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Optimal Supplemental Chromium Concentration for Alleviating Heat Stress in Broiler Chickens: A Meta-analysis

H. Kim, K. W. Lee, & B. G. Kim*
Department of Animal Science and Technology, Konkuk University, Seoul 05029, Republic of Korea
*Corresponding author: bgkim@konkuk.ac.kr
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ABSTRACT

The objective of the present work was to determine the optimal supplemental chromium (Cr) concentration for alleviating the detrimental effects of heat stress on the growth performance of broilers using the literature data. A total of 53 observations from 22 experiments that assessed the growth performance of broilers fed various doses of Cr under heat stress were collected. The control groups received no supplemental Cr, whereas supplemental Cr concentrations ranged from 200 μg/kg to 2,000 μg/kg of diet. The sources of Cr were Cr chloride (n= 12), Cr-amino acid chelate (n= 14), and Cr picolinate (n= 27). The relative change (Δ %) of average daily gain (ADG) between broilers fed a Cr-supplemented diet and those fed a control diet was calculated. To compare ΔADG among Cr sources, the source was considered a fixed variable, while the experiment and the supplemental Cr concentration were considered random variables. The ΔADG was not different among the sources. Polynomial contrast analysis indicated that ΔADG increased quadratically (p<0.05) as the dietary Cr concentration increased. The optimum supplemental Cr concentration was estimated using a one-slope broken-line model with the fixed variable of supplemental Cr and a random variable of experiment based on the NLMIXED procedure of SAS. The optimum supplemental Cr concentration to maximize ΔADG in broilers under heat stress was 687 μg/kg (SE= 137, R^2= 0.70, and p<0.001). Taken together, the optimum Cr supplemental concentration in broiler diets to alleviate the detrimental effects of heat stress on body weight gain is 687 μg/kg, regardless of the source of Cr.

Keywords: average daily gain; broiler chicken; chromium; heat stress

INTRODUCTION

Trivalent chromium (Cr) is an essential micronutrient for broilers (Huang *et al.*, 2016), and Cr is known to regulate glucose levels by potentiating the actions of insulin, thereby regulating the metabolism of lipids, proteins, and carbohydrates toward anabolic processes (Brooks *et al.*, 2016; Feng *et al.*, 2021a). One of the major stressors in broiler production systems is heat stress caused by high environmental temperatures particularly in tropical regions of the world, which can adversely affect growth performance (Samanta *et al.*, 2008; Norain *et al.*, 2013), carcass traits (Toghyani *et al.*, 2012; Huang *et al.*, 2016), blood biochemical parameters (Moeini *et al.*, 2011; Akbari & Torki, 2014), disease susceptibility (Hamidi *et al.*, 2016; Hajializadeh *et al.*, 2017), or a combination of these responses. In addition, heat stress has been known to have a negative impact on growth performance in laying hens (Kim *et al.*, 2021a; Kim *et al.*, 2022a) and pigs (Kim *et al.*, 2009; Serviento *et al.*, 2020).

Dietary supplementation of Cr has been reported to alleviate the detrimental effects of heat stress on growth performance in poultry by mitigating stress-induced immunosuppression (Norain *et al.*, 2013; Jahanian & Rasouli, 2015). However, in some studies, supplemental Cr had no significant effects on growth performance in heat-stressed broilers (Habibian *et al.*, 2013). Additionally, the effects of the source of Cr on alleviating efficacy were inconclusive in previous studies (Toghyani *et al.*, 2012; Sahin *et al.*, 2017). Furthermore, the optimal supplemental Cr concentration for alleviating the detrimental effects of heat stress on growth performance in broilers has been inconsistent among studies (Sahin *et al.*, 2002; Toghyani *et al.*, 2012; Ebrahimzadeh *et al.*, 2013). The effects of the source and concentration of supplemental Cr have not always been reproducible, and individual studies may have false-positive results (type I errors) or may fail to detect small effects (false-negative, type II errors). A meta-analysis can combine growth performance data from multiple studies on the same topic, increase the precision of estimating the effects of supplemental Cr, and clarify the discrepancies among the literature (Lee, 2018). Therefore, the objectives of this meta-analysis were to analyze the effects of Cr sources on the growth performance of heat-stressed broilers and to determine the optimum supplemental Cr concentration for alleviating the detrimental influence of heat stress on the growth performance of broilers.

MATERIALS AND METHODS

Data Collection and Processing

A dataset was generated by conducting an extensive search of online databases using the following keywords: broiler chicken, chromium, growth performance, heat stress, and poultry. The studies identified through the literature search were then manually screened based on their title and experimental information. The collected data included the ingredient and analyzed the composition of diets, ambient temperature, sources of Cr, supplemental Cr concentrations, experimental duration, and growth performance (Table 1). Growth performance included average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F). In cases where G:F was reported as a feed conversion ratio, the values were converted to G:F. A total of 53 observations from 22 experiments in 16 literature sources that empirically evaluated the growth performance of broilers under heat stress were collected. Data for carcass traits and serum metabolites were also collected, and all carcass trait data were converted as a percentage of body weight. The broilers were given ad libitum access to feed and water throughout the experiments. The ambient temperature ranged from 32 °C to 36 °C (34.1 ± 1.3 °C), and the experimental duration ranged from 21 to 49 days (37.8 ± 9.7 days). The dietary treatments within each experiment were divided into control and treatment groups, with the control group receiving no supplemental Cr, whereas the treatment group received supplemental Cr concentrations ranging from 200 μg/kg to 2,000 μg/kg of diet. The nutritional values and environmental conditions in the control and treatment groups were identical within each experiment.

The relative change of measurements in broilers fed a Cr-supplemented diet compared to the control group was calculated using the following equations:

Actual difference= measurement of treatment group – measurement of control group,

Relative change (Δmeasurement %)= actual difference/ measurement of control group × 100

A total of 53 observations for relative growth performance were used for further statistical analyses (Table 1). The sources of Cr evaluated were Cr chloride

Table 1. Ambient temperature, experimental duration, and effects of supplemental chromium (Cr) sources and doses on the relative changes (Δ) of average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) in broilers fed a Cr-supplemented diet compared with the control group with no supplemental Cr under heat stress

Ambient temperature (°C)	Sources of Cr[1]	Doses of Cr (μg/kg)	Duration (days)	Relative changes (%)[2]			Reference
				ΔADG	ΔADFI	ΔG:F	
34.0	Cl	400/2,000	42	3.75	–0.75	4.52	Huang *et al.* (2016)
33.0	Cl	500/1,000/1,500	42	5.80	5.52	0.25	Toghyani *et al.* (2012)
33.0	Cl	600/1,200	49	3.82	1.59	2.22	Ghazi *et al.* (2012)
33.0	Cl	600/1,200	28	2.40	0.88	1.51	Habibian *et al.* (2013)
33.0	Cl	800/1,200	42	10.56	2.02	8.55	Moeini *et al.* (2011)
34.2	Cl	2,000	35	13.38	5.01	7.96	Norain *et al.* (2013)
33.0	AA	200/400/800	42	5.71	11.48	–5.13	Ebrahimzadeh *et al.* (2013)
34.0	AA	200	42	6.91	3.03	3.76	Sahin *et al.* (2017)
34.0	AA	400/2,000	42	3.79	2.28	1.47	Huang *et al.* (2016)
35.0	AA	500/1,000	42	22.32	10.87	10.28	Jahanian & Rasouli (2015)
33.0	AA	600/1,200	49	7.82	6.17	1.57	Ghazi *et al.* (2012)
33.0	AA	600/1,200	28	1.62	–1.48	3.20	Habibian *et al.* (2013)
33.0	AA	800/1,200	42	18.13	2.73	14.99	Moeini *et al.* (2011)
35.0	Pic	200/400	42	7.56	1.04	6.45	Sands & Smith (1999)
32.8	Pic	200/400/800/1,200	42	14.61	8.16	5.89	Sahin *et al.* (2002)
34.0	Pic	200	42	3.37	1.41	1.93	Sahin *et al.* (2017)
32.0	Pic	400	42	3.24	0.34	2.89	Sahin *et al.* (2003)
34.0	Pic	400/2,000	42	3.01	0.35	2.65	Huang *et al.* (2016)
33.0	Pic	500/1,000/1,500	37	3.92	4.61	–0.64	Toghyani *et al.* (2006)
35.5	Pic	500/1,000	40	11.58	0.82	10.71	Samanta *et al.* (2008)
36.0	Pic[3]	500/500/1,000/1,000/ 1,500/1,500	21	21.20	9.46	10.68	Hajializadeh *et al.* (2017)
36.0	Pic[3]	500/500/1,000/1,000/ 1,500/1,500	21	13.21	–2.47	16.02	Hamidi *et al.* (2022)

Note: [1]Cl= Cr chloride, AA= Cr-amino acid chelate, Pic= Cr picolinate. [2]Values for relative changes were the mean relative change values for one or more doses of Cr within an experiment. [3]These experiments were conducted simultaneously to determine the effects of Cr picolinate and Cr picolinate nanoparticles.

(n= 12), Cr-amino acid chelate (n= 14), and Cr picolinate (n= 27).

Statistical Analysis

The 53 observations for the relative change of growth performance were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA). To compare the effects of Cr sources on the relative change of growth performance, the source was considered a fixed variable, whereas the experiment and the supplemental Cr concentration were considered random variables. The least squares means of each Cr source were calculated, and pairwise comparisons were conducted using the least significant difference method, with Tukey's adjustment if an effect was significant. To evaluate the linear and quadratic effects of supplemental Cr concentrations on the relative change of growth performance, pre-planned polynomial contrasts were used, with Cr concentrations as a fixed variable, and the experiment and the source of Cr as random variables. The least squares means of each Cr concentration were then calculated.

Due to the quadratic response of ΔADG to the increasing supplemental Cr concentrations, a broken-line analysis was conducted for ΔADG. The optimal supplemental Cr concentrations for mitigating the detrimental effects of heat stress on ΔADG in broiler chickens were estimated using a one-slope broken-line analysis, employing the NLMIXED procedure of SAS (Robbins *et al.*, 2006). In the one-slope broken-line models, the experiment was considered a random variable. An individual Cr-supplemented diet in the treatment group was the experimental unit and statistical significance was considered at a p-value less than 0.05.

Mean, standard deviation, and 95% confidence interval were calculated for growth performance, carcass traits, and serum metabolites regardless of the sources and doses of supplemental Cr. The data were analyzed using the UNIVARIATE procedure of SAS, and significance was declared for a p-value less than 0.05 (2-tailed), when the 95% confidence interval did not include zero.

RESULTS

The ΔADG, ΔADFI, and ΔG:F were not affected by the sources of Cr (Table 2). The ΔADG increased quadratically ($p<0.05$) with increasing dietary Cr concentration (Table 3). The optimum supplemental Cr concentration to maximize ΔADG in broilers under heat stress was 687 μg/kg (SE= 137, R^2= 0.70, and $p<0.001$) based on one-slope broken-line analysis (Figure 1).

Supplementing Cr in broiler diets under heat stress improved ($p<0.05$) ΔADG, ΔADFI, and ΔG:F regardless of the sources and doses of Cr (Table 4). Additionally, Cr supplementation increased ($p<0.05$) the relative changes of carcass yield, dressing yield, the weight of Bursa of Fabricius, thymus, and breast meat; however, it decreased ($p<0.05$) the weight of heart and abdominal fat. Broilers fed the Cr-supplemented diet showed greater ($p<0.05$) relative changes in insulin, total protein, and Cr concentrations in serum compared with those fed the control diet. Supplementation of Cr in broiler diets under heat stress led to decreased ($p<0.05$) relative changes of glucose and corticosterone concentrations in serum regardless of the sources and doses of Cr.

DISCUSSION

Broiler chickens subjected to heat stress exhibit lower growth performance compared with those kept under a thermoneutral environment (Samanta *et al.*, 2008; Norain *et al.*, 2013). In our meta-analysis, we identified 5 studies that investigated the effects of ambient temperatures on growth performance in broilers fed a

Table 2. The relative changes (Δ) of average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) in broilers fed a chromium (Cr)-supplemented diet compared with the control group with no supplemental Cr under heat stress

Variables, %	Sources of Cr			SEM[1]	p-value
	Cr chloride	Cr-amino acid chelate	Cr picolinate		
n	12	14	27	-	-
ΔADG	7.40	10.81	10.33	2.25	0.365
ΔADFI	3.53	5.44	3.50	1.37	0.240
ΔG:F	4.04	5.08	6.53	1.94	0.629

Note: [1]SEM = standard error of the means.

Table 3. The relative changes (Δ) of average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) in broilers fed a chromium (Cr)-supplemented diet compared with the control group with no supplemental Cr under heat stress

Variables, %	Dietary Cr concentration, μg/kg									SEM[1]	p-values	
	200	400	500	600	800	1,000	1,200	1,500	2,000		Linear	Quadratic
n	5	7	8	4	4	8	7	6	4	-	-	-
ΔADG	4.80	4.50	11.05	3.76	15.06	14.73	8.96	14.27	7.88	3.31	0.167	0.023
ΔADFI	3.31	2.89	3.61	1.90	6.67	5.46	3.47	4.83	2.31	2.32	0.959	0.276
ΔG:F	1.50	1.58	7.26	1.93	8.45	8.59	5.31	9.16	5.43	2.99	0.156	0.104

Note: [1]SEM = standard error of the means.

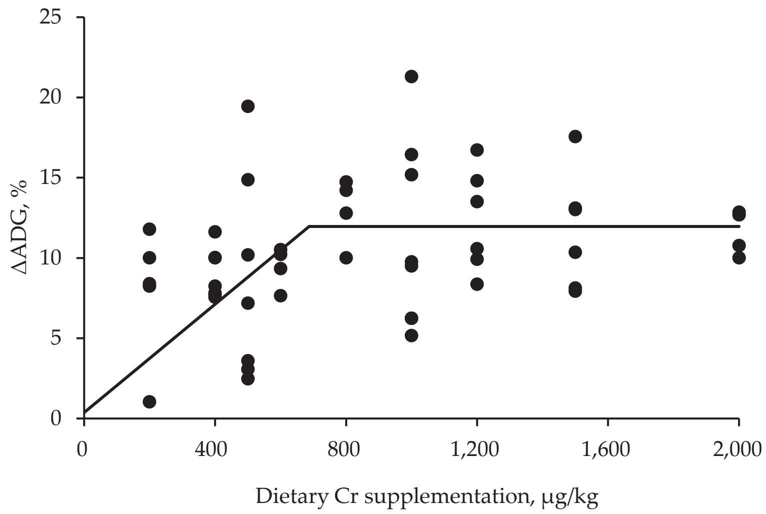

Figure 1. A one-slope broken-line analysis for the relative change of average daily gain (ADG) in broilers fed a chromium (Cr)-supplemented diet compared with a control group with no supplemental Cr diet (ΔADG, %), according to dietary Cr supplementation (µg/kg; as-fed basis) in broilers under heat stress (n= 53). The one-slope broken-line model indicated that the minimum dietary Cr supplementation to maximize ΔADG in broilers under heat stress was 687 µg/kg (SE= 137). The break-point was estimated based on the following equation: ΔADG= 11.96 + 0.017 × (X – 687) where X is less than 687 (R^2= 0.70 and $p<0.001$).

Table 4. The relative changes (Δ) of growth performance, carcass traits, and serum metabolites in broilers fed a chromium (Cr)-supplemented diet compared with the control group with no supplemental Cr under heat stress[1]

Variables, %	n	Mean	SD[2]	95% Confidence interval (CI)[3]		p-value
				Lower CI	Upper CI	
Growth performance						
ΔAverage daily gain	53	10.01	7.97	7.82	12.21	< 0.001
ΔAverage daily feed intake	53	3.82	4.91	2.46	5.17	< 0.001
ΔGain to feed ratio	53	6.04	6.90	4.14	7.93	< 0.001
Carcass traits, % of body mass						
ΔCarcass yield	29	1.74	3.70	0.33	3.14	0.017
ΔDressing yield	12	3.19	2.93	1.33	5.06	0.003
ΔLiver weight	37	0.64	6.36	−1.48	2.76	0.544
ΔHeart weight	25	−5.59	11.23	−10.22	−0.95	0.020
ΔPancreas weight	13	3.56	10.58	−2.84	9.95	0.249
ΔSpleen weight	21	17.68	44.46	−2.56	37.92	0.083
ΔBursa of Fabricius weight	16	20.44	22.61	8.40	32.49	0.003
ΔThymus weight	10	25.02	16.60	13.15	36.90	0.001
ΔAbdominal fat weight	31	−14.98	11.82	−19.31	−10.64	< 0.001
ΔBreast meat weight	17	8.07	6.83	4.56	11.58	< 0.001
ΔLeg weight	10	1.15	6.36	−3.40	5.70	0.581
Serum metabolites						
ΔCholesterol	29	−2.55	18.62	−9.63	4.54	0.467
ΔGlucose	23	−8.04	8.60	−11.75	−4.32	< 0.001
ΔTriglyceride	22	10.99	35.74	−4.86	26.83	0.164
ΔCorticosterone	16	−15.23	9.00	−20.02	−10.43	< 0.001
ΔInsulin	13	16.95	21.98	3.67	30.23	0.017
ΔTotal protein	15	4.84	7.99	0.41	9.26	0.034
ΔHigh-density lipoprotein	14	0.10	5.98	−3.35	3.56	0.949
ΔLow-density lipoprotein	14	1.08	5.40	−2.04	4.20	0.469
ΔSerum Cr concentration	6	48.74	18.61	29.21	68.26	0.001

Note: [1]Means and statistical parameters were calculated regardless of the sources and doses of supplemental Cr. [2]SD= standard deviation. [3]Significance from 0 (2-tailed) was declared when 95% CI did not include zero ($p<0.05$).

control diet without Cr supplementation. The studies included a positive control group kept under thermoneutral conditions and a negative control group subjected to a tropical or hyperthermic environment (Sands & Smith, 1999; Samanta *et al.*, 2008; Jahanian & Rasouli, 2015; Hajializadeh *et al.*, 2017; Hamidi *et al.*, 2022). The results indicated that broilers kept under a hyperthermic environment had decreased ADG by 24.0% (standard deviation= 7.2% data not shown), ADFI by 13.5% (3.8%), and G:F by 11.9% (10.0%) compared with those under thermoneutral conditions. The hypothalamus plays a crucial role in regulating energy balance and feed intake by manipulating the endocrine system and body temperature (Berthoud, 2002). Heat stress affects the rostral cooling center of the hypothalamus, which simulates the medial satiety center, inhibiting the lateral appetite center and resulting in reduced feed intake (Silanikove, 2000). This reduction in feed intake affects the center of satiety in the hypothalamus, resulting in a decrease in the heat increment associated with digestion and absorption of feed and nutrient metabolism. Lower G:F induced by heat stress is attributed to physiological and immunological adaptations in birds, and a part of the consumed energy is used for heat dissipation, resulting in impaired G:F (Jahanian & Rasouli, 2015). The combination of reduced feed consumption and efficiency explains the decreased ADG in broilers kept under heat stress.

The empirical studies utilized in this meta-analysis employed various feed formulations, growth phases, including starter, grower, and finisher, as well as initial body weight and experimental duration. As a result, the growth performance of broilers fed the control group and the actual difference in growth performance between broilers fed the treatment and control groups varied. In 22 experiments, ADG and ADFI of broilers fed the control group under heat stress ranged from 30.4 g/d to 60.2 g/d (SD= 8.8 g/d) and 50.9 g/d to 137.7 g/d (SD = 24.7 g/d), respectively. To combine and compare the 22 experiments, we employed the relative change, which is a unitless value calculated as the actual difference divided by the growth performance of broilers fed the control group, to minimize biases among the experiments. The use of relative changes is widely employed in meta-analyses for nutritional research in pigs and poultry (Cowieson & Roos, 2013; Kim *et al.*, 2021b).

Sources of Cr can be categorized into inorganic sources (Cr chloride) and organic sources, including Cr nicotinate, Cr yeast, Cr-amino acid chelate, and Cr picolinate (Huang *et al.*, 2016). In most studies, organic Cr has been added in the form of Cr-amino acid chelate or Cr picolinate (Samanta *et al.*, 2008; Moeini *et al.*, 2011; Ghazi *et al.*, 2012; Toghyani *et al.*, 2012; Habibian *et al.*, 2013; Huang *et al.*, 2016). Of the 4 studies employed in this meta-analysis that simultaneously compared the alleviating effects of Cr chloride and Cr-amino acid chelate on growth performance, none showed significant differences in ADFI between the 2 sources (Moeini *et al.*, 2011; Ghazi *et al.*, 2012; Habibian *et al.*, 2013; Huang *et al.*, 2016). Average daily feed intake in heat-stressed broilers fed the Cr-amino acid chelate-supplemented diet was numerically increased at –2.3%, 0.7%, 3.0%, and 4.5% (mean= 1.48%) compared with those fed the Cr chloride-supplemented diet.

The ΔADG increased as the dietary Cr concentration increased. In our study, only 2 experiments used the polynomial contrast to determine the effects of increasing supplemental Cr levels on ADG in heat-stressed broilers, and the results showed that ADG increased linearly (Sahin *et al.*, 2002) and quadratically (Samanta *et al.*, 2008) as the doses of Cr increased. Our results agreed with a previous meta-analysis in which Cr picolinate significantly increased the ADG of broilers under heat stress but had no significant effect on ADFI and G:F (Feng *et al.*, 2021b). Heat stress conditions can lead to immunosuppression and oxidative stress by reducing antioxidant defenses and increasing the production of free radicals, and these factors are associated with the harmful effects of heat-stressed broilers (Norain *et al.*, 2013; Jahanian & Rasouli, 2015). Dietary supplementation of Cr has been known to alleviate the detrimental effects of heat stress on growth performance in poultry by ameliorating stress-induced immunosuppression (Norain *et al.*, 2013; Jahanian & Rasouli, 2015). Additionally, dietary Cr supplementation increases insulin plasma concentration and regulates glucose level by potentiating the insulin, acting as an insulin cofactor and enabling proper metabolic transformations of carbohydrates, proteins, and lipids towards the anabolic side (Feng *et al.*, 2021b). Furthermore, Cr supplementation could increase the digestion and absorption of feeds by improving intestinal morphology in stressed broilers, thereby increasing the amount of nutrients available for growth (Bunglavan *et al.*, 2014). A combination of one or more factors by Cr supplementation ameliorated the reduced body mass gain in heat-stressed broilers.

To determine the optimal nutrient concentration, the broken-line analysis has been widely employed (Choi & Kim, 2019; Kim *et al.*, 2022b). Our study found that the optimal supplemental Cr concentration to maximize ΔADG in broilers under heat stress was 687 μg/kg based on one-slope broken-line analysis. Spears *et al.* (2019) reported that Cr supplementation at 400 μg/kg and 2,000 μg/kg over the normal life span of broilers (49 days) did not adversely affect performance and mortality. Moreover, Cr supplementation at 2,000 μg/kg did not affect Cr concentrations in breast muscle and skin with adhering fat. Therefore, the optimal Cr supplemental dose of 687 μg/kg to alleviate the detrimental effects of heat stress on ADG may have no toxicity on broilers and may not present a human health concern.

Serum glucose, corticosterone, and total protein concentrations are stress indicators in broilers. The concentration of serum glucose and corticosterone has been reported to increase, whereas that of serum total protein has been reported to decrease in broilers under heat stress (Sahin *et al.*, 2002). In the present work, Cr supplementation alleviated the changes in serum glucose, corticosterone, and total protein concentrations by heat stress based on multiple studies, indicating that Cr effectively mitigates heat stress. Chromium supplementation increases circulating insulin concentrations, regulating glucose levels by acting as an insulin cofactor and enabling proper metabolic transformations of carbohydrates, proteins, and lipids towards the anabolic side (Feng *et al.*, 2021b). Additionally, Cr supplementation can manipulate the carcass composition to yield more lean meat, thus increasing protein accretion in broilers (Samanta *et al.*, 2008). In the present study, Cr supplementation increased carcass and dressing yields of broilers by 1.7% and 3.2%, respectively, whereas it decreased abdominal fat weight by 15.0%. Moreover, Cr supplementation increased the weight of breast meat by 8.1%, confirming that the broilers fed the Cr-supplemented diet retain more protein and weight gain.

CONCLUSION

The growth performance of broilers fed Cr-supplemented diets is not affected by the source of Cr under heat stress. The relative change of weight gain in broilers subjected to a hyperthermic environment quadratically increases as dietary Cr concentration increases. The optimal Cr supplemental concentration in broiler diets to alleviate the detrimental effects of heat stress on body weight gain is 687 μg/kg, regardless of the source of Cr.

CONFLICT OF INTEREST

B. G. Kim serves as an editor of the Tropical Animal Science Journal but has no role in the decision to publish this article. We also certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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