Nutrient Requirements—Research Communication

Chromium Supplementation Can Alleviate Negative Effects of Heat Stress on Egg Production, Egg Quality and Some Serum Metabolites of Laying Japanese Quail

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ABSTRACT This study was conducted to determine the effects of Cr (chromium picolinate, CrPic) supplementation at various levels (0, 200, 400, 800 or 1200 μg/kg of diet) on egg production, egg quality and serum concentrations of insulin, corticosterone and glucose in laying Japanese quail (*Coturnix coturnix japanica*) reared under conditions of heat stress (32.5°C). Laying Japanese quail (n = 150; 45 d old) were divided into five groups of 30 birds. The quail were fed either a control diet containing 965 μg Cr/kg diet or the control diet supplemented with 200, 400, 800 or 1200 μg of Cr/kg diet. Increased supplemental chromium increased body weight (P = 0.05, linear), feed intake (P = 0.05, linear), egg production (P = 0.01, linear) and also improved feed efficiency (P = 0.01, linear). Increased supplemental chromium linearly increased egg weight (P = 0.01), eggshell thickness, egg specific gravity (P = 0.05) and Haugh unit (P = 0.01). Serum insulin concentration increased linearly (P = 0.01), whereas corticosterone and glucose concentration decreased linearly (P = 0.05) as dietary chromium increased. The best results were obtained with 1200 μg Cr/kg diet, and chromium supplementation at such a level can be considered to be protective management practice in a quail diet, reducing the negative effects of heat stress. J. Nutr. 132: 1265–1268, 2002.

KEY WORDS: • chromium • eggs • insulin • corticosterone • Japanese quail

High ambient temperature reduces feed intake, live weight gain, egg production, egg quality and feed efficiency (1,2), thus negatively influencing the performance of poultry. Hurwitz et al. (3) suggested that the decrease in growth rate was due in part to the decrease in feed intake. Plasma corticosterone concentration also increases during heat stress (4). In addition, Donkoh (1) reported reduced plasma protein and markedly increased blood glucose concentrations during heat stress. Such ambient temperatures decrease serum vitamin and mineral concentrations in poultry as well as humans (2,5,6,7). Heat stress has also been shown to increase mineral excretion (2,8,9). Temperatures >30°C represent heat-stressed conditions for birds (6).

Several methods are available to alleviate the effect of high environmental temperature on poultry performance. Because it is expensive to cool animal buildings, such methods focus mainly on manipulating the diet. Cr is used in the poultry diet because of the reported benefits of Cr supplementation to laying hens (5,10,11) during cold and heat stress and because chromium is reduced during environmental stress. The primary role of Cr in metabolism is to potentiate the action of insulin through its presence in an organometallic molecule, the glucose tolerance factor (GTF)[2] (5,7,12). Insulin metabolism influences lipid peroxidation (13); Cr, as an insulin potentiator, is therefore postulated to function as an antioxidant (14). Moreover, Cr is thought to be essential for activating certain enzymes and for stabilizing proteins and nucleic acids (7,15,16). Cr deficiency can disrupt carbohydrate and protein metabolism, reduce insulin sensitivity in peripheral tissues and impair growth rate (17,18). Some minerals such as Cr can also be supplemented to reduce the negative effects of environmental stress (10,19,20,21). In a previous study, we observed that supplemental Cr significantly alleviated the cold stress–related decrease in performance, suggesting that adding Cr to diets may alleviate cold stress conditions in laying hens (10,11). By evaluating the effects of supplemental Cr on performance, it is possible to gain an understanding of the metabolic changes in heat-stressed poultry. Therefore, the objective of this study was to evaluate the effects of Cr (postulated to function as antioxidant) supplementation on egg production, egg quality and serum concentrations of insulin, corticosterone and glucose in laying Japanese quail reared under conditions of heat stress (32.5°C).

MATERIALS AND METHODS

Animals, diets and experimental design. Japanese quail (*Coturnix coturnix japanica*) (n = 150; 45 d old) obtained from Uluova Quail Farm, Elazig, Turkey were used in the study. The experiment was in accordance with animal welfare and ethics, and was conducted under protocols approved by the Veterinary Control and Research Institute of Elazig, Turkey. The birds were fed either a basal diet containing 17% crude protein (CP) and 12.4 MJ/kg metabolizable energy (ME), or the control diet supplemented with 200, 400, 800 or 1200 μg Cr/kg

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[2] Abbreviations used: ACTH, adrenocorticotropic hormone; CP, crude protein; CrPic, chromium picolinate, GTF, glucose tolerance factor; HU, Haugh unit; ME, metabolizable energy.

TABLE 1

Ingredients and chemical composition of the basal diet fed to laying Japanese quails

Ingredient	*g/100 g*
Ground corn	58.62
Soybean meal	26.72
Vegetable oil (Sunflower)	3.80
Limestone	8.60
Dicalcium phosphate	1.60
Vitamin + mineral premix[1]	0.25
DL-Methionine	0.10
Sodium chloride	0.35
ME,[2] *MJ/kg*	12.4
Chemical analyses, dry matter (DM) basis	
Crude protein,[3] %	17.00
Calcium,[3] %	3.53
Phosphorus,[3] %	0.65
Chromium,[3] *μg/kg*	965

[1] Mix supplied per kg of diet: retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; *dl*-α tocopheryl acetate, 1.25 mg; menadione sodium bisulfite, 2.5 mg; thiamine-hydrochloride, 1.5 mg; riboflavin, 3 mg; D-pantothenic acid, 5 mg; pyridoxine hydrochloride, 2.5 mg; vitamin B-12, 0.0075 mg; folic acid, 0.25 mg; niacin, 12.5 mg., Mn ($MnSO_4 \cdot H_2O$), 50 mg; Fe ($FeSO_4 \cdot 7H_2O$), 30 mg; Zn (ZnO), 30 mg; Cu ($CuSO_4 \cdot 5H_2O$), 5 mg; I (KI), 0.5 mg; Se (Na_2SeO_3), 0.15 mg; Co ($CoCl_2$-$6H_2O$), 0.1 mg; choline chloride, 125 mg.

[2] ME, metabolizable energy, Calculated from the tabular values (22).

[3] Analyzed value.

diet. The basal diet was formulated using NRC guidelines (22) and analyzed to contain 965 μg Cr/kg diet. Chromium picolinate (CrPic) was used as Cr source. Ingredients and chemical composition of the basal diet are shown in **Table 1**.

The birds were randomly assigned to caging units, 30 birds each. Water and the diets were consumed by the birds ad libitum throughout the experiment. The bird house was lit for 17 h/d. During the experiment, the temperature and humidity of the hen house were measured four times a day (0600, 1200, 1800 and 2400 h). The average ambient relative humidity inside the hen house was 40.8 ± 6.3%. The mean value of daily temperature in the hen house was 32.5 ± 3.7°C. The experiment was conducted between June 30 and September 20, 2001.

Performance variables and egg quality. Body weights were recorded at the beginning and at the end of the study. Feed consumption was measured weekly. The number of eggs and egg weights were recorded daily. Egg quality measurements were conducted monthly using all eggs of 1 d from all treatments. Indices of egg quality included specific gravity, egg shell thickness and Haugh unit (HU). Specific gravity of eggs was determined by using the saline flotation method of Hempe et al. (23). NaCl salt solutions were made in incremental concentrations of 0.005 in the range from 0.1065 to 0.1120 g/L. Haugh units were calculated using the HU formula (24) based on the height of albumen determined by a micrometer and egg weight (Saginomiya, TLM-N1010, Tokyo, Japan). Shell thickness was determined by taking the mean value of the thickness measured at three locations on the egg (air cell, equator and sharp end) using a dial pipe gauge (Mitutoyo, 0.01–20 mm, Tokyo, Japan).

Sample collection and laboratory analysis. At the end of the experiment, 10 birds were randomly chosen from each treatment and slaughtered; blood samples were taken, centrifuged at 1500 × *g* for 10 min, and sera were collected and stored at −20°C. Serum samples were thawed at room temperature, and insulin and corticosterone concentrations were determined. Serum insulin concentration was determined via RIA using a commercially available heterologous kit (IMMULITE 2000, no. L2KIN6, DPC, Los Angeles, CA). The maximum binding for [^{125}I] insulin was 24.8%. The sensitivity was 305 pmol/L at 80% binding. RIA for corticosterone concentration was performed using a commercially available kit (IMMULITE 2000). All measurements for each hormone assay were performed in a single run to avoid interassay variation. Serum glucose concentrations were measured using biochemical analyzer (Technicon RA-XT, New York, NY). For Cr content analysis, basal diet samples were wet-digested in triplicate as described by Chang et al. (25) and were read using atomic absorption spectrometer with a graphite furnace (Shimadzu AA-660-GFA-4B-P/N 204−03154-02, Kyoto, Japan). Chemical analysis of the diet was conducted using AOAC procedures (26).

Statistical analyses. The data were analyzed using the General Linear Models procedure of SAS software (27) ($P < 0.05$). Linear, quadratic and cubic polynomial contrasts (regression) were used to evaluate treatment effects.

RESULTS

Increasing supplemental Cr increased body weight ($P = 0.05$, linear), feed intake ($P = 0.05$, linear), egg production ($P = 0.01$, linear) and also improved feed efficiency ($P = 0.01$, linear) (**Table 2**). Supplemental Cr also influenced egg quality (**Table 3**). Increased Cr linearly increased egg weight ($P = 0.01$), eggshell thickness ($P = 0.01$), egg specific gravity and HU ($P = 0.05$). Serum insulin concentration increased ($P = 0.01$), whereas corticosterone concentration decreased linearly ($P = 0.05$) as dietary Cr increased. Serum glucose concentration decreased ($P = 0.01$) when dietary Cr increased.

DISCUSSION

In the present study, Cr supplementation improved the performance variables including live weight, feed intake, egg production and feed efficiency as well as egg quality in laying Japanese quail reared under heat stress conditions (32.5°C) (Tables 2, 3). Growth rate and feed efficiency decrease when the ambient temperature is above the thermoneutral zone (18–22°C) (6). Stressors such as high environmental temperature induce a cascade of neural and hormonal events beginning with hypothalamic stimulation and the production of corticotropin releasing factor, which stimulates the anterior pituitary to produce adrenocorticotropic hormone (ACTH) and ending with stimulation of adrenal cortical tissue by

TABLE 2

Effects of chromium supplementation on performance in laying Japanese quails reared under conditions of heat stress (32.5°C)[1]

Treatment	Feed intake	Final body weight	Feed efficiency[2]	Quail-day egg production
	g/bird	*g*		%
Chromium *μg/kg*				
0	26.2	214	1.75	63.5
200	27.6	215	1.66	74.3
400	29.5	225	1.61	84.6
800	31.6	228	1.55	87.2
1200	33.1	234	1.52	89.4
Pooled SEM	3.50	4.33	0.24	3.36
		Probabilities		
Polynomial contrasts				
Linear	0.05	0.01	0.003	0.006
Quadratic	0.28	0.09	0.20	0.46
Cubic	0.56	0.35	0.29	0.52

[1] Values are means, $n = 30$.

[2] Kg feed consumed/kg egg production.

TABLE 3

Effects of chromium supplementation on egg quality in laying Japanese quails reared under conditions heat stress (32.5°C)[1]

Treatment	Egg weight	Specific gravity	Eggshell thickness	Haugh unit[2]
	g		*μm*	
Chromium *μg/kg*				
0	10.5	1.0871	21.8	82
200	10.9	1.0875	22.6	86
400	11.6	1.0879	22.9	89
800	12.0	1.0885	23.1	92
1200	12.7	1.0889	23.5	95
Pooled SEM	0.25	0.002	0.5	2.6
		Probabilities		
Polynomial contrasts				
Linear	0.05	0.01	0.05	0.01
Quadratic	0.33	0.03	0.08	0.46
Cubic	0.46	0.51	0.63	0.55

1 Values are means, $n = 30$.
2 Haugh unit, $100 \times \log (H + 7.57 - 1.7 \times W^{0.37})$; H, albumen height, mm; W: egg weight, g.

ACTH to increase production and release of corticosteroids, primarily corticosterone, in birds (2). High ambient temperatures also decrease serum and tissue vitamin and mineral concentrations in poultry as well as humans (5). In addition, stress increases Cr mobilization from tissues and its excretion (5,28), and thus may exacerbate a marginal Cr deficiency or an increased Cr requirement. Similar to results of the present study, Sahin et al. (10) reported that 400 μg/kg of diet Cr picolinate supplementation increased egg production by 11% in laying hens reared at a low ambient temperature (6.9°C). The data reported in the present study indicate that the egg production response to Cr supplementation is far higher under heat stress than cold stress. Cr has been shown to increase performance in poultry in several studies (11,21,29,30). Cr is involved in protein metabolism (20) and is thought to have a role in nucleic acid metabolism because an increase in stimulation of amino acid incorporation into liver protein in vitro was observed (31). Okada et al. (32) showed an interaction of Cr with DNA templates that resulted in a significant stimulation of RNA synthesis in vitro. The oligopeptide low-molecular-weight Cr-binding protein (chromodulin) tightly binds four chromic ions before the oligopeptide obtains a conformation required for binding to the tyrosine kinase active site of the insulin receptor (33). The oligopeptide chromodulin binds chromic ions in response to an insulin-mediated chromic ion flux, and the metal-saturated oligopeptide can bind to an insulin-stimulated insulin receptor, activating the receptor's tyrosine kinase activity. Thus, chromodulin appears to play a role in an autoamplification mechanism in insulin signaling. In addition, release of chromium from Cr picolinate for use in cells requires reduction of the chromic center, a process that can lead potentially to the production of harmful hydroxyl radicals (33,34).

In the present study, insulin serum concentration increased whereas corticosterone concentration decreased with increasing dietary Cr (**Table 4**). This is a typical metabolic relationship between insulin (anabolic) and corticosterone (catabolic), having opposite effects to one another in metabolism. The presence of Cr in the diet did not change this relationship. In addition, increasing dietary Cr linearly increased the insulin serum concentration, indicating chromium's physiologic role to act as an insulin potentiator. Similar to results of the present study, Sahin et al. (10) found that Cr supplementation increased serum insulin concentration while markedly decreasing corticosterone concentration in laying hens at low ambient temperature. Rosebrough and Steele (35) have also considered Cr to be a cofactor for insulin activity and necessary for normal glucose utilization and animal growth. The relationship between Cr and insulin in the present study is in agreement with those reported by other researchers (5,35,36). Similar to results of the present study, Chang and Mowat (37) and Moonsie-Sheageer and Mowat (36) also reported significant decreases in blood serum cortisol in stressed calves fed a diet supplemented with Cr. In the present study, serum glucose concentrations increased as dietary Cr decreased. Increasing concentration of corticosterone paralleled increases in serum glucose concentration. This result was probably due to the greater catabolic effect (or concentration) of corticostrone, yielding more glucose in the serum. Similar to results of the present study, Sahin et al. (12) found that Cr supplementation markedly decreased blood glucose and cholesterol concentrations in Japanese quail under thermoneutral zone. Cr is essential for normal glucose metabolism; it is a component of GTF that works with insulin to move glucose into cells for energy generation. Insulin regulates metabolism of carbohydrate, fat and protein, stimulating amino acid uptake and protein synthesis as well as glucose utilization (38). Rosebrough and Steele (35) reported that turkeys fed a diet supplemented with chromium had greater liver glycogen levels as a result of the increased activity of the enzyme glycogen synthetase, and Cr increased glucose transport by increasing insulin activity. In the present study, increased insulin concentration should have increased glucose utilization, thus improving live weight gain, feed efficiency and carcass qualities. Similarly, Cupo and Donaldson (39) reported that Cr supplementation (20 mg/kg $CrCl_3 \cdot 6H_2O$) increased the rate of glucose utilization by 16%.

Supplemental dietary Cr, particularly at 1200 μg/kg diet, may offer a potential protective management practice in preventing heat stress in laying Japanese quail.

TABLE 4

Effects of chromium supplementation on serum concentrations of corticosterone, insulin and glucose concentrations in laying Japanese quails reared under conditions of heat stress (32.5°C)[1]

Treatments	Corticosterone	Insulin	Glucose
	mmol/L	*pmol/L*	*mmol/L*
Chromium *μg/kg*			
0	2300	198	12.7
200	1900	204	12.5
400	1800	215	12.1
800	1600	226	11.8
1200	1400	231	11.5
Pooled SEM	57	5.3	0.09
		Probabilities	
Polynomial contrasts			
Linear	0.01	0.01	0.01
Quadratic	0.56	0.50	0.33
Cubic	0.73	0.81	0.52

1 Values are means, $n = 10$.

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