

Effect of Organic and Inorganic Chromium Supplementation on Meat Quality of Heat-Stressed Broiler Chicks

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Abstract: This study examined the effect of different levels of dietary organic and inorganic chromium (Cr) on meat quality of broiler chicks reared under heat stress condition. Four hundred and twenty Ross male chickens in heat stress condition ($33\pm 3^{\circ}\text{C}$) were allocated to seven treatments in a completely randomized design. Treatments were supplemented with 0 (control), 500, 1000 or 1500 $\mu\text{g kg}^{-1}$ Cr in the form of Cr nicotinate and Cr chloride. Twelve chicks from each treatment were slaughtered at 42 d, to evaluate moisture, protein, lipid, pH and lipid oxidation of thigh and breast meat. Moisture, lipid and pH of meat were not affected by supplemental Cr. Breast meat protein was significantly ($p<0.05$) increased by the Cr supplementation especially organic Cr. Storage time increased lipid oxidation of meat ($p<0.01$). Lipid oxidation of breast and thigh muscle for two days of storage were affected by supplemental Cr and decreased ($p<0.05$). Results of the present study showed that supplementation of diet with Cr can improve the meat quality of broiler chicks in heat stress condition.

Key words: Chicken, Heat stress, Organic and inorganic chromium, Meat quality

INTRODUCTION

Heat stress has long been recognized as having a detrimental effect on broiler production efficiency and meat yield^[1,2]. Exposure to high ambient temperatures has been reported to cause undesirable changes in meat characteristics in broilers^[3-5]. Trivalent Cr is an essential element in the animal body^[6] and is involved in carbohydrate, lipid, protein and nucleic acid metabolic functions^[7]. Cr is also a cofactor of insulin, promoting insulin activity^[8] and enhancing amino acid uptake into muscular cells for protein synthesis^[9]. Stress increased urinary excretion of Cr and may exacerbate a marginal Cr deficiency^[10,11]. Dietary Cr supplementation has been reported to have a positive effect on meat quality^[12,13] and carcass traits of broiler chicks in natural^[14,15] or heat stress condition^[16]. Certain organic Cr sources are suggested to be utilized more efficiently than inorganic Cr sources^[7]. However, studies designed to compare the effectiveness of organic and inorganic sources of Cr on meat quality of broiler chicks are few.

With the heightened awareness of meat quality in the poultry industry and possible supplementation of Cr in broiler diets, the effects of Cr on meat quality need to be more clearly defined. Thus, the purpose of this study was to investigate the effects of two sources of Cr, Cr nicotinate and Cr Chloride, on the meat quality of broiler chicks in heat stress condition.

MATERIALS AND METHODS

Animals and diets: From July 11 to Aug 20th, four hundred and twenty one-day-old commercial Ross male chicks were reared under heat stress condition. During the experiment, house's temperature was measured four times a day (0600, 1200, 1800 and 2400) and the mean value of daily temperature in the house was kept $33\pm 3^{\circ}\text{C}$. Birds were randomly allotted by body weight to one of seven treatments (four replicate pens of fifteen chicks per pen) in a completely randomized design. Broiler chicks were housed in floor pens (length 120 cm×width 120 cm×height 80 cm) equipped with feeders and waterers. Chicks were maintained on a 23 h light

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and 1 h dark schedule and allowed ad libitum access to experimental diets and water. The dietary treatments consisted of the basal diet supplemented with 0 (control), 500, 1000 and 1500 $\mu\text{g kg}^{-1}$ Cr of diet in the form of Cr nicotinate (contain 12.25% Cr) and Cr chloride ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$, contain 18% Cr) respectively as organic and inorganic sources. The birds were fed a maize-soybean meal starter diets until 21 d of age followed by a finishing diet from 21-42 day. Ingredients and chemical composition of the starter and finisher basal diets are shown in Table 1. The basal diets were formulated to meet or exceed the nutrient requirements of broilers by the National Research Council^[17]. Cr contents were 3.45 and 3.96 mg kg^{-1} in starting and finishing basal diets, respectively, as measured by atomic absorption spectrometer with a graphite furnace (Perkin-Elmer, AAnalyst 600, USA).

Sample collection: On day 42 of the trial, three broilers from each pen were selected according to average body weight within the pen following a 12 h fasting, were weighed individually, killed and eviscerated (abdominal fat pad, liver, intestines, proventriculus, gall bladder, spleen, oesophagus and full crop). Some muscles from the breast and thigh were immediately stored at -20°C for assessing crude fat and crude protein and others were stored individually in plastic bags at 4°C in refrigerator for 2 and 6 days for analysis of meat lipid oxidation.

Table 1: Composition of the basal diets

Ingredients (%)	Starter	Finisher
Corn	51.88	56.19
Soybean meal, CP 44%	39.8	34.6
Soybean oil	4.47	5.75
Dicalcium phosphate	1.56	1.18
Calcium carbonate	1.22	1.35
Salt	0.4	0.35
Vitamin premix ¹	0.25	0.25
Mineral premix ²	0.25	0.25
DL-Methionine	0.17	0.08
Calculated composition		
Metabolizable energy (Kcal Kg^{-1})	3050	3200
Crude protein (%)	21.92	20
Calcium (%)	0.953	0.900
Available phosphorus (%)	0.429	0.350
Methionine+Cysteine (%)	0.858	0.720
Lysine (%)	1.205	1.077
Chromium analyzed (mg kg^{-1})	3.45	3.96

- 1: Vitamin premix contains followings in 2.5 kg: vitamin A, 9000000 IU, vitamin D3, 2000000 IU, vitamin E, 18 g, vitamin k3, 2 g, thiamine 1.8 g, riboflavin, 6.6 g, panthothenic acid, 10 g, vitamin B6, 3 g, vitamin B12, 15 mg, niacin, 30 g, biotin, 100 mg, folic acid, 1 g, choline chloride, 250 g, Antioxidant 100 g
 2: Mineral premix contains followings in 2.5 kg: manganese, 100 g, zinc, 100 g, iron, 50 g, copper, 10 g, Iodine 1 g, selenium 200 mg

Moisture, crude fat and protein measurement: In order to determine the moisture content, the sample (5 g) was dried at 105°C for 24 h^[18].

Intramuscular fat content was determined according to the AOAC (1990) (Soxhlet procedure). The sample was dehydrated (2 g) and subjected for 75 min to a $40\text{-}60^\circ\text{C}$ petroleum ether circuit at 80°C ^[18].

The crud protein was determined following the Kjeldahl method. The sample (0.25 g) was digested with sulphuric acid (10 mL) and a catalyst (1 g) (4% copper sulphate (II) penta hydrate, 3% selenium and 86% potassium sulphate), distilled with 40% sodium hydroxide (75 mL) for 5 min collected in an excess of boric acid and valued with hydrochloric acid 0.1N.

pH value: At 12 h after slaughter the breast and thigh muscle pH was measured with a Knick digital pH meter (Broadly Corp., Santa Ana, CA, USA) after homogenization of 1 g of raw muscles for 30 sec in 10 mL of 5 M iodoacetate^[19].

Lipid oxidation: Lipid oxidation was monitored by measuring Thio Barbituric Acid Reactive Substances (TBARS) using the method described by Strange *et al.*^[20] Twenty grams of minced muscles were blended with 50 mL of cold 20% trichloroacetic acid (TCA) for 2 min. The blender contents were rinsed with 50 mL of water, mixed together and filtered through a Whatman #1 filter and TCA extract was prepared. Then 5 mL aliquot of the TCA extract were mixed with 5 mL of 0.01 M 2-thiobabituric acid and were heated in a boiling water bath for 30 min. After cooling under running tap water for 10 min, the color development, measured as absorbance at 532 nm, was identical when either color development procedure was used with standard solution of tetraethoxypropane or with TCA extracts of meat. Absorbance at 532 nm is reported as the TBARS value. Oxidation products were quantified as malondialdehyde equivalents ($\text{mg malonaldehyde kg}^{-1}$ meat).

Statistical analysis: Data were analyzed by analysis of variance procedures appropriate for a completely randomized design using the GLM procedures of SAS^[21]. Significant differences ($p < 0.05$) among treatment means were determined using Duncan's new multiple range test.

RESULTS AND DISSCUSION

Moisture, lipid and protein: Effects of Cr supplementation on moisture, intramuscular fat and protein of breast and thigh meat are summarized in

Table 2: Effects of organic and inorganic Cr supplementation on moisture, protein, lipid and pH of thigh and breast meat

	Control	Cr chloride ($\mu\text{g kg}^{-1}$)			Cr nicotinate ($\mu\text{g kg}^{-1}$)			SE
		500	1000	1500	500	1000	1500	
Breast								
Moisture (%)	73.3	73.1	73.3	74.1	73.2	73.9	73.2	0.457
Protein (%)	20.72 ^d	22.1 ^{cd}	22.98 ^{ab}	21.35 ^{cd}	23.5 ^a	22.58 ^{ab}	23.08 ^{ab}	0.403
Lipid (%)	0.994	1.05	1.31	1.14	1.25	1.19	1.21	0.265
pH	5.73	5.95	5.94	5.80	5.83	5.83	5.89	0.088
Thigh								
Moisture (%)	73.2	73.4	73.1	74.1	73.6	73.2	74.4	0.699
Protein (%)	17.27	17.40	17.87	18.15	18.30	17.75	18.76	0.570
Lipid (%)	5.96	6.50	6.57	5.30	6.17	5.12	4.96	0.737
pH	6.17	6.37	6.33	6.31	6.26	6.20	6.22	0.065

^{a-b}: Means within the same row without common superscripts differ significantly ($p < 0.05$)

Table 3: Effects of organic and inorganic Cr supplementation on the lipid oxidation of thigh and breast meat (mg malonaldehyde kg^{-1} meat) following different refrigerated storage

Treatments	Thigh		Breast	
	Day 2	Day 6	Day 2	Day 6
Control	0.935 ^a	1.418	0.492 ^a	0.562
Cr chloride ($\mu\text{g kg}^{-1}$)				
500	0.856 ^{ab}	1.465	0.334 ^b	0.458
1000	0.659 ^{bc}	1.625	0.392 ^{ab}	0.474
1500	0.696 ^{abc}	1.133	0.326 ^b	0.481
Cr nicotinate ($\mu\text{g kg}^{-1}$)				
500	0.663 ^{bc}	1.333	0.356 ^b	0.462
1000	0.638 ^{bc}	1.377	0.264 ^b	0.441
1500	0.568 ^c	1.293	0.305 ^b	0.431
SE	0.079	0.120	0.044	0.057

^{a-b}: Means within the same column without common superscripts differ significantly ($p < 0.05$)

Table 2. Dietary Cr source and level had no effect ($p > 0.05$) on moisture and intramuscular fat content of breast and thigh meat. Breast meat protein was significantly ($p < 0.05$) increased by the Cr supplementation (Table 2). When the two Cr sources compared, broilers that consumed Cr nicotinate had more breast protein content than the broilers that consumed Cr chloride. Protein content of thigh muscle was also higher but not significantly ($p > 0.05$) in Cr supplemented groups compared with birds receiving no Cr supplementation.

pH value: Breast and thigh muscle pH was not significantly influenced by the source and level of supplemental Cr (Table 2). However, muscle pH tended to increase with supplemental Cr especially in Cr chloride groups.

Lipid oxidation: The effects of supplemental organic and inorganic Cr on lipid oxidation of thigh and breast muscle are presented in Table 3. The results of oxidative stability of breast and thigh meat stored under refrigerated conditions show that oxidation of both, the breast and thigh meat, occurred slowly and followed a

linear increase in oxidation with length of storage. TBARS value of thigh muscle in each storage time was higher than breast muscle ($p < 0.05$). The breast and thigh meat of birds fed the organic and inorganic Cr showed a reduction ($p < 0.05$) of TBARS values at two days of storage, especially in organic Cr at level of 1500 $\mu\text{g kg}^{-1}$. The data showed that, although the effects of Cr source and supplementation level on TBARS on the 6th days of storage, were not significant ($p > 0.05$), there was a trend for decreasing in lipid oxidation in thigh and breast meat.

Moisture, lipid and protein: Moisture and lipid of thigh and breast meat were not affected by Cr supplementation (Table 2). These results were similar to observation of Mooney and Cromwell^[22] when pigs were fed by Cr picolinate or Cr chloride, tissue fat and moisture were not affected. However, Kim *et al.*^[23] reported in broiler chicks, supplementing the Cr picolinate in the diets decreased fat content of the carcass. The Cr has been found to exert inhibitory effects on *in vitro* lipogenic activity in chick and pig adipose tissue^[23,24]. However Lambert and Jacquemin^[25] reported insulin inhibits gluconeogenesis and depresses adipocyte lipolysis by reducing the activities of adenylate cyclase and hormone-sensitive lipase.

In the present study Supplemental Cr increased muscle protein (Table 2). Amayta *et al.*^[12] observed an increase of the protein level in muscles of broilers fed a diet supplemented with Cr in the form of Cr chloride or Cr yeast. Samanta *et al.*^[13] reported meat protein accretion improved in broiler fed organic Cr under heat stress condition. Also increasing in protein levels in the carcass and liver of broilers given Cr picolinate were observed^[14,23]. Cr has been shown to potentiate insulin action by enhancing its binding to the target cell receptors and also by improving its post receptor signaling. Insulin is known as primary hormone regulating glucose cellular absorption and utilisation. In

chickens, insulin is also known to increase the protein synthesis, efficiency of amino acid transport and diminished protein degradation rate^[26,27]. The mechanism of insulin action on protein metabolism is not clarified yet, but it was shown by Bigot *et al.*^[28] that S6K1 in chickens, known as potent regulator of protein synthesis in mammals, is activated by insulin. The present investigation revealed that the effects of organic Cr is more than inorganic on meat quality. It has been reported in swine that supplementation of organic Cr utilized more efficiently than inorganic Cr sources^[22,29].

pH value: The muscle pH tended to increase in Cr supplemented groups (Table 2). In agreement with our results, increasing in muscle pH was reported in broilers^[12] and pigs^[30] fed Cr. However, Matthews *et al.*^[31] observed the pH of loin muscle 24 h after slaughter was not affected by Cr in pig. Stress before slaughter can lead to increased muscle glycogen breakdown and glycolysis after slaughter and then the increased muscle lactic acid lowers the pH of meat. The mechanism of dietary Cr to influence pH of the muscle after slaughter could be explained by the roles of Cr to reduction the stress-induced catecholamine secretion^[13,16] and then inhibited glycogen breakdown and glycolysis.

Lipid oxidation: Oxidation of lipids is a major cause of deterioration in the quality of meat and can directly affect many meat characteristics such as flavor, color, texture, nutritive value and safety of the meat^[32]. The balance between antioxidants and prooxidants and the composition of skeletal muscle influences the susceptibility of muscle lipids to oxidation^[33].

According to Table 3 the higher extent of lipid oxidation in thigh meat than breast meat was due to the variation on the heme pigment of the two meats. According to Jones^[34], the concentration of heme pigments in breast and thigh meat of broilers is 0.5 and 1.7 mg g⁻¹, respectively. The oxidation of the pigment may have catalyzed the lipid oxidation, as reported by Monahan *et al.*^[35]. In the present study, lipid oxidation was affected by supplemental Cr (Table 3). It is well known that Cr plays an important role as integral component of the Glucose Tolerance Factor (GTF), which potentiate the action of insulin and regulate fat metabolism^[36]. It has been well recognized that insulin metabolism influences lipid peroxidation^[37]. Cr is insulin cofactor, therefore postulated to function as an antioxidant^[38]. According to antioxidant theory^[39], when the concentrations of antioxidant vitamins (vitamin C and E) decrease, lipid peroxidation increases in the plasma and tissues, leading to damage of cell

membranes. Sahin *et al.*^[40] reported supplemental Cr resulted in an increase in serum concentrations of vitamin C and E and decrease in malonaldehyde concentration in serum of heat-stressed broiler chicks. Preuss *et al.*^[38] reported decreased hepatic TBARS formation upon supplementation of Cr picolinate and nicotinate in rats. Similarly Anderson *et al.*^[41] also reported the potential beneficial antioxidant effects of the individual and combined supplementation of Cr and Zn in Tunisian adult subjects with type 2 diabetes mellitus for 6 months. It seems, studies on Cr and its effect on meat oxidative are scarce. However, Sahin *et al.*^[42] reported, when Japanese quails were fed by Cr picolinate, malondialdehyde (MDA) concentration in serum decreased.

CONCLUSION

It was concluded from the present study that, supplementing the Cr in the broiler diets, especially organic Cr at level of 1500 µg kg⁻¹ may influence the meat quality in terms of protein content and oxidative stability in broiler chicks reared under heat stress condition.

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