supplementation progressively reduced oxidative stress with increase in its concentration in broiler diet (Rao *et al*., 2012).

Taking these into consideration, adding Cr can improve antioxidant properties of poultry and alleviate oxidative damage caused by heat stress. According to our experiment, at 14th day dietary supplementation of Cr could decrease serum MDA level significantly compared with HS group. At 21th day, serum SOD levels in CrPic group were significantly higher than those in HS group. And at 35th day, adding CrPic tended to alleviate the decrease of Serum T-AOC levels caused by HS. Besides, CrPic would remarkable improve Serum SOD levels compared to the controls. As a consequence, our results have confirmed the previous views.

CONCLUSIONS

It is concluded that dietary supplemental Cr at 0.2 mg /kg from CrPic had no influence on body mass and feed efficiency. In addition, dietary Cr supplementation has a positive effect on breast meat quality, intestinal health and antioxidant ability of ducks under heat stress conditions.

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RONG LI; YING ZHOU; YAN LI; LIANG GUO; YIFENG ZHANG & ZHILI QI. Efectos de la suplementación con picolinato de cromo en el rendimiento de crecimiento, morfología del intestino delgado y estado antioxidante en patos bajo condiciones de estrés calórico. *Int. J. Morphol., 36(1)*:226-234, 2018.

RESUMEN: Se evaluó los efectos del cromo (Cr) dietético suplementario sobre el rendimiento del crecimiento, la calidad de la carne, la morfología intestinal, la expresión del ARNm Hsp70 en la mucosa y el estado antioxidante de los patos criados bajo condiciones de estrés por calor. Todos los patos se dividieron aleatoriamente en tres grupos: grupo control (control, 23 ± 2 °C), grupo de estrés térmico (HS, 32 ± 2 °C) y grupo de picolinato de Cr (CrPic, 32 ± 2 °C, 0,2 mg Cr / kg). El alimento y el agua desionizada destilada estuvieron disponibles *ad libitum* durante la fase experimental de 35 días. Las muestras se recogieron los días 14, 21 y 35 para determinar los valores biológicos y hematológicos. Los resultados mostraron que el estrés térmico o la suplementación dietética de Cr no tuvieron una influencia distinta en el rendimiento del crecimiento (P> 0,05), en comparación con los controles. Los patos alimentados con 0,2 mg de Cr / kg de dieta tuvieron un mayor pH final (pHu) (P <0,05) que el grupo HS. En el día 14, la relación de la altura de las vellosidades a la profundidad de la cripta (V / C) en el grupo CrPic aumentó significativamente (P <0,05) en relación a la del grupo de HS en el yeyuno. El estrés por calor incrementó notablemente la expresión del ARNm de Hsp70 en el yeyuno en comparación con los controles (P <0,05). Mientras que la expresión del ARNm de Hsp70 en el grupo CrPic se redujo significativamente en comparación con HS (P <0,05). En el día 21, la relación V / C del íleon en el grupo CrPic aumentó significativamente en comparación con el grupo HS (p <0,05). Los niveles séricos de SOD en el grupo CrPic fueron significativamente más altos que los del grupo HS (P <0,05). En el día 35, la expresión de ARNm de Hsp70 y los niveles séricos de T-SOD en el grupo CrPic aumentaron significativamente en comparación con los controles (P <0,05). T-AOC en el grupo HS disminuyó significativamente en comparación con los controles (P <0,05). Los resultados indican que la suplementación dietética de Cr no influye en el rendimiento de crecimiento de los patos, pero tiene un efecto positivo en la calidad de la carne, en la morfología del intestino delgado, y también regula la expresión de ARNm de Hsp70 en condiciones de estrés calórico y mejora el estado antioxidante.

PALABRAS CLAVE: Cromo; Estrés por calor; Pato; Intestino delgado; Hsp70.

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