



Effects of Cr-methionine supplementation on growth performance, relative organ weight, immune hormones, and meat quality of broiler chicks under heat stress

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ABSTRACT

The experiment was conducted to observe the effects of dietary supplementation with Cr-Met supplementation on growth performance, relative organ weight, immune hormones, and meat quality in broilers under heat stress. Male broilers (672; BW = 46±5 g) were randomly allotted into one of the following 4 dietary treatments: (1) CON (basal diet), (2) C1 (basal diet + 20 mg/kg Cr-Met), (3) C2 (basal diet + 30 mg/kg Cr-Met), (4) C3 (basal diet + 50 mg/kg Cr-Met). Organic chromium in the form of Cr-Met was used. From d 8 to 21, BW gain was greater in C1 diet, and FCR was better in C2 and C3 treatments than in CON diet. The birds fed diets supplemented with Cr-Met had higher WBC and RBC level at 30 days old compared with those fed CON diet. The parameters of drop loss at 7 days, the highest presented in C2 diets, the lowest appeared in C1 diets. As the concentrations of adding Cr-Met increasing, the L (lightness) of meat was greater. We are able to observe the highest a (redness) in C1 diets, the lowest a in C3 diets, respectively. In addition, no distinctions were discovered from the data of hormones (BUN, creatinine, lymphocyte) and relative organ weights (spleen, liver breast, bursa of Fabricius, gizzard), abdominal fat and the pH of meat. In conclusion, Cr-Met fed in diets is beneficial for growth performance, immunity, under heat stress conditions. Besides, it had some effects on meat color and drop loss.

Key words: Blood characteristic, Broiler chicks, Chromium, Growth performance, Meat quality

The heat stress causes high mortality, decreased feed consumption, poor body weight gain and meat quality in broiler chickens, and poor laying rate and egg weight and shell quality in laying hens (Donkoh 1989, Siegel 1995, Howlader and Rose 1987, Marsden and Morris 1987, Shane 1988, Yahav 2000a). Vitamin E and Cr are mainly used as anti-heat stress substance. Especially, Cr is able to alleviate the effect of high and low ambient temperature on the performance of poultry (Sahin 2001). McCarty (1993) documented that chromium is a cofactor of insulin, which regulates energy production, muscle tissue deposition, fat metabolism and cholesterol utilization. Cr also has beneficial effects on meat quality. Sazzad (1997) confirmed the viewpoint in broilers. Under heat stress, Cr reduced mortality of broiler (Sazzad 1998). The increases in lymphocyte count and serum IgG level were observed in broiler chicks fed Cr supplementation (Majid 2007). The exact mechanism by which Cr enhances the immune system is not known.

An appropriate recommendation on the chromium requirement for poultry was not made by NRC (1994); and most of the poultry diets are of plant origin having low chromium. Majid (2008) demonstrated that organic chromium has a better effect on meat quality. Mowat (1997)

and Olin (1994) reported that chromium from organic complex is absorbed more efficiently than inorganic compounds. Methionine is recognized as the first-limiting amino acid of poultry in corn-soybean diet. The form of Cr-Met is one of the sources of organic Cr compounds, which has greater value to be focused on. However, data of Cr-Met in broilers is still scarce, and more information is needed. Therefore this study was conducted to evaluate its effects on growth performance, relative organ weight, immune hormones, and meat quality in broilers at a high ambient temperature (32°C)

MATERIALS AND METHODS

Two-day-old male Arbor Acres (BW of 46.3±5 g) broiler chicks (672) obtained from a commercial hatchery were randomly assigned into 4 dietary treatment groups by body weight in a randomized complete block design. (1) CON (basal diet), (2) C1 (basal diet + 20 mg/kg Cr-Met), (3) C2 (basal diet + 30 mg/kg Cr-Met), (4) C3 (basal diet + 50 mg/kg Cr-Met). There were 14 chicks per coop, 12 replications per treatment. All birds in house with stainless steel pens of identical size (1.75 m × 1.55 m) with concrete floors covered with clean rice bran in area that was provided with continuous light, were given free access to water and mash feed. During the experiment, house's temperature was regulated around 32°C. All diets were formulated to meet or exceed the nutritional requirements of the broilers

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Table 1. Diet composition (as-fed basis)

Ingredients (%)	Starter ¹	Finisher ¹
Corn	55.42	62.98
Soybean meal (CP 48%)	28.25	24.61
Corn gluten meal (CP 60%)	6.50	3.50
Soybean oil	5.50	4.89
Dicalcium phosphate	2.46	2.29
Limestone	0.89	0.75
Salt	0.20	0.20
DL-Methionine (98%)	0.17	0.17
L-Lysine-HCl (78%)	0.21	0.21
Vitamin premix ²	0.20	0.20
Trace mineral premix ³	0.20	0.20
Calculated composition, %		
ME, MJ/kg	12.97	12.76
CP	22.00	19.00
Lysine	1.10	1.00
Ca	1.00	0.90
Available P	0.80	0.75
Met+Cys	0.89	0.84
Analytical composition, %		
CP	22.30	20.10
Ca	1.00	0.91
Met+Cys	0.88	0.85
Available P	0.45	0.40
Cr (ug/kg)	330	320

¹Starter diets, provided during weeks 0 to 1; grower diets, provided during weeks 2 to 5.²Provided per kg of diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D₃, 37.5 mg of vitamin E, 2.55 mg of vitamin K₃, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B₆, 24 µg of vitamin B₁₂, 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin and 13.5 mg of pantothenic acid.³Provided per kg of diet: 37.5 mg Zn (as ZnSO₄), 37.5 mg of Mn (MnO₂), 37.5 mg of Fe (as FeSO₄•7H₂O), 3.75 mg of Cu (as CuSO₄•5H₂O), 0.83 mg of I (as KI), and 0.23 mg of Se (as Na₂SeO₃•5H₂O).

reported (NRC 1994). Chromium contents were 330 and 320 ug/kg in starter and finisher diets, respectively, as measured by atomic absorption spectrophotometer. The initial and final diet compositions are shown in Table 1. All birds used in this trial were handled in accordance with the guidelines set forth by the Animal Care and Use Committee of Dankook University.

Sampling and measurements

The broilers were weighed by pen and feed intake (FI) was recorded on d 0, 7, 21, and 28. This information was then used to calculate body weight gain (BWG), and feed conversion rate (FCR). At the end of the experiment, 15 broilers were randomly selected from each treatment (3 birds per pen) and blood samples were selected from the wing vein into a sterile syringe and stored at -4°C. The white blood cells (WBC), red blood cells (RBC) and

lymphocyte counts in the whole blood were then determined using an automatic blood analyzer. Samples for serum analysis were then centrifuged at 3,000 ×g for 15 min, after which the serum was separated. The immunoglobulin G (IgG) was analyzed using nephelometry. Commercial kits were used to measure serum concentrations of creatinine and blood urea nitrogen. After blood collection, the same broilers were weighed individually and slaughtered by cervical dislocation. The stomach, breast meat, bursa of Fabricius, liver, spleen, and abdominal fat were then removed by trained personnel and weighed. The breast muscles were stored at -20°C for the following analysis. Organ weight was expressed as a percentage of BW.

The breast muscle Hunter L* (lightness), a* (redness), and b* (yellowness) values were determined using chromameter. Duplicate pH values for each sample were measured using a pH meter.

Statistical analysis

The average weekly data were statistically analyzed by one-way ANOVA using GLM procedure of SAS (SAS 1998), with the pen as the experimental unit. Before conducting statistical analysis of the microbial counts, the value was transformed logarithmically. Differences among all treatments were separated by Duncan's multiple test, with a P < 0.05 indicating significance.

RESULTS AND DISCUSSION

Only during the phase 2 (d8-21), the supplementation of Cr-Met had a positive influence (P<0.05) on BWG and FCR (Table 2). Nevertheless, the highest BWG (P<0.05) was observed at supplementation of 20 ug/kg Cr-Met. As the concentrations of Cr-Met rising, BWG was lower, and no difference between C2 and C3 was observed here.

Table 2. Effects of Cr-methionine on growth performance of broilers chickens¹

Items	CON ¹	C1 ¹	C2 ¹	C3 ¹	SE ²
<i>Phase 1 (d 1-7)</i>					
BWG, g	146	143	140	138	4.7
FI, g	170	154	154	158	5.8
FCR	1.164	1.076	1.1	1.145	0.05
<i>Phase 2 (d 8-21)</i>					
BWG, g	617 ^b	649 ^a	637 ^{ab}	629 ^{ab}	6.9
FI, g	937	918	902	943	15.3
FCR	1.519 ^a	1.414 ^b	1.416 ^b	1.499 ^{ab}	0.03
<i>Phase 3 (d 21-28)</i>					
BWG, g	696	711	758	758	26.9
FI, g	1,458	1,525	1,524	1,421	36.1
FCR	2.146	2.156	1.998	1.905	0.1
<i>Overall (d 0-28)</i>					
BWG, g	1,463	1,505	1,527	1,528	27.1
FI, g	2,564	2,597	2,581	2,522	37.4
FCR	1.777	1.725	1.685	1.659	0.04

¹Abbreviation: CON, basal diet; C1, basal diet + Cr-Met 20 mg/kg; C2, basal diet+ Cr-Met 30 mg/kg; C3, basal diet+ Cr-Met 50 mg/kg. ²Pooled standard error.

Table 3. Effects of Cr-methionine on growth performance of broilers chickens¹

Items	CON ¹	C1 ¹	C2 ¹	C3 ¹	SE ²
<i>Epinephrine, pg/mL</i>					
d 14	2,356	2,459	2,395	2,553	67
d 21	2,126	2,352	2,348	2,301	91
d 28	2,351	2,452	2,403	2,415	55
d 30	2,456	2,483	2,401	2,435	44
<i>Norepinephrine, pg/mL</i>					
d 14	3,456	3,519	3,452	3,617	60
d 21	3,358	3,415	3,395	3,402	34
d 28	3,458	3,562	3,642	3,510	101
d 30	3,328	3,399	3,475	3,421	72

¹ Abbreviation: CON, basal diet; C1, basal diet + Cr-Met 20 mg/kg; C2, basal diet+ Cr-Met 30 mg/kg; C3, basal diet+ Cr-Met 50 mg/kg. ²Pooled standard error.

Table 4. Effects of Cr-methionine on blood profile of broilers chickens¹

Items	CON ¹	C1 ¹	C2 ¹	C3 ¹	SE ²
<i>BUN, mg/dL</i>					
d 21	3.9	3.8	3.9	3.9	0.39
d 30	3.2	2.9	2.9	2.8	0.32
<i>Creatinine, mg/dL</i>					
d 21	0.35	0.38	0.38	0.37	0.03
d 30	0.33	0.35	0.35	0.33	0.03
<i>WBC, 10³/ul</i>					
d 21	371	343	349	347	12.6
d 30	320.3 ^b	382.2 ^{ab}	425.5 ^a	395.1 ^{ab}	28.6
<i>RBC, 10⁶/ul</i>					
d 21	2.31 ^a	2.01 ^c	2.17 ^{ab}	2.13 ^{bc}	0.05
d 30	2.08 ^b	2.22 ^{ab}	2.25 ^a	2.18 ^{ab}	0.04
<i>Lymphocyte, %</i>					
d 21	81	82	76	78.6	3.46
d 30	73	66	62.8	65.2	3.57

^{a,b,c}Means in the same row with different superscripts differ (P<0.05).¹ Abbreviation: CON, basal diet; C1, basal diet + Cr-Met 20 mg/kg; C2, basal diet+ Cr-Met 30 mg/kg; C3, basal diet+ Cr-Met 50 mg/kg. RBC, red blood cells; WBC, white blood cells. ²Pooled standard error.³Value presented as percentage of total white blood count.

Correspondingly, the highest FCR (P<0.05) was presented at supplementation of 40 mg/kg Cr-Met. In the other phases, there was no significant (P>0.05) effect on growth performance.

The Cr-Met had a positive effect on WBC (P<0.05) and RBC (P<0.05) respectively (Table 4). Besides, RBC (P<0.05) decreased in broilers fed Cr- Met on 21 days. The influence of Cr- Met on BUN, creatinine (P>0.05) and lymphocyte was not statistically significant; and Cr-Met also did not affect the concentration of epinephrine. Compared with the control group, the content of

norepinephrine increased slightly (P>0.05). The statistical analysis did not show significant effect (Table 3).

Relative organ weight and meat quality

There was no effect on relative organ weight caused by the dietary treatment (Table 5). The effects of Cr-Met on meat quality are given in Table 6. On 7 days meat light increased by raising the levels of Cr-Met (P<0.05). Drip loss was affected by Cr-Met (P<0.05). The highest drip loss was presented at C2. The lowest drip loss was observed at C1. Adding Cr-Met did not lead to effects on pH (P>0.05).

Hossain (1998) put forward that supplementation of Cr from Cr-yeast at a level of 300, 600 ug/kg did not affect body weight, feed consumption, and feed conversion ratio of the broilers from 0 to 21 days. In contrast, Amir (2010) reported that Cr at 200, 400, 800 ug/kg improved the performance of broilers, especially, FI and BWG, under commercial condition. It is evident that growth rate and feed efficiency decrease when ambient temperature goes above the thermoneutral (weeks 1 through 6, with decreasing maximum from 32°C to 25°C and minimum

Table 5. Effects of Cr-methionine on meat quality of broilers chickens¹

Items	CON ¹	C1 ¹	C2 ¹	C3 ¹	SE ²
Spleen, %	0.1	0.1	0.1	0.1	0.01
Liver, %	2.88	2.82	2.89	2.71	0.14
Breast, %	16.2	16.4	16.1	15.9	0.73
Bursa of Fabricius, %	0.15	0.16	0.15	0.14	0.01
Abdominal fat, %	1.38	1.44	1.47	1.49	0.20
Gizzard, %	1.10	0.96	1.02	0.93	0.10

¹ Abbreviation: CON, basal diet; C1, basal diet + Cr-Met 20 mg/kg; C2, basal diet+ Cr-Met 30 mg/kg; C3, basal diet+ Cr-Met 50 mg/kg. ²Pooled standard error. ^{a,b}Means in the same row with different superscripts differ (P<0.05).

Table 6. Effects of Cr-methionine on meat quality of broilers chickens¹

Items	CON ¹	C1 ¹	C2 ¹	C3 ¹	SE ²
pH	6.29	6.65	6.21	6.29	0.34
<i>Drip loss, %</i>					
d 1	1.26	0.74	1.32	1.03	0.31
d 3	3.32	1.58	2.93	2.58	0.73
d 5	6.39	5.67	7.26	6.29	0.76
d 7	8.40 ^{ab}	5.26 ^b	9.59 ^a	7.07 ^{ab}	0.97
<i>Meat color³</i>					
L*	50.6 ^b	50.2 ^b	53.9 ^a	54.1 ^a	0.66
a*	16.7 ^{ab}	17.1 ^a	14.6 ^{bc}	13.3 ^c	0.69
b*	11.0	11.3	11.6	12.0	0.93

^{a,b,c}Means in the same row with different superscripts differ (P<0.05).¹ Abbreviation: CON, basal diet; C1, basal diet + Cr-Met 20 mg/kg; C2, basal diet+ Cr-Met 30 mg/kg; C3, basal diet+ Cr-Met 50 mg/kg. ²Pooled standard error. ³L* indicates lightness, a* indicates redness, b* indicates yellowness.

from 28°C to 19°C; relative humidity 49% to 58%) zone (Ensminger 1990). In addition, stress increase chromium excretion (Anderson 1994) and thus may exacerbate a marginal Cr deficiency or an increased Cr requirements. Cr is able to alleviate this condition in Japanese quails, which improved feed efficiency and body weight, as indicated by Sahin (2002). It was in agreement with the report of Kazim (2002) who demonstrated that Cr supplementation with either 200, 400, 800, or 1,200 µg/kg of diet. Increased supplemental chromium resulted in an increase in body weight and improved feed efficiency. In the present study, we got the similar outcome on 21 days. The observed BWG improved, in the stressful conditions, might have resulted from anti-stress role of Cr-Met. Control group in stress, was bound to consume more energy. Consequently, C group fed Cr-Met, had a more economic FCR, and a higher BWG. While the quantity of the supplementation Cr-Met increasing, the effects on BWG, FCR were more and more nonsignificant. The results may be due to adding Cr-Met exceeded the demands of broilers. The pervious researches did not reveal an appropriate recommendation on the chromium requirement for poultry. Kazim (2002) suggested that a supplementation of diet with Cr-Pic at 1,200 µg/kg can alleviate the detrimental effects of heat stress in broiler. Nevertheless, it was at 20mg/kg that the optimal dietary concentration of Cr-Met presented. Besides, the FCR of both experiments 1.39, 1.41 was similar at 21 days. The results may be caused by the different resources of Cr. Thereby more researches are needed to be conducted.

As we know, BUN directly reflected the utilization of nitrogen of feeds; and creatinine is an index of protein deposition. Chromium, as an integral component of the glucose tolerance factor (GTF), plays a vital role in the maintenance of normal glucose tolerance (Schwartz and Mertz 1957, Baselt 2000). Consequently, Cr can accelerate protein deposition, and decrease the quantity of BUN and creatinine. Yukiko (2005) demonstrated this opinion, whose that study showed the serum ratios of BUN were significantly smaller in Cr fed mice than in the control mice. However, Dhia *et al.* (2010) got the opposite result on broiler chickens. It is similar to the present experiment, that BUN and creatinine were not affected by different levels of Cr-Met. The results showed that the dietary Cr-Met had no effect on BUN and creatinine, on the other hand thinking, which may reveal that the Cr-Met levels added in diets were safe for birds and had nondeleterious effects on kidney function of chickens (Mostafa 2007); both epinephrine and norepinephrine levels were important indicators to reflect the degree of stress, which accelerate catabolism of tissues, glycogen decomposition, making triglyceride lipase active to expedite triglyceride degradation, leading to blood sugar increase and metabolic enhancement (Vitus 1998). Unfortunately, we could not determine this from this data. The results may be due to the fact that Cr in basal diets was enough to affect epinephrine and norepinephrine in control group. The mechanism is unknown. Therefore, further

studies are required to assess the values.

Cr has ability to expedite production of hemoglobin and hematopoietic (Daniel 1983). Heat stress has a negative response on RBC (Donkoh 1989), that is probably a consequence of increasing the quantity of water in the blood of poultry. And it lowered hematocrit significantly. Hemodilution leads to the decrease in concentration of hemoglobin. As for WBC, the heat stress also makes WBC lower (Mashaly 2004), and impairs immunity. The study of Toghyani (2007) indicated that supplement of chromium picolinate was beneficial for some hematological values including RBC, WBC. Our result is parallel with Toghyani (2007) which showed that Cr-Met assisted broilers keep a relatively natural level of RBC, WBC, guarantee metabolic balance and enhanced immunity.

The effects of Cr on carcass characteristics varied. Some researchers believe Cr increases the breast yield and the percentage of bursa of Fabricius and spleen and decreases abdominal fat-pad thickness (Anderson 1989). In contrast, the finding of Jackson (2008) and Uyanik (2004) reported that supplementation with Cr did not have consistent effects on carcass traits, which is in conformity with our results. This is probably a consequence of the quantity of Cr-Met. especially organic Cr fed in diet resulting differently, it seems that the interactions between organic ingredient and substance *in vivo*, impact the effects of Cr on carcass characters. Specific mechanism is unclear, the further experiment is needed for confirmation.

Meat color usually determines the economic value of production. Because, color is an important quality attribute that influences consumer acceptance of many food products, including poultry meat. The data of this study showed that the different levels of Cr-Met affected the meat color, including L* and a*. Therefore, we can adjust L* and a* to meet the market requirements for meat color by adding the different levels Cr-Met. Additionally, there is a negative correlation between light and WHC (Bardut 1997). Pieterzak (1997) obtained similar conclusions. Hence, an appropriate quantity of Cr-methionine fed in diets, is capable to ameliorate WHC indirectly. However, the specific level, demanded further studies to determine.

It can be concluded that dietary Cr-Met supplementation had obvious effects on growth performance, immunity, under heat stress conditions. Besides, it had some effects on meat color and drop loss. However, it is necessary to determine the influence of Cr-Met on broilers, as well as other livestock, on account of the limited data on Cr-Met.

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