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ORIGINAL PAPER

Effects of chromium and chromium+vitamin C combination on metabolic, oxidative, and fear responses of broilers transported under summer conditions

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Abstract A total of 240 female broilers (42 days old) were randomly assigned to four groups with six replicates and fed either a basal diet (two control groups) or a basal diet supplemented with either 1,200 μg Cr^{+3} from chromium (Cr) methionine/kg (Cr group) or 1,200 μg Cr^{+3} from Cr methionine plus 800 mg vitamin C (Vit C)/kg of diet (Cr+Vit C group). After 7 days on the dietary treatment, all groups except one of the controls were transported for 3 h under the summer conditions. Performance parameters were not influenced by dietary treatments. The plasma concentrations of insulin, triiodothyronine, triglyceride, and the ratio of triiodothyronine/thyroxin were decreased and the ratio of glucose/insulin was increased due to transport process. Road transportation also increased the plasma concentrations of protein, cholesterol, aspartate aminotransferase, and creatine kinase and decreased the concentration of low-density lipoprotein cholesterol in the Cr+Vit C group. The pretransport concentrations of insulin and triiodothyronine were highest in the Cr+Vit C group. The concentration of phosphorous was lower in the Cr group than that in the other groups after transport. No significant effects of dietary treatments were observed on the other biochemical parameters. Transport increased malondialdehyde concentration in the control group and did not change plasma total antioxidant capacity and erythrocyte glutathione peroxidase activity. Either in combination or alone, Cr increased plasma total antioxidant capacity (before transport $P \leq 0.05$, after transport $P=0.07$) but did not affect the concentration of malondialdehyde and activity of glutathione peroxidase. The duration of tonic immobility (TI) was similar between nontransported control chicks and transported chicks without any supplements. Pretreatment with Cr+Vit C significantly reduced the duration of TI.

Keywords Transport · Chromium · Vitamin C · Blood metabolites · Antioxidant status · Tonic immobility

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Introduction

Birds transported to the slaughterhouse can be exposed to a variety of stressors including handling by humans, gathering, loading and unloading, thermal extremes, noise and vibration, mixing with unfamiliar birds, overcrowding, daylight, feed and water deprivation, and exposure to new pathogens (Weeks 2007; Vosmerova et al. 2010). Transport is an important economic factor because it can cause physical, psychological, and physiological stresses that affect health, well-being, performance of birds, and, finally, the quality of the final product (Weeks 2007; Minka and Ayo 2010; Vosmerova et al. 2010). Several nutritional strategies such as supplementation of diets with one or more vitamins (Ajakaiye et al. 2010; Minka and Ayo 2010; Aktas et al. 2011), minerals (Kegley et al. 1997; Apple et al. 2005; Aktas et al. 2011), pre- and probiotics (Ghareeb et al. 2008; Huff et al. 2010), and feeding of semi-synthetic diet (Nijdam et al. 2006) have been evaluated to ameliorate the severity of thermal and other stresses associated with transport in animals. Vitamin C (Vit C), also known as L-ascorbic acid, has numerous physiological activities, and many of them are derived from its reducing properties that enable it, along with α-tocopherol, to protect the cell from oxidative stress (Whitehead and Keller 2003). Birds are

normally able to synthesize Vit C; this ability is thought to be inadequate or Vit C requirements may be increased under certain circumstances such as heat stress and transport, and, therefore, supplementation with Vit C may be efficient to overcome any deficiency and increase resistance to stress (Sahin et al. 2003a, b; Whitehead and Keller 2003; Zulkifli 2003; Gursu et al. 2004; Minka and Ayo 2010). However, Konca et al. (2009) found no positive effect of supplemental Vit C on growth performance of turkeys reared under summer conditions. It was proposed that a low-molecular-weight-chromium (Cr) (LMWCr)-binding substance, also known as chromodulin, is the most popular candidate for the biologically active form of Cr^{3+}. Chromodulin is an oligopeptide molecule consisting of glycine, cysteine, aspartate, and glutamate that binds with high affinity to four chromic ions. It amplifies insulin signaling through maintaining the active conformation of insulin receptors and cause greater glucose uptake (Anderson 2003). Stress associated with physical trauma, diet rich in carbohydrates, exercise, lactation, infection, transportation, and high ambient temperatures may increase excretion of Cr and consequently may cause a compromised Cr status (Chang and Mowat 1992; Anderson 2003; Sahin et al. 2003a). Positive physiological and production responses to supplemental Cr especially as organic Cr can be perceived more efficiently under stressful conditions, such as transportation and thermal stresses (Chang and Mowat 1992; Sahin et al. 2002a, b, 2003b). However, Akbari and Torki (2013) and Habibian et al. (2013) reported that supplementation with Cr did not influence growth performance of heat-stressed (HS) broilers. A number of nutritional relationships between Cr and Vit C have been reported. Sahin et al. (2002a) demonstrated that either Vit C or Cr supplementation obviously increased the serum concentrations of Vit C, Cr, Vit E, Fe, Zn, Mn, and digestibility of nutrients in laying hens under low ambient temperature, and further improvements in these regards were observed when Vit C and Cr was supplemented together. Regarding antioxidant property, there is a positive synergistic effect of vitamin C and Cr against oxidative stress (Sahin et al. 2002a, 2003b; Perai et al. 2014). It was documented that hyperglycemia or decreased insulin level reduced the rate of ascorbic acid uptake into the cells (Kapeghian and Verlangieri 1984). Through amplifying the action of insulin, Cr may indirectly enhance the intracellular availability of Vit C (Sahin et al. 2002a). Several studies have been conducted on the effects of supplemental Vit C as an ameliorating agent against road transportation stress in poultry (Zulkifli 2003; Ajakaiye et al. 2010; Minka and Ayo 2010). It was shown that pretreatment with Vit C solution for 24 h reduced the ratio of heterophil/lymphocyte and the duration of tonic immobility (TI), as indicator of fear response, in transported broilers (Zulkifli 2003). Minka and Ayo (2010) also reported that administration of vitamins C and E prior to transport markedly modified fear-related behavior in transported pullets. To our knowledge, no research has been carried out to assess the effects of supplemental Cr as organic Cr on broilers responses to transport. Therefore, this study was conducted to investigate the effects of supplementation of Cr methionine alone or in combination with Vit C during the last week before slaughter on plasma metabolites, antioxidant status, and fear-related behavior of broilers transported by road for 3 h under summer conditions.

Materials and methods

Birds, management, and diets

One-day-old mix-sexed Ross 308 broiler chicks were obtained from a local hatchery and reared on floor pens covered with sterilized and contaminant-free wood shavings. Birds were fed broiler starter (22 % crude protein (CP); 3,025 kcal/kg metabolizable energy (ME)) until 10 days of age, broiler grower (21 % CP; 3,150 kcal/kg ME) from 11 to 24 days of age, and broiler finisher (19 % CP; 3,200 kcal/kg ME) from 25 to 49 days of age. Feed and water were provided ad libitum, and lighting was continuous. The initial room temperature was set at approximately at 32 °C and, thereafter, was gradually reduced based on normal management practice to 21 °C by 28 days of age. On day 42, 240 female broiler chickens were weighed and assigned randomly to four groups with six replicates of 10 birds each. Average body mass was 2,223±23 g. Two groups received a corn-soybean-based diet (control groups). The remaining two groups were fed either on basal diet supplemented with 1,200 μg Cr^{+3} from Cr methionine/kg of diet (Cr group) or basal diet supplemented with 1,200 μg Cr^{+3} from Cr methionine and 800 mg Vit C/kg of diet (Cr+Vit C group) for 1 week. On day 49, all chicks (except one of the controls) were caught manually and placed in plastic crates (0.80×0.60×0.32 m). Birds from each replicate were placed in one crate. The crates were then loaded randomly into an open truck and transported for 3 h (1,000 to 1,300) with an average speed of 60 km/h. The truck had no roof and its floor was made of metal. During transportation, the thermal microenvironments experienced by the broilers in the crates were achieved by recording the ambient temperature and relative humidity with the aid of a wet- and dry-bulb thermometer (TES-1364, Taiwan, China). The average environmental temperature and relative humidity during transportation were 31.5±1.1 °C and 35.0±3.7 %, respectively (Table 1). After transport, the birds remained in their crates. Feed and water were supplied until transport, and no feed and water were provided during transport intervention (3 h of transportation with 45 min of recovery). The nontransported control chicks remained in their pens and had no access to feed and water. These birds are only used to measure tonic immobility (TI). All animal research procedures were assessed and approved by the Animal Care Committee of the Ferdowsi University of Mashhad.

Table 1 Ambient temperature and relative humidity during the transportation period

Hour of transportation (h)	Dry-bulb temperature (°C)	Relative humidity (%)
0.5	28.7	44.3
1	31.1	37.0
2	32.2	31.6
3	34.0	27.1
Mean±SEM	31.5±1.1	35±3.7

Performance

Body mass and feed consumption of each pen were recorded prior to transport at 49 days of age.

Tonic immobility

Twenty chicks per group were tested individually for TI immediately after the transport intervention. Chicks were caught randomly and carried in an inverted manner to a separate neighboring room for TI measurements. The procedure described by Zulkifli (2003) was used.

Serum metabolites

One bird from each pen was randomly chosen prior to and immediately after the transport intervention (that which was not used for bleeding before the transport and for TI measurement) and bled by venipuncture within 30 s. The blood samples were collected into EDTA-treated tubes. The tubes were centrifuged at 1,800×*g* for 15 min to obtain plasma, which was kept at −20 °C in Eppendorf test tubes until the analyses were performed. The buffy coat was carefully removed and then the sedimented cells were washed three times by resuspending in isotonic phosphate-buffered saline, followed by recentrifugation and removal of the supernatant fluid and buffy coats. The washed erythrocytes were then lysed with nine volumes of ice-cold distilled water to prepare 10 % erythrocyte hemolysates. The hemolysates were stored at −70 °C for later analysis. The plasma concentrations of insulin, triiodothyronine (T_3), and thyroxin (T_4) were measured using radioimmunoassay with commercial kits and an automatic gamma counter (BioSource International, Camarillo, CA, USA). The plasma glucose, total protein, albumin, phosphorous, triglyceride, cholesterol, uric acid concentrations, and enzyme activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and creatine kinase (CK) were determined using the BioSystems kits and associated procedures (BioSystems S.A. Costa Brava 30, 08030 Barcelona, Spain). The plasma concentrations of high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were analyzed using Pars Azmoon kits (Pars Azmoon Co; Tehran, Iran). Globulin content was achieved by subtracting albumin from total protein.

Antioxidant parameters

Total plasma antioxidant capacity was determined by the *ferric reducing-antioxidant power* (FRAP) *assay* based on the method of Benzie and Strain (1999). Levels of plasma *malondialdehyde* (MDA), an indicator of lipid peroxidation, were determined by the method described by Yoshioka et al. (1979). The principle of the method is the spectrophotometric measurement of the color formed by the reaction of thiobarbitaric acid (TBA) with MDA. Briefly, 2.5 ml of 20 % trichloroacetic acid and 1.0 ml of 0.67 % TBA were added to 0.5 ml plasma, and the mixture was heated in a boiling water bath for 30 min. After cooling, 4 ml of *n*-butanol was added, and the mixture was vortexed. Following this process, centrifugation was performed at 3,000 rpm for 10 min, and the absorbance of the upper *n*-butanol layer was read at 535 nm. Values were compared to a series of standard solutions (1,1,3,3-tetramethoxypropane). Results were expressed as nanomole per milliliter of plasma. The activity of erythrocyte *glutathione peroxidase* (GPx) was measured in erythrocyte hemolysates using a commercially available kit (Ransel test kit, Randox Laboratories Ltd., UK) according to Paglia and Valentine (1967).

Statistical analyses

For all variables tested, normality was assessed by means of a Shapiro-Wilk test, stem-and-leaf plots, and normal probability plots using the SPSS statistical software (SPSS 1993). The distributions of the plasma triglyceride, HDL-C, ALP, T_3, MDA concentrations, and ratios of glucose/insulin (G/I), albumin/globulin (A/G) and T_3/T_4, and TI were skewed, and appropriate transformations were applied using the statistical package Unistat (StatSoft 2009). Data obtained within each time point were analyzed with the GLM procedure of the SAS (SAS 2003) as a completely randomized design. The significance of mean differences was determined using the least squares means. Data from two time points for each group were compared with *t* test if the parametric conditions existed; otherwise, the nonparametric Mann–Whitney test was performed (SPSS 1993). Data are presented in the tables as means±SEM. Statements of significance were based on $P \leq 0.05$.

Results

Performance

The initial body mass was not different among treatments at 42 days of age. Either alone or in combination, Cr had no significant effect on final body mass, body mass gain, feed intake, and feed conversion ratio (Table 2).

Blood metabolites

The concentrations of insulin, T3, and ratio of T_3/T_4 were significantly decreased, and the ratio of G/I was significantly increased due to transport process. Transport had no significant effect on the glucose and T_4 concentrations. The Cr+Vit C group had the highest insulin and T_3 concentrations prior to transport. The concentrations of glucose and T_4 and the ratios of T_3/T_4 and G/I were not influenced by the treatments (Table 3).

The plasma activities of AST and CK were significantly increased in the Cr+Vit C group due to transport. Transport did not significantly affect the plasma activities of ALP and ALT and significantly decreased phosphorous concentration in the Cr group. Treatments did not significantly change the activities of ALP, ALT, AST, and CK at any sampling times. The level of phosphorous was lower in the Cr group than that in the control and Cr+Vit C groups after transport (Table 4).

The protein level was significantly increased in the Cr+Vit C group following transport. Neither transport nor treatments had significant effects on the albumin, globulin, and uric acid concentrations and on the A/G ratio (Table 5).

The triglycerides were significantly decreased due to transport. Transport increased the cholesterol level and decreased the LDL-C level in the Cr+Vit C group. Plasma HDL-C concentration and LDL-C/HDL-C ratio were not significantly changed by transport. Treatments had no significant effect on plasma lipid profile (Table 6).

Table 2 Effect of treatments on the performance parameters of broiler chickens from 42 to 49 days of age

	Control	Cr	Cr+Vit C	*P* value
Final body mass, g	2,747±20	2,721±7	2,761±51	0.72
Body mass gain, g/day	75.8±3.2	72.2±1.2	77.0±6.9	0.77
Feed intake, g/chick/day	205.7±5.7	197.0±1.6	204.5±5.0	0.42
Feed conversion ratio, g/g	2.73±0.08	2.73±0.05	2.72±0.15	0.99

Means±SEM (n=6)

Control basal diet with no supplements, *Cr* control diet+1,200 μg chromium methionine/kg, *Cr+ Vit C* control diet+1,200 μg of chromium methionine/kg+800 mg of vitamin C/kg

Table 3 Effects of chromium and chromium+vitamin C supplementations on the glucose and hormone concentrations of broilers transported under summer conditions

	Control	Cr	Cr+Vit C	*P* value
Glucose, mg/dL				
Before Trans	333±10	318±10	321±4	0.43
After Trans	343±12	315±16	351±15	0.22
P value	0.54	0.88	0.08	
Insulin, μIU/mL				
Before Trans	$8.37±2.55^{bx}$	$8.54±3.06^{bx}$	$19.3±3.17^{ax}$	0.03
After Trans	$1.73±0.34^{y}$	$2.1±0.20^{y}$	$2.24±0.52^{y}$	0.60
P value	0.05	0.006	0.006	
G/I ratio				
Before Trans	$42.4±9.9^{y}$	$42.9±10.6^{y}$	$19.0±3.0^{y}$	0.10
After Trans	$272.6±83.4^{x}$	$156.7±16.2^{x}$	$197.0±85.0^{x}$	0.34
P value	0.006	0.0001	0.006	
T_3, ng/dL				
Before Trans	$101.7±9.5^{bx}$	$108.3±7.0^{bx}$	$148.3±19.2^{ax}$	0.05
After Trans	$33.2±11.6^{y}$	$36.7±8.3^{y}$	$61.5±18.3^{y}$	0.42
P value	0.001	0.0001	0.008	
T_4, μg/dL				
Before Trans	0.55±0.08	0.60±0.07	0.50±0.07	0.64
After Trans	0.55±0.14	0.50±0.13	0.52±0.05	0.95
P value	1.00	0.53	0.82	
T_3/T_4 ratio				
Before Trans	$0.205±0.037^{x}$	$0.166±0.027^{x}$	$0.328±0.067^{x}$	0.10
After Trans	$0.078±0.032^{y}$	$0.073±0.020^{y}$	$0.125±0.049^{y}$	0.90
P value	0.03	0.02	0.03	

Means±SEM (n=6)

Control basal diet with no supplements, *Cr* control diet+1,200 μg chromium methionine/kg, *Cr+ Vit C* control diet+1,200 μg of chromium methionine/kg+800 mg of vitamin C/kg, *Trans* transport, *G/I* glucose/insulin, *T_3* triiodothyronine, *T_4* thyroxin

[a,b] Means within the same row with no common superscripts differ significantly ($P \leq 0.05$)

[x,y] Means within the same parameter and column with no common superscripts differ significantly ($P \leq 0.05$)

Antioxidant status

Transport significantly increased plasma MDA concentration in the control group. The FRAP value and the GPx activity were not affected by transport. The plasma concentration of MDA and the erythrocyte activity of GPx were not significantly changed by treatments. Either in combination or alone, Cr significantly increased the FRAP value before transport (Table 7).

Tonic immobility

The duration of TI was not significantly different between nontransported control chicks and transported control chicks.

Table 4 Effects of chromium and chromium+vitamin C supplementations on the enzyme activities and phosphorous concentration of broilers transported under summer conditions

	Control	Cr	Cr+Vit C	*P* value
ALP, U/L				
Before Trans	373±105	469±150	232±70	0.39
After Trans	241±65	337±163	427±72	0.24
P value	0.31	0.34	0.08	
ALT, U/L				
Before Trans	13.5±0.8	15.2±1.3	12.0±2.3	0.39
After Trans	15.7±1.3	13.8±1.5	16.2±0.95	0.42
P value	0.20	0.53	0.12	
AST, U/L				
Before Trans	234.0±6.8	243.4±24.2	210.3±18.9^{y}	0.43
After Trans	268.0±13.3	266.3±32.5	303.0±14.3^{x}	0.37
P value	0.06	0.60	0.003	
CK, U/L				
Before Trans	8,951±1,676	6,388±1,548	4,826±972^{y}	0.14
After Trans	6,830±1,199	4,856±1,016	8,924±945^{x}	0.07
P value	0.36	0.46	0.02	
Phosphorous, mg/dL				
Before Trans	6.65±0.23	6.74±0.12^{x}	6.73±0.24	0.96
After Trans	6.76±0.26^{a}	6.12±0.20^{by}	6.86±0.17^{a}	0.05
P value	0.77	0.05	0.66	

Means±SEM (n=6)

Control basal diet with no supplements, *Cr* control diet+1,200 μg chromium methionine/kg, *Cr+ Vit C* control diet+1,200 μg of chromium methionine/kg+800 mg of vitamin C/kg, *Trans* transport, *ALP* alkaline phosphatase, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *CK* creatine kinase

^{a,b} Means within the same row with no common superscripts differ significantly (P≤0.05)

^{x,y} Means within the same parameter and column with no common superscripts differ significantly (P≤0.05)

Table 5 Effects of chromium and chromium+vitamin C supplementations on some blood metabolites of broilers transported under summer conditions

	Control	Cr	Cr+Vit C	*P* value
Total protein, g/L				
Before Trans	32.8±0.98	33.2±1.24	32.6±0.51^{y}	0.91
After Trans	32.8±2.33	32.0±1.67	36.2±1.35^{x}	0.23
P value	1.00	0.59	0.05	
Albumin, g/L				
Before Trans	18.0±0.86	17.7±1.02	17.3±0.49	0.84
After Trans	18.8±1.89	16.7±0.88	19.7±1.05	0.30
P value	0.63	0.48	0.07	
Globulin, g/L				
Before Trans	14.8±0.60	16.8±1.17	15.2±0.97	0.30
After Trans	14.0±0.52	15.3±0.88	16.5±0.92	0.12
P value	0.32	0.33	0.36	
A/G ratio				
Before Trans	1.15±0.04	1.07±0.09	1.10±0.13	0.53
After Trans	1.28±0.10	1.09±0.04	1.21±0.10	0.18
P value	0.40	0.83	0.50	
Uric acid, mg/dL				
Before Trans	5.29±0.28	5.67±0.29	5.40±0.21	0.80
After Trans	4.32±1.05	4.31±0.60	6.17±0.65	0.19
P-value	0.36	0.07	0.49	

Means±SEM (n=6)

Control basal diet with no supplements, *Cr* control diet+1,200 μg chromium methionine/kg, *Cr+ Vit C* control diet+1,200 μg of chromium methionine/kg+800 mg of vitamin C/kg, *Trans* transport, *A/G* albumin/globulin

^{x,y} Means within the same parameter and column with no common superscripts differ significantly (P≤0.05)

The combined supplementation of Vit C and Cr significantly reduced the duration of TI in broilers. Neither transport nor treatments had a significant effect on the number of induction to induce TI (Table 8).

Discussion

The present study indicates that the supplementation of Cr alone or in combination with Vit C had no effect on feed intake, body mass gain, and feed conversion ratio of broilers were fed with corn-soybean-based diet at the last week of age. There have been many studies on the effects of feeding Vit C to poultry. It is demonstrated from previous studies that dietary supplementation of Vit C generally did not exhibit any beneficial effect on the performance of broilers reared under nonstressful conditions (Whitehead and Keller 2003). It seems that the benefits of Vit C supplementation to poultry would be more obvious under stressful conditions (Whitehead and Keller 2003). However, Konca et al. (2009) and Torki et al. (2014) did not observe a positive effect of supplemental Vit C on performance parameters of turkeys and laying hens under heat stress conditions, respectively. Data on the effect of supplemental Cr on growth performance have been contradictory. The results of the present study are similar to the findings of Akbari and Torki (2013) and Habibian et al. (2013) in broilers. However, Sahin et al. (2003b) reported that dietary supplementation with Vit C and Cr alone or in combination improved performance traits of HS broilers. The lack of a consistent response to supplemental Vit C or Cr may be related to species, strain, and age of birds; levels of Vit C or Cr; nutrient composition of basal diets; stress condition; type and severity of stress; length of the experiments; and managerial conditions (Whitehead and Keller 2003; Lindemann 2007; Konca et al. 2009; Habibian et al. 2013). Nevertheless, other factors such as Cr status of subjects and

Table 6 Effects of chromium and chromium+vitamin C supplementations on the plasma lipid profile of broilers transported under summer conditions

	Control	Cr	Cr+Vit C	*P* value
Triglyceride, mg/dL				
Before Trans	125.5±9.7^{x}	131.2±17.2^{x}	118.8±10.3^{x}	0.89
After Trans	31.5±3.4^{y}	34.8±5.4^{y}	40.2±7.1^{y}	0.77
P value	0.0001	0.004	0.0001	
Cholesterol, mg/dL				
Before Trans	130.3±7.1	121.7±5.3	115.8±4.9^{y}	0.24
After Trans	125.7±7.4	122.8±6.6	141.3±7.1^{x}	0.17
P value	0.66	0.89	0.01	
HDL-C, mg/dL				
Before Trans	32.0±5.3	44.5±8.5	33.2±3.3	0.41
After Trans	32.7±5.3	37.0±3.6	41.2±8.0	0.61
P value	0.93	0.44	0.38	
LDL-C, mg/dL				
Before Trans	26.3±2.5	26.3±3.1	28.3±1.7^{x}	0.81
After Trans	22.3±1.4	17.8±2.6	20.7±1.5^{y}	0.27
P value	0.19	0.06	0.007	
LDL-C/HDL-C ratio				
Before Trans	0.91±0.13	0.66±0.08	0.89±0.09	0.19
After Trans	0.77±0.11	0.48±0.04	0.65±0.15	0.23
P value	0.43	0.08	0.20	

Means±SEM (n=6)

Control basal diet with no supplements, *Cr* control diet+1,200 μg chromium methionine/kg, *Cr+ Vit C* control diet+1,200 μg of chromium methionine/kg+800 mg of vitamin C/kg, *Trans* transport, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

[x,y] Means within the same parameter and column with no common superscripts differ significantly ($P \leq 0.05$)

Cr source may influence the final response of poultry to Cr supplementation (Lindemann 2007; Habibian et al. 2013).

In the present study, neither transport nor dietary treatments had significant effect on the plasma glucose concentration. This finding is similar to that of Kegley et al. (1997) who reported that transport did not change glucose concentration in the control and Cr nicotinic-supplemented steers. Nijdam et al. (2006) also found no significant effect of transport on the concentration of glucose in broilers. However, it has previously been reported that transport stress decreased (Huff et al. 2008; Zhang et al. 2009) or increased (Al-Aqil and Zulkifli 2009; Huff et al. 2010) blood glucose concentration. This is a conventional metabolic relationship between insulin (anabolic) and corticosteroids (catabolic), having opposite influence to each other under high temperatures. High ambient temperatures increased corticosterone concentration but decreased that of insulin in broilers (Sahin et al. 2003b; Dai et al. 2011). The results of the present study demonstrated that transportation under summer conditions decreased the concentration of insulin and therefore increased the ratio of G/I. Similarly, Apple et al. (2005) reported that transport caused a significant decrease in the insulin concentration of control and magnesium-administrated pigs. However, Zhang et al. (2009) found that the concentration of insulin was not changed due to transport treatments in the broilers. It was proposed that Cr may potentiate the action of insulin through

Table 7 Effects of chromium and chromium+vitamin C supplementations on the antioxidant parameters of broilers transported under summer conditions

	Control	Cr	Cr+Vit C	*P* value
MDA, nmol/ml				
Before Trans	0.355±0.034^{y}	0.362±0.016	0.337±0.051	0.30
After Trans	0.522±0.090^{x}	0.403±0.014	0.458±0.047	0.49
P value	0.05	0.09	0.11	
GPx, U/l				
Before Trans	326±21	310±24	357±32	0.46
After Trans	300±36	353±47	373±36	0.43
P value	0.56	0.42	0.74	
FRAP, μmol/l				
Before Trans	314±36^{b}	411±33^{a}	412±11^{a}	0.05
After Trans	273±47	340±45	438±48	0.07
P value	0.50	0.23	1.00	

Means±SEM (n=6)

Control basal diet with no supplements, *Cr* control diet+1,200 μg chromium methionine/kg, *Cr+ Vit C* control diet+1,200 μg of chromium methionine/kg+800 mg of vitamin C/kg, *Trans* transport, *MDA* malondialdehyde, *GPx* glutathione peroxidase, *FRAP* ferric reducing-antioxidant power

[a,b] Means within the same row with no common superscripts differ significantly ($P \leq 0.05$)

[x,y] Means within the same parameter and column with no common superscripts differ significantly ($P \leq 0.05$)

Table 8 Effects of transport and treatments on tonic immobility reaction of broilers

	Tonic immobility	
Group	Duration	Induction
Transported		
Control	269±46.8^{a}	1.65±0.17
Cr	323±55.0^{a}	1.42±0.16
Cr + Vit C	140±40.8^{b}	1.70±0.18
Nontransported control	288±46.5^{a}	1.45±0.11
P value	0.03	0.64

Means±SEM (n=20)

Control basal diet with no supplements, *Cr* control diet+1,200 μg chromium methionine/kg, *Cr+ Vit C* control diet+1,200 μg of chromium methionine/kg+800 mg of vitamin C/kg

[a,b] Means within the same column with no common superscripts differ significantly ($P \leq 0.05$)

increasing the binding activity of insulin-sensitive cell receptors (Anderson 2003). In the present study, pretransport level of insulin was highest in the broilers supplemented with Cr+Vit C. These results are similar to the findings of Habibian et al. (2013) who observed no positive effects of Cr chloride or Cr methionine supplementation on the concentrations of glucose and insulin in HS broilers. However, Sahin et al. (2003b) reported that supplementation of Vit C and Cr picolinate (CrPic) alone or in combination decreased the corticosterone and glucose concentrations and increased the insulin concentration in HS broilers.

The thyroid hormones, T_3 and T_4, regulate body temperature and metabolic homeostasis (Lara and Rostagno 2013). They play an important role in acclimation and are used as indicators of heat stress (Lin et al. 2006). The T_3 concentrations have been observed to decline in heat stress conditions (Lin et al. 2006; Dai et al. 2011; Lara and Rostagno 2013), whereas results of high temperatures on T_4 concentrations are contradictory with studies reporting reduction (Dai et al. 2011), elevation (Lin et al. 2006), or no change (Mack et al. 2013). Lin et al. (2006) exposed broiler chickens to acute heat stress (32 °C for 3 and 6 h). The plasma concentration of T_3 and the ratio of T_3/T_4 were significantly decreased by heat treatment at both time points in comparison with a control treatment of 21 °C, whereas the concentration of T_4 markedly increased after 6 h of heat exposure. As follows from the results, the concentration of T_3 and the ratio of T_3/T_4 were decreased and the concentration of T_4 was not changed by transport. These results were partly similar to those reported by Nijdam et al. (2005) who reported that transport caused a decrease in the levels of T_3 and T_4 in broilers. In another study, Zhong et al. (2011) found no significant effect of transport on the T_3 and T_4 concentrations in sheep. In contrast, Nijdam et al. (2006) reported that transport did not affect the concentration of T_3 in broilers. In the present study, the concentration of T_3 was significantly higher in the Cr+Vit C group than that in the control and Cr groups before the transport. The concentration of T_4 and the ratio of the T_3/T_4 were not changed by dietary treatments. These results are different from the findings of Sahin et al. (2003b) who demonstrated that application of Vit C and CrPic increased the T_3 and T_4 levels in broiler chickens under heat stress. They also observed further improvements in these regards when Vit C and CrPic were supplemented together.

Creatine kinase is an intracellular enzyme whose plasma concentration is frequently used as an indicator of skeletal muscle damage associated with trauma, nutritional myopathies, exercise-induced muscle injury, or congenital myopathies (Hoffmann and Solter 2008). In the present study, transport significantly increased the plasma activity of CK in the Cr+Vit C group. The activity of AST, an indicator of damage to liver and skeletal and cardiac muscles (Hoffmann and Solter 2008), was also increased in the Cr+Vit C group by transport. Similarly, Huff et al. (2008, 2010) reported that transport stress increased the activities of CK and AST in turkeys. These increases in the CK and AST activities suggests that the transported Cr+Vit C group may have experienced some muscle damage and physical stress. In this experiment, transport had no effect on the activity of ALT. The activities of CK, AST, and ALT were unaffected by treatments in this study, which are in agreement with the results of Perai et al. (2014) who reported that supplementation of Cr chloride and Vit C did not change the activities of CK, AST, and ALT in transported broilers. Supplemental Vit C alleviated the negative effect of heat stress on the activity of CK and did not change the activities of AST and ALT in quails (Gursu et al. 2004). In the present study, transport did not obviously change plasma activity of ALP and caused a marked decrease in the plasma phosphorous concentration of the Cr group. These results are partly consistent with the results of Huff et al. (2008, 2010) in turkeys. However, Ajakaiye et al. (2010) found a significant decrease in serum ALP activity in the control and Vit C-treated layer hens transported by road. Results of this study demonstrate that Cr either alone or in combination did not change the plasma activity of ALP and treatment of Cr alone greatly decreased phosphorous concentration after transport termination. Similarly, Imik et al. (2013) reported that neither heat stress nor Vit C affect activity of ALP and concentration of phosphorous.

Transport has previously been shown to result in dehydration and manifested itself as a hyperproteinemia (Minka and Ayo 2012). In the present study, total protein content was increased in the Cr+Vit C-treated broilers by transport which is in line with previous report in turkeys (Huff et al. 2010). This result is partly inconsistent with the results of Ajakaiye et al. (2010) who reported that road transportation increased the total protein concentration in the control and Vit C-treated laying hens. On the other hand, Minka and Ayo (2012) reported no significant change in the total protein concentration of goats administrated with Vit C and transported by road during the hot-dry conditions. Vosmerova et al. (2010) reported that the total protein concentration in broilers was decreased after 70 and 130 km of transport at summer temperatures. The results of this study indicate that the concentrations of albumin and globulin and the ratio of A/G were not significantly changed by transport process. Nevertheless, an insignificant elevation in the albumen concentration was observed in the transported Cr+Vit C broilers. These results favorably compared with those of Zhong et al. (2011) who observed no significant effect of transport on the albumin and globulin concentrations of sheep. In another study, Huff et al. (2008) found that transport stress decreased the concentration of albumin without affecting the concentration of protein in turkeys. Dietary treatments had no significant effects on the plasma total protein, albumin, and globulin concentrations and

on the A/G ratio. Similarly, supplementation with Vit C did not change the concentrations of total protein, albumin, and globulin in broilers reared under heat stress conditions (Imik et al. 2013). In contrast, Gursu et al. (2004) reported that supplemental Vit C increased the total protein and albumin contents in HS quails. In the present study, the concentration of uric acid was not significantly changed by any of the factors studied. Similarly, Nijdam et al. (2006) reported that transport did not change uric acid concentration in broiler chickens. However, Vosmerova et al. (2010) and Huff et al. (2010) reported that concentration of uric acid was significantly increased after transport in broilers and turkeys, respectively. Perai et al. (2014) reported that supplemental Vit C or Cr chloride did not change uric acid concentration in transport-stressed broilers.

The reduction in the triglycerides obtained in the present study is in agreement with the finding of Vosmerova et al. (2010), who found a significant decrease in the plasma triglycerides of broilers transported during summer conditions. They observed no significant effect of transport on triglyceride concentrations at winter and fall temperatures. Nijdam et al. (2005) conducted two experiments to investigate the combined effects of feed withdrawal and transport on the stress and energy metabolism of broilers and concluded that feed withdrawal during transport was main factor caused a significant reduction in triglycerides. In contrast, Nijdam et al. (2006) reported that transport process had no effect on plasma triglycerides in broilers. The concentration of cholesterol was increased and the concentration of LDL-C was decreased in the Cr+Vit C group due to transport. Similarly, Al-Aqil and Zulkifli (2009) reported that level of cholesterol increased in broilers transported for 6 h. However, Huff et al. (2008) reported that transport reduced plasma cholesterol concentration in turkeys. In this study, plasma lipid profile was not affected by dietary treatments. Similarly, Perai et al. (2014) reported that supplemental Vit C and Cr chloride had no significant effect on triglyceride, total cholesterol, LDL-C, and HDL-C concentrations of transported broilers. On the other hand, Gursu et al. (2004) reported that Vit C supplementation reduced serum triglyceride, total cholesterol, and HDL-C concentrations in HS quails. In a previous study, Moeini et al. (2011) reported that Cr supplementation especially in organic form increased serum HDL-C concentration and decreased serum triglyceride, total cholesterol, and LDL-C concentrations in HS broilers.

MDA is the end-product of lipid peroxidation and can used as indicator of oxidative stress (Lin et al. 2006). In this study, oxidative stress arises by increased MDA concentration after the transport in the control group when compared to the pretransit concentration. Treatment with Cr or Cr+Vit C prevented the increase of MDA levels in the Cr and Cr+Vit C groups after transport. These results suggest that lipid peroxidation associated with transport has been prevented via supplementation of either Cr alone or Cr+Vit C combination. It has previously been reported that transport-induced lipid peroxidation in lambs (Zhong et al. 2011), cattle (Chirase et al. 2004; Aktas et al. 2011), and camels (Nazifi et al. 2009). Heat stress and other stressful stimuli present during transport may increase levels of glucocorticoids and adrenaline-induced pathways of aerobic energy production which induce free radical generation as indicated by MDA concentration (Nazifi et al. 2009). No significant effect of treatments was found on the concentration of MDA in this study. In the present study, the GPx activity and total antioxidant power were assayed to determine the responses in enzymatic and non-enzymatic antioxidant systems, respectively. The results obtained in this study indicate that neither transport nor treatments had significant effect on the activity of GPx. FRAP value was not influenced by transport. The results of the present study are partly inconsistent with the findings of Chirase et al. (2004), who reported that transport stress markedly increased serum MDA concentration and lowered serum total antioxidant capacity in beef cattle. The results of this study support earlier results of Lin et al. (2006), who reported that exposing broilers to acute heat stress (32 °C, 6 h) increased plasma MDA concentration and did not change plasma activity of superoxide dismutase (SOD) and level of FRAP. Altan et al. (2003) reported that heat stress (38±1 °C for 3 h at 36 and 37 days of age) caused significant increases in the activities of catalase, GPx, and SOD and concentration of MDA. Following the results, the level of FRAP was higher in the treated groups as compared with that of the control group (before transport $P \leq 0.05$, after transport $P = 0.07$). This increase in FRAP value may be explained by alteration of antioxidant vitamins and minerals status by Cr and Vit C supplementation. Sahin et al. (2003a) reported that heat stress reduced serum vitamins C, E, A, retention of Zn, Cr, and increased MDA concentration in HS Japanese quails and these negative effects were attenuated by supplemental Vit C. Supplementation with Cr has increased concentrations of vitamins E and C and decreased concentration of MDA in HS broilers (Sahin et al. 2003b).

The prolonged or severe fear can markedly reduce poultry's welfare. Tonic immobility was proposed as a useful indicator of general fearfulness in poultry (Jones 1986). It previously has been shown that transport significantly prolonged duration of TI and increased susceptibility to TI reaction (Ghareeb et al. 2008; Minka and Ayo 2010). In the present study, duration of TI was not significantly different between nontransported control chicks and transported chicks without any additives, indicating that transport had no a significant effect on the fear response of broilers. This finding is in agreement with those of Zulkifli et al. (1999) and Prieto and Campo (2010) who reported no significant effect of heat stress on the duration of TI in broiler chickens but disagree with the result previously obtained in broilers

(Altan et al. 2003). Variation in genetic background, age, stress severity, and prior experiences could be responsible for the inconsistency in the results observed (Zulkifli et al. 1999; Prieto and Campo 2010). The results of this study also demonstrate that duration of TI was significantly shorter in the Cr+Vit C-supplemented broilers compared with that of the other groups. It has been reported that Vit C markedly reduced the duration of TI in transported broilers (Zulkifli 2003) and pullets (Minka and Ayo 2010). No significant effect was observed in this study on the number of inductions to induce TI, neither due to transport nor to treatments.

In conclusion, transportation of broilers for 3 h under summer conditions decreased the plasma concentrations of triglyceride, insulin, and T_3 and the ratio of T_3/T_4 and increased the ratio of glucose/insulin. The plasma concentrations of AST, CK, and total protein were increased in the Cr+Vit C group due to transport process. Additionally, plasma concentration of MDA, as an oxidative biomarker, was increased in the control broilers by transport. Supplementation with Cr or Cr+Vit C combination prevented oxidative stress in the transport-stressed broilers and increased plasma total antioxidant capacity. This study also indicated that transportation performed under summer conditions had no significant effect on fear-related behavior of broilers. Cr+Vit C supplementation reduced the duration of tonic immobility in broilers. Further research is warranted to investigate the effectiveness of these supplements under more stressful conditions.

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