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The Effect of Different Levels of Organic and Inorganic Chromium Supplementation on Production Performance, Carcass Traits and Some Blood Parameters of Broiler Chicken Under Heat Stress Condition

Mohammad Mehdi Moeini · Akram Bahrami · Shahab Ghazi · Mohammad Reza Targhibi

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Abstract A total of 250 broilers in a completely randomized design selected to evaluate the effect of different levels of chromium (Cr) supplementation on performance, carcass traits and some blood parameters of heat-stressed broiler chicks. All birds were kept under heat stress temperature (33±3°C) and divided into five treatments groups. Each treatment consisted of five pens with 10 birds in each pen. The basal diets were supplemented with 0 ppb (control), 800 ppb Cr-L-Met (T_1), 1,200 ppb Cr-L-Met (T_2) or 800 ppb $CrCl_3$ (T_3), and 1,200 ppb $CrCl_3$ (T_4). The feed intake and body mass were measured at 10, 21, and 42 days of age. Blood samples were collected from two birds in each replicates to determine biological and hematological values at 28 and 42 days of age. There were no significant difference in mass gain and feed conversion of broilers that received Cr supplementations compared with controls. The serum glucose concentration decreased in broilers received organic chromium methionine supplements compared with other treatments groups. Slight but not significant increases were observed in serum high-density lipoprotein (HDL) concentration of treated groups than controls while the mean serum HDL concentration was significantly higher in T_2 group compared with control group. Serum low-density lipoprotein level decreased in broiler received organic Cr supplements ($p<0.05$).

Keywords Chromium · Performance · Broiler · Blood parameters · Heat stress

Introduction

High ambient temperature reduces feed intake, live weight gain, and feed efficiency [42], thus negatively influencing the performance of broilers. Several methods are available to alleviate the effect of high environmental temperature on the performance of poultry. Because it is expensive to cool animal buildings, such methods are focused mostly on

M. M. Moeini (✉) · A. Bahrami · S. Ghazi · M. R. Targhibi
Animal Science Department, Razi University, Kermanshah, Iran
e-mail: mmoeini@razi.ac.ir

dietary manipulations. In this respect, trivalent chromium is used in the poultry diet because of the reported benefits of chromium supplementation in broiler under heat stress [38, 39]. Dietary chromium supplementation has been shown to positively affect the growth rate and feed efficiency of growing poultry [12, 21, 30], particularly in birds reared under heat or cold stress [36, 38, 41]. Heat stress condition increased chromium metabolism from the tissues that is irreversibly excreted through the urine [6, 23]. Chromium is a component of an oligopeptide low molecular weight Cr-binding substance, chromodulin, functioning as a part of the insulin signaling auto-amplification mechanism [46]. Insulin has been shown to increase the glucose and amino acid uptake into muscle cells in order to regulate energy production, muscle tissue deposition, fat metabolism, and cholesterol utilization. If glucose cannot be utilized by body cells due to a low insulin level, it is converted into fat and stored in fat cells. Furthermore, if adequate amino acids cannot enter the cells, muscles cannot be built [4]. Moreover, chromium deficiency can disrupt the carbohydrate and protein metabolisms, reduce the insulin sensitivity in peripheral tissues, and also impair the growth rate [32, 37, 38].

National Research Council [28] has recommended an intake of 50 to 200 microgram Cr per day of trivalent chromium for adult humans. This value was replaced in 2002 with an adequate intake of 30 μg Cr. However, an appropriate recommendation on the chromium requirement of poultry has not been made [29, 30] and most poultry diets are basically composed of plant-origin ingredients, usually low in Cr [14]. Dietary Cr supplementation has been shown to positively affect the growth rate and food efficiency in growing poultry [12, 21, 36]. In most of the cases, beneficial effects were obtained in poultry and swine species when Cr concentration in the diet was within a range of 100 to 400 ppb [17, 24, 25].

Researches with animals has confirmed that chromium from dietary organic complexes, such as chromium picolinate and chromium methionine (Cr-Met) is absorbed more efficiently than chromium from inorganic forms [30]. The reasons for the lower availability of inorganic sources of Cr^{3+} are numerous and probably related to the formation of insoluble chromic oxide, the binding to natural-chelating agents in feedstuffs (such as Phytate), and the interference by ionic forms of other elements as well as slow or no conversion of inorganic chromium to the bioactive form [7].There are studies designed to compare the effectiveness of organic and inorganic sources of Cr on broiler performance in heat stress condition. The effects of the supplemental Cr on serum glucose [21, 40], cholesterol [33, 40] proteins [9, 33, 45], minerals [10, 33] and cortisol [10, 16] levels have been evaluated in various animal species, but variable results were obtained. The present study was conducted to assess the effects of different sources and different levels of chromium supplementations on production traits and some blood metabolite of chicks in heat-stressed condition.

Materials and Methods

Two hundred fifty 1-day-old broiler chicks (ROSS 308) were used in the study in a completely randomized design. The birds were randomly assigned to five treatment groups, five replicates of 10 birds each. The birds in all of group were given the basal diet including maize-soybean meal. Dietary treatment was supplemented with 0 ppb (control), 800 ppb (T_1), 1,200 ppb (T_2) organic chromium in the form of Cr-L-Met (contain 10% Cr) or 800 ppb (T_3), and 1,200 ppb (T_4) inorganic chromium in the form of chromium chloride ($CrCl_3{\cdot}6H_2O$, contain 18% Cr). The temperature of bird's house measured four times a day (0600, 1200, 1800, and 2400 hours). The mean value of daily temperature during experiment was 33±3°C. The birds were fed with the starter diet (20.91% protein, 2,947.5 kcal ME kg^{-1}) for 10 days, followed by a grower diet (19.86% protein, 3027 kcal ME kg^{-1}), from days 10 to 21, and finishing diet (18.05% protein, 3,118.5 kcal ME kg^{-1}), from days 21 to 42. The ingredients and chemical composition of diets are shown in Table 1.

The basal diets in mash form were formulated to meet or exceed (5%) the catalog of ROSS 308. Respective amount of chromium were first blended with 10 g dicalcium phosphate and then, with a larger amount of the basal diets, were homogeneously mixed. Chromium contents were 3.42, 3.95, and 4.23 ppb in starter, grower, and finishing basal diets, respectively, as measured by atomic absorption spectrometer with a graphite furnace (Perkin–Elmer, AAnalyst 600, USA). Fresh water was provided ad libitum. A continuous and proportionate supply of light was provided to the birds throughout the experiment

Body mass was determined at 10, 21, and 42 days of age. Feed consumption, body mass gain and feed conversion were measured in different periods. At 42 days of age, two birds were chosen randomly from each replicate and slaughtered, and the abdominal fat pad, liver, heart, and pancreas were removed, weighed, and expressed as a percentage of live body mass. At 28 and 42 days of age, blood samples were collected from the wing vein of two birds in each replicate. On day 42, bursa of Fabricius, spleen, and thymus were collected, weighed, and expressed as the grams per 100 g of live body mass. The blood samples separated by centrifugation at 2,500×*g* for 15 min following at 1-h incubation at

Table 1 Composition of the basal diets

Ingredients (%)	Starter	Grower	Finisher
Corn seed	58	60	65
Soybean meal, CP 44%	36	32	27
Soybean oil	1	2	2.5
Fish meal	1	2	2
Dicalcium phosphate	1.4	1.3	1.07
Calcium carbonate	1.25	1.3	1.03
Salt	0.4	0.4	0.4
Vitamin premix[a]	0.25	0.25	0.25
Mineral premix[b]	0.25	0.25	0.25
DL-methionine	0.13	0.12	0.11
HCL-lysine	0.05	0.04	0.04
Nutrients composition			
Metabolizable energy (kcal kg^{-1})	2,947.5	3,027	3,118.5
Crude protein (%)	20.91	19.86	18.05
Calcium (%)	0.435	0.57	0.800
Available phosphorus (%)	0.505	0.618	0.46
Methionine+cysteine (%)	0.94	0.84	0.76
Lysine (%)	1.342	1.271	1.109
Chromium analyzed (mg kg^{-1})	3.42	3.95	4.23

[a] Vitamin premix contains the following in 2.5 kg: vitamin A, 9,000,000 IU; vitamin D3, 2,000,000 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

[b] Mineral premix contains the following in 2.5 kg: manganese, 100 g; zinc, 100 g; iron, 50 g; copper, 10 g; iodine, 1 g; selenium, 200 mg

room temperature and stored at −20°C until the analysis of blood parameters. The concentrations of serum glucose, triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, total cholesterol (Pars Azmoon, Tehran, Iran) and insulin (Immulite 2000, no. L2kin6, Dpc, Los Angeles, CA) were measured using commercial kits as indicated.

Data were calculated by analysis of variance procedures appropriate for a completely randomized design using the SPSS 16 [43]. Means were determined using comparative multiple range test (Duncan test).

Results

The effects of supplemental chromium on performance of broilers are presented in Table 2. No statistically significant effect was observed on body mass and body mass gain of broilers at 42 days of age ($P>0.05$). Feed intake and feed conversion (feed intake/gain) of broilers were not affected by different levels of Cr supplementations (Table 2).

Table 3 shows the effects of different levels of organic and inorganic Cr supplementation on carcass traits of broiler chickens under heat-stressed condition. Carcass yield, abdominal fat, heart, liver, and pancreas weight were not affected by Cr supplementations ($P>0.05$).

The effects of supplemental dietary Cr on blood parameters at the 28th and 42nd days of the experiment are given in Tables 4, 5, and 6. The concentration of insulin increased

Table 2 Effect of supplemental chromium on performance of broilers (mean±SD)

Item	Control	T_1	T_2	T_3	T_4	SEM
Body mass (g)						
10 days	159.8±47.1	163.7±27.9	162.7±51.4	166.4±47.8	165.9±62.9	10.11
21 days	622.7±44.8	678.2±31.9	672.9±31.0	676.6±12.1	647.0±45.3	77.95
42 days	2059.7±364	2430.5±268	2479.0±367	2485.1±362	2142.7±604	8.73
Mass gain (g/day)						
0–10 days	11.53±0.50	12.11±0.59	12.03±0.85	12.06±0.48	12.00±0.78	0.12
10–21 days	44.05±3.94	47.70±3.45	47.28±2.69	46.38±1.19	45.99±5.82	0.73
21–42 days	45.11±9.70	56.29±7.21	58.07±10.40	58.75±9.31	46.88±14.94	2.27
0–42 days	39.19±3.91	45.90±4.79	46.69±5.32	46.46±4.91	40.20±8.10	1.22
Feed intake (g/day)						
0–10 days	27.90±2.95	28.05±2.26	27.30±1.93	26.29±1.69	27.74±1.75	0.39
10–21 days	68.80±4.73	70.65±4.46	71.26±2.75	69.43±2.10	67.86±3.35	0.70
21–42 days	105.92±9.1	110.73±7.14	114.00±5.83	111.78±2.71	113.92±8.35	1.4
0–42 days	84.79±4.81	86.23±7.81	87.98±5.20	84.99±2.32	88.01±5.55	1.0
Feed/gain (g/g)						
0–10 days	2.23±0.08	2.27±0.12	2.24±0.21	2.18±0.11	2.20±0.11	0.02
10–21 days	1.56±0.03	1.48±0.03	1.50±0.04	1.50±0.05	1.51±0.06	0.01
21–42 days	2.15±0.21	1.98±0.15	2.00±0.26	1.90±0.25	2.18±0.33	0.05
0–42 days	2.09±0.17	1.88±0.10	1.89±0.17	1.82±0.17	2.04±0.24	0.03

Means within the same row without common letters differ significantly ($P<0.05$). Control, T1=800 ppb Cr-L-Met, T2=1,200 ppb Cr-L-Met, T3=800 ppb $CrCl_3$, T4=1,200 ppb $CrCl_3$

SEM standard error of the mean

Table 3 Effect of supplemental chromium on the carcass traits of broilers (percent)

Item	Control	T_1	T_2	T_3	T_4	SEM
Carcass	66.14±6.2	62.8±7.8	61.7±8.6	66.5±6.3	70.8±4.1	1.20
Abdominal fat	2.36±0.56	1.74±0.73	1.63±0.34	2.02±0.36	1.86±0.60	0.111
Heart	0.43±0.04	0.38±0.02	0.38±0.04	0.39±0.04	0.38±0.05	0.008
Liver	1.54±0.24	1.49±0.10	1.46±0.12	1.52±0.15	1.54±0.10	0.029
Pancreas	0.17±0.07	0.14±0.03	0.16±0.01	0.14±0.01	0.18±0.05	0.009

Means within the same row without common letters differ significantly ($P<0.05$). Control, T1=800 ppb Cr-L-Met, T2=1,200 ppb Cr-L-Met, T3=800 ppb $CrCl_3$, T4=1,200 ppb $CrCl_3$

significantly in T_2 group compared with other groups. Although supplemental Cr had no effects on serum glucose concentration in 28 days of age ($P>0.05$), serum glucose concentration decreased in broiler that received organic Cr in 42 days of age ($P<0.01$) (Table 4).

The effects of supplemental dietary Cr on total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides concentrations at the 28th day and the 42nd day of the experiment are given in Tables 5 and 6. The serum total cholesterol content was significantly decreased in chicken fed organic Cr on the 21st days and on the 42nd days compared with control group ($P<0.05$). Similarly, the mean of the LDL cholesterol and triglycerides levels significantly decreased in chicken fed organic Cr ($P<0.01$), whereas the mean of the HDL cholesterol was significantly increased at 42nd day of age ($P<0.05$). The effects of supplemental Cr on weight of lymphoid organs are shown in Table 7. The weights of lymphoid organs were not affected in chicks fed dietary treated with different levels of Cr supplementation.

Discussion

No statistically significant effect was observed on body mass, body mass gain, and feed intake of broilers at 42 days of age ($P>0.05$). The growth rate and feed efficiency decreased when ambient temperature moves beyond the thermoneutral zone [13], and decreases in

Table 4 Effect of supplemental chromium on serum insulin and glucose concentration of broilers at 28 and 42 days of age (mean±SD)

Item	Insulin (ng/ml)		Glucose (mg/dl)	
	28 days	42 days	28 days	42 days
Control	0.36±0.05c	0.36±0.04c	261.67±15. 50	229.00±5.00a
T_1	0.46±0.05bc	0.53±0.04ab	247.67±15. 63	201.33±5.13c
T_2	0.93±0.04a	0.63±0.05a	232.67±8. 62	197.67±7.37c
T_3	0.40±0.1c	0.43±0.05bc	251.33±6.65	213.67±6.65b
T_4	0.80±0.1ab	0.46±0.05bc	244.00±17. 34	203.67c±4.16b
SEM	0.07	0.027	3.87	3.37

Means within the same row without common letters differ significantly ($P<0.05$). Control, T1=800 ppb Cr-L-Met, T2=1,200 ppb Cr-L-Met, T3=800 ppb $CrCl_3$, T4=1,200 ppb $CrCl_3$

Table 5 Effects of supplemental Cr on some lipid parameters in the serum of broiler chickens (mean±SD)

Item	Total cholesterol (mg/dl)		HDL (mg/dl)	
	28 days	42 days	28 days	42 days
Control	143.5±23.04a	163.00±6.48a	27.50±2.64	33.75±2.50b
T_1	127.5±7.32ab	141.75±5.31b	30.75±2.36	36.25±1.70ab
T_2	111.0±7.07b	138.50±16.70b	32.00±1.41	38.00±0.81a
T_3	136.5±3.31a	151.75±4.78a b	29.50±2.08	35.00±0.81b
T_4	135.0±7.25a	143.75±6.99b	30.50±2.08	35.50±1.29b
SEM	3.46	2.71	0.55	0.45

Means within the same row without common letters differ significantly ($P<0.05$). Control, T1=800 ppb Cr-L-Met, T2=1,200 ppb Cr-L-Met, T3=800 ppb $CrCl_3$, T4=1,200 ppb $CrCl_3$

growth rate were partly of the result of the decrease in feed intake [15]. In other studies, Sahin et al. [39] reported that an increase in supplemental chromium (200, 400, 800, or 1200 μg/kg Cr picolinate) resulted in an increase in body mass and feed efficiency in broilers reared under heat stress condition. Lien et al. [21] reported that dietary supplements of 1,600 or 3,200 μg kg^{-1} Cr as chromium picolinate markedly improved the mass gain of broiler chicken. Kim et al. [17] also observed that 1,600 μg/kg Cr picolinate supplementation increased the mass gain and feed intake without affecting feed conversion in broilers. Sands and Smith [41] also showed that the supplementation of chromium picolinate increased the growth rate without affecting the feed intake in broilers reared under environmental stress. Sahin et al. [38] found a significant decrease in live mass gain and feed efficiency in broiler reared under heat stress which was alleviated by dietary chromium and vitamin C supplementation. Mean cumulative feed intake revealed no significant difference between groups. This finding favorably compared with those earlier reports of Motozono et al. [26] who reported that inclusion of chromium picolinate at 0, 200, and 400 ppb had no effect on feed intake of broilers at 6 weeks of age. Sahin et al. [36] and Amatya et al. [3] expressed similar opinion about the inclusion of organic chromium in chicken diet.

The observations of the present study did not fully support the earlier reports [17, 24, 25] with respect to the beneficial role of organic sources of Cr in different livestock species

Table 6 Effects of supplemental Cr on some lipid parameters in the serum of broiler chickens (mean±SD)

Item	LDL (mg/dl)		Triglyceride (mg/dl)	
	28 days	42 days	28 days	42 days
Control	76.00±3.36a	83.25±1.25a	128.25±29.56a	68.75±4.75a
T_1	65.50±2.08cd	79.75±0.95b	95.25±7.93bc	52.50±6.24c
T_2	60.75±4.42d	78.25±0.95b	87.00±10.89c	50.00±1.63c
T_3	72.50±2.51ab	82.00±1.41a	119.25±2.21a	66.00±2.94ab
T_4	70.50±3.69bc	81.75±0.95a	114.00±4.96ab	60.25±3.30b
SEM	1.40	0.47	4.58	1.86

Means within the same row without common letters differ significantly ($P<0.05$). Control, T1=800 ppb Cr-L-Met, T2=1,200 ppb Cr-L-Met, T3=800 ppb $CrCl_3$, T4=1,200 ppb $CrCl_3$

Table 7 The effects of chromium supplementation on weight of lymphoid organs (grams per 100 g of body mass) (mean±SD)

Treatments	Bursa of Fabricius	Thymus	Spleen	Mean body mass (g)
Control	0.102±0.03	0.139±0.02b	0.066±0.01	2059±364.82
T1	0.121±0.03	0.181±0.03ab	0.044±0.01	2430±268.59
T2	0.132±0.01	0.208±0.04a	0.052±0.01	2479±367.34
T3	0.107±0.02	0.148±0.03ab	0.060±0.02	2485±362.17
T4	0.117±0.04	0.192±0.07ab	0.052±0.01	2142±302.32
SEM	0.006	0.009	0.003	8.732

Means within a row with different letters differ ($P<0.05$). The number of samples in each treatment is equal to 10 chicks. Control, T1=800 ppb Cr-L-Met, T2=1,200 ppb Cr-L-Met, T3=800 ppb $CrCl_3$, T4=1,200 ppb $CrCl_3$

SEM standard error of the mean

including broiler chickens. The present results, rather, were not in agreement with those of Nam et al. [27] who reported that inorganic supplements of Cr ($CrCl_3 \cdot 6H_2O$) were equally as effective as organic Cr supplements (Cr picolinate) in improving the performances of broiler chickens. Moreover, these researchers also reported that food conversion efficiency in broiler birds was improved more effectively by chromium chloride than was achieved by Cr picolinate supplementation, and Cr absorption from chromium picolinate is within error of that from CrCl3.

Dietary chromium supplementation increased the insulin plasma concentration, indicating chromium's physiological role to empower the insulin acting as an insulin cofactor [36]. However, Sahin et al. [16] reported that dietary chromium did not affect insulin plasma concentration in pregnant but does in newborn and weaned growing rabbits under the thermoneutral zone. The relationship between chromium supplementation and insulin in the present study is in agreement with those reported by other researchers ([4, 8, 35, 36]). Rosebrough and Steele [35] have also stated chromium as a cofactor for insulin activity is necessary for normal glucose utilization. However, Kristin et al. [18] reported that the chromium content of the diet had no effect on body mass or food intake of Rat. Similarly, the chromium content of the diet had no effect on glucose levels in glucose tolerance or insulin tolerance tests, but a distinct trend toward lower insulin levels under the curve after a glucose challenge was observed with increasing chromium content in the diet. Mineral absorption and excretion changed with animal needs and animal intake, and urinary chromium loss is actually controlled by chromium intake and chromium absorption, which is by passive diffusion. The diabetic animals absorb more chromium, resulting in loss of more chromium in the urine. Thus, chromium supplementation has been shown to lead to increased insulin sensitivity in response to a stress [46].

Elevated concentrations of corticosterone are parallel to increases in serum glucose concentrations. Decreases of glucose concentrations in the current study may be attributed to decreased glucocorticoid secretion, which increases glucogenesis. Similar to the results of the present study, Sahin et al. [36] found that chromium supplementation markedly decreased blood glucose concentrations in Japanese quails. Chromium is essential for normal glucose metabolism, and insulin regulates the metabolism of carbohydrate, fat, and protein, stimulating amino acid uptake and protein synthesis [11, 33, 45]. Page et al. [33] found an increase in serum protein levels with chromium supplementation which was agreed with the findings of Chang and Mowat [9]). These effects may be the result of increased amino acid synthesis in the liver via insulin, which enhanced the incorporation of several amino acids into the protein.

The changes in insulin and glucose concentration in the Cr-Met treatments group could be from stimulating the amino acid and protein synthesis as well as glucose utilization [45]. Rosebrough and Steele [35] reported that turkeys fed a diet supplemental with chromium had greater liver glycogen levels as a result of increasing activity of the enzyme glycogen synthetase and chromium increased glucose transport by increasing insulin activity. Similarly, Cupo and Donaldson [12] reported that chromium supplementation (20 ppm of $CrC_3{\cdot}6H_2O$) increased the rate of glucose utilization by 16%. Numerous studies show evidence that Cr is essential for lipid metabolism and reducing the risk of parthenogenesis. For example, rats and rabbits fed on a Cr-deficient diet had increased total cholesterol and aortal lipid concentrations and showed increased plaque formation [1, 2]. The effects of dietary manipulation methionine on plasma homocysteine and high-density lipoprotein (HDL) cholesterol levels in wild-type mice demonstrated that the HDL production rate was significantly reduced in mice fed the methionine diet [47]. The changes in lipid parameters in the Cr-Met treatments group might be due to the effect of methionine as the methyl group. Cr supplementation has decreased the total cholesterol in their blood. An increase of HDL cholesterol [34] and a decrease in total cholesterol, LDL cholesterol, and triacylglycerols [20] have been observed in humans after Cr supplementation. On the other hand, Cr supplementation was not proven to have any effect in other human trials ([5, 31, 44]).

The results of the current study indicate that Cr supplementation, particularly at 1,200 ppb of organic chromium, profitably affected the lipid parameters in growing broiler chicken. Similarly, the mean of LDL cholesterol and triglycerides level decreased, whereas the mean of the HDL cholesterol significantly increased. This result is in agreement with other reports [19, 21], but Cupo and Donaldson [12] demonstrated that Cr had no effect on serum cholesterol content in chicks fed 20 mg/kg Cr as CrCl3. Likewise, Uyanik et al. [45]) reported that total and LDL cholesterol decreased, whereas HDL cholesterol significantly increased after supplementation of 400 g/kg inorganic Cr in lambs, but triglycerides were lower than the control with marked differences in the 400 g/kg Cr group. Furthermore, McNamara and Valdez [22] studied the action of chromium propionate on lipogenesis and lipolysis in adipose tissues in Holstein dairy cows from 21 days prepartum to 35 days postpartum. Chromium increased the net synthesis of fat in the adipose tissue and decreased the net release. This might be acting through linkage of chromodulin with the insulin receptor and the increased glucose flux into the adipocyte. Chromium supplementation increased the ratio of bursa of Fabricius to body mass and liver. At the present study, the masses of lymphoid organs were not affected in chicks fed dietary treated with different levels of Cr supplementation.

Conclusions

As a result of this study, supplementation with Cr (1,200 ppb Cr-Met) might have some positive effects on some aspect of blood parameters and broilers performance when can be used as additives in heat-stressed broiler chickens diet. Further studies would be helpful by adding different levels of Cr supplements to clarify the nutritional, therapeutic, and physiological effects of Cr on metabolism and performance, with regards to varied management conditions, including different stress factors, dietary ingredients, and nutrient content.

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