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Effects of Supplemental Vitamin C and Chromium on Metabolic and Hormonal Responses, Antioxidant Status, and Tonic Immobility Reactions of Transported Broiler Chickens

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Abstract Three hundred female broilers were assigned to five groups with six replicates and were fed with either a basal diet (two control groups) or the basal diet supplemented with 800-mg vitamin C/kg (Vit C group), 1,200-μg Cr^{+3} from chromium (Cr) chloride/kg (Cr group) or 800-mg Vit C and 1,200-μg Cr^{+3} from Cr chloride/kg (Vit C+Cr group) from 42 to 49 days of age. Treatments did not affect performance. Transport decreased insulin level in the control and Cr groups and increased glucose/insulin (G/I) ratio in the groups. The level of insulin was higher in the Vit C+Cr group than those in the control and Cr groups after the transport. The G/I ratio was lowest in the Vit C+Cr group after the transport. The transport significantly decreased triiodothyronine (T_3) concentration in the groups except the Vit C+Cr group and only increased thyroxin (T_4) concentration in the Vit C+Cr group. The T_3/T_4 ratio was significantly decreased in the groups except the Cr group by transport. The T_3/T_4 ratio was greatest in the Vit C+Cr group before the transport. Alkaline phosphatase activity was decreased in the Vit C+Cr group due to transport. Transport decreased triglyceride levels in the groups and also decreased low-density lipoprotein cholesterol levels in the Vit-C-supplemented groups. Transport increased malondialdehyde concentration in the control and Vit C groups and also increased glutathione peroxidase (GPx) activity in the Cr-fed groups. The GPx activity was higher in the Vit C+Cr group than those in the control and Cr groups after the transport. Ferric reducing/antioxidant power (FRAP) value was decreased in the Vit C and Cr groups by transport. Either alone or in combination, Cr increased the FRAP value before the transport. Neither transport nor treatments had significant effects on the duration of tonic immobility (TI) and number of inductions to induce TI.

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

Introduction

Transportation is an extremely stressful process for commercial broiler chickens, which includes several potential traumatic events (catching, handling, crating, loading, transportation itself, and unloading). The broilers may exposed to a variety of potential stressors, including feed and water deprivation, physical contact with humans, social disruption, motion, vibration, acceleration, daylight, unfamiliar noise, overcrowding, and thermal extremes. These conditions may elicit both stress and fear responses and may result in behavioral changes and physiological changes such as hematological, enzymatic and hormonal, and physical injuries such as bruising, broken bones, and even mortality [1–4]. Vitamin C (Vit C), also known as L-ascorbic acid, has various physiological functions, and many of them are derived from its reducing characteristics that enable it, along with α-tocopherol, to protect the cell from oxidative damages. Thus, Vit C is a primary water-soluble antioxidant in plasma and tissues. Although chickens are able to synthesize adequate amounts of

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Vit C endogenously under normal conditions, this ability is inadequate, or Vit C requirements may be increased under stressful conditions such as low or high ambient temperatures and during transportation [5]. Conducted studies with poultry confirmed that supplementation of Vit C to diet generally had beneficial effects on birds under stressful conditions [5]. However, Lohakare et al. [6] reported that supplemental Vit C improved the performance of broilers reared at commercial-growing conditions. It was proposed that chromium (Cr) may function as part of the oligopeptide low-molecular-weight chromium (LMWCr)-binding substance which can bind to insulin-activated insulin receptors and results in stimulating their tyrosine kinase activity. This process activates the insulin receptor kinase and potentiates the actions of insulin [7]. Responses to supplemental Cr have been contradictory among reports, and the beneficial physiological and production effects of supplemental Cr can be perceived more efficiently under environmental, physiological, and pathological stresses [8, 9]. However, some previous literatures demonstrated that the supplementation of Cr improved growth performance and carcass characteristics [10, 11] and blood lipid profiles [12, 13] of poultry under non-stressful conditions. Recently, superanutritional levels of Cr were proposed to have a pharmacological effect rather than a nutritional effect [14]. Some researchers did not find any positive effect of supplemental Cr on growth performance and carcass traits [15–17] and blood lipid profiles [10, 18] of birds in normal conditions. A number of nutritional relationships exist between Cr and Vit C. Sahin et al. [19] reported that either the supplementation of Cr or Vit C increased the serum concentrations of both Vit C and Cr in laying hens reared at low ambient temperature and also observed a synergistic effect between Vit C and Cr on digestibility of nutrients, serum vitamin E, serum Fe, Zn, and Mn concentrations. It is well documented that Vit C and Cr have antioxidant properties, and previous studies demonstrated that concurrent use of them enhanced antioxidant capacity [16, 19]. Hyperglycemia or decreased insulin level diminished the rate of ascorbic acid uptake into the cells [20, 21]. Through augmenting the effectiveness of insulin, Cr may indirectly improve the intracellular availability of Vit C [22]. Limited research has been conducted to determine the effects of supplemental Vit C on broilers prepared for slaughter. Zulkifli [23] found that supplementation of 1,200 ppm of Vit C for 24 h before transportation reduced heterophil/lymphocyte ratio and tonic immobility (TI) duration in transported broilers without affecting the number of inductions to induce TI. No research has been conducted to determine the effects of supplemental Cr on broilers prepared for slaughter. Thus, the objective of this study was to evaluate the effects of Vit C and Cr supplementation during the last week before slaughter on performance, plasma metabolites, hormonal responses, antioxidant status, and TI reaction of transported broilers.

Materials and Methods

Birds, Management, and Diets

Day-old mix-sexed Ross 308 broiler chickens were purchased from a local hatchery and reared on floor pens covered with sterilized and contaminant-free wood shavings. Light was provided continuously (24 h) throughout the rearing period, and the initial room temperature was set at approximately 32 °C and then gradually reduced based on normal management practices until reaching 21 °C on 28 days of age, which was designated as the thermoneutral zone. All the birds were provided ad libitum access to water and a corn–soybean meal starter diet with 22 % crude protein (CP) and 3,025 kcal/kg metabolizable energy (ME) from 0 to 10 days of age, and a grower diet with 21 % CP and 3,150 kcal/kg ME from 11 to 24 days of age. A finisher diet was fed from 25 to 49 days of age (Table 1). On day 42, 300 female broiler chickens were weighed and allocated randomly to five groups with six replicates of ten birds each. Average body mass was 2,228± 15 g. Birds were fed either on a basal diet (two control groups) or the basal diet supplemented with 800-mg Vit C/kg of diet (Vit C group), 1,200-μg Cr^{+3} from Cr chloride ($CrCl_3.6H_2O$)/kg of diet (Cr group) or 800-mg Vit C and 1,200-μg Cr^{+3} from Cr chloride/kg of diet (Vit C+Cr group) for 1 week. On day 49, all birds (except the nontransported control group) were caught manually and placed in plastic

Table 1 Ingredient and nutrient composition of finisher basal diet

Ingredients (%)	
Corn	61.25
Soybean meal	30.67
Dicalcium phosphate	1.20
Limestone	1.23
Vitamin and mineral premix[a]	0.50
Salt	0.30
Vegetable oil	4.52
DL-Methionine	0.24
L-Lysine HCl	0.10
Calculated analysis	
Metabolizable energy (kcal kg^{-1})	3,200
Crude protein (%)	19
Calcium (%)	0.85
Available phosphorus (%)	0.42
Lysine (%)	1.09
Methionine+cysteine (%)	0.86

[a] Supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D3, 9,790 IU; vitamin E, 121 IU; B12, 20 μg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 μg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg

crates (0.80×0.60×0.32 m). Ten birds from each pen 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 average environmental temperature and relative humidity in the vehicle during transportation were 31.5 °C and 35 %, respectively. After transport, the birds remained in the crates. Feed and water were not deprived until transport, and no feed or water was supplied during the transport and recovery periods. Birds not subjected to the transport remained in their pens, and they had no access to feed and water. This group was only used to measure TI. All animal research procedures were assessed and approved by the Animal Care Committee of Ferdowsi University of Mashhad.

Performance

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

Tonic Immobility

A total of 100 birds (20 chicks per each group) was tested individually for TI immediately after the transport intervention (3-h transportation with 45-min recovery). Chicks were caught randomly, carried in an inverted manner to a separate neighboring room, and subjected to TI measurements. The procedure described by Zulkifli et al. [24] 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 blood samples were drawn from the brachial vein within 30 s and collected into EDTA-treated tubes. The tubes were centrifuged at 1,800 g for 15 min to obtain plasma, which was stored at −20 °C in Eppendorf test tubes until further analysis. The buffy coat was removed, and the erythrocytes were carefully sampled from the bottom of the tubes. They 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 kept at −70 °C for later analysis. Plasma insulin, triiodothyronine (T_3), and thyroxin (T_4) concentrations were measured using radioimmunoassay with commercial kits and an automatic gamma counter (BioSource International, Camarillo, CA, USA). Plasma concentrations of glucose, total protein, albumin, phosphorus, triglyceride, cholesterol, uric acid and enzyme activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and creatine kinase (CK) were measured using the BioSystems kits and associated procedures (BioSystems S.A., Costa Brava 30, 08030 Barcelona, Spain). Plasma high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) concentrations were measured 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 according to Benzie and Strain [25]. Levels of plasma malondialdehyde (MDA), an indicator of lipid peroxidation, were measured by the method of Yoshioka et al. [26]. The principle of the method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA. For this purpose, 2.5 ml of 20 % trichloroacetic acid and 1.0 ml of 0.67 % TBA were added to 0.5-ml plasma in each tube, and the tubes were kept in a boiling water bath for 30 min prior to being rapidly cooled. Subsequently, 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 intensity of the upper *n*-butanol phase was read at 535 nm. Values were compared to a series of standard solutions (1,1,3,3-tetramethoxypropane). Results were expressed as nmol/ml of plasma. The glutathione peroxidase (GPx) activity in erythrocyte hemolysate was measured using a commercially available kit (Ransel test kit, Randox Laboratories Ltd., UK) according to the method of Paglia and Valentine [27].

Statistical Analyses

The assumption of normality of the results was checked by means of a Shapiro–Wilk test, stem-and-leaf plots, and normal probability plots using the SPSS statistical software [28]. The distributions of the plasma ALP, T_3, insulin, MDA concentrations, glucose/insulin (G/I) ratio, T_3/T_4 ratio, and TI were skewed, and appropriate transformation was performed using the statistical package Unistat [29]. The transformed data were used for ANOVA, although actual means were presented in the tables. Data from before and after the transport were analyzed separately by ANOVA using the GLM procedure of SAS software as a completely randomized design [30]. The significance of mean differences was determined using the least squares means. Data from before and after the transport for each group were compared with *t* test if the parametric conditions existed, otherwise, the nonparametric Mann–Whitney test was performed [28]. Data are presented in the tables as means±SEM. Differences were considered significant at $P \leq 0.05$.

Table 2 Effects of supplemental vitamin C and chromium on the performance parameters of broiler chickens from 42 to 49 days of age

	Control	Vit C	Cr	Vit C+Cr	*P* value
Final body mass (g)	2,747±20	2,765±21	2,797±35	2,736±19	0.33
Body mass gain (g/day)	75.8±3.2	77.4±2.5	82±5.1	71.7±2.2	0.24
Feed intake (g/chick/day)	205.7±5.7	205.8±6.0	210.7±6.0	199.8±3.8	0.63
Feed conversion ratio (g/g)	2.73±0.08	2.66±0.05	2.60±0.11	2.79±0.06	0.36

Means±SEM (n=6)

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

Results

Performance

The initial body mass was not different among treatments at 42 days of age. Supplementation of Vit C, Cr, or their combination had no significant effects on the final body mass, body mass gain, feed intake, and feed conversion ratio (Table 2).

Blood Metabolites

Transport significantly increased glucose concentration in the Vit C+Cr group. Plasma glucose concentration was not significantly different among treatments at both sampling times. The level of insulin was significantly decreased in the control and Cr groups by transport. No significant difference was observed in the insulin level among treatments before the

Table 3 Effects of supplemental vitamin C and chromium on glucose and hormone concentrations

	Control	Vit C	Cr	Vit C+Cr	*P* value
Glucose (mg/dl)					
Before trans	333±10	324±11	352±16	311±7^{a}	0.10
After trans	343±12	343±13	318±9	342±7^{b}	0.29
P value	0.54	0.28	0.08	0.01	
Insulin (μIU/ml)					
Before trans	8.37±2.55^{b}	7.03±2.29	9.88±1.65^{b}	13.05±3.39	0.40
After trans	1.73±0.34^{a,c}	1.95±0.33^{c,d}	1.25±0.22^{a,c}	3.62±0.81^{d}	0.02
P value	0.05	0.06	0.004	0.11	
G/I ratio					
Before trans	42.4±9.9^{a}	42.0±9.4^{a}	34.6±5.1^{a}	26.0±8.1^{a}	0.47
After trans	272.6±83.4^{b,d}	224.7±58.6^{b,d}	280.3±31.0^{b,d}	107.5±20.9^{b,c}	0.02
P value	0.006	0.01	0.006	0.008	
T_3 (ng/dl)					
Before trans	101.7±9.5^{b}	118.0±15.6^{b}	110.0±5.8^{b}	127.0±32.2	0.63
After trans	33.2±11.6^{a}	39.5±11.5^{a}	42.7±9.7^{a}	64.2±19.6	0.42
P value	0.001	0.003	0.004	0.07	
T_4 (μg/dl)					
Before trans	0.55±0.08^{c,d}	0.68±0.10^{d}	0.58±0.08^{d}	0.32±0.06^{a,c}	0.03
After trans	0.55±0.14	0.44±0.09	0.37±0.08	0.63±0.10^{b}	0.31
P value	1.00	0.11	0.09	0.02	
T_3/T_4 ratio					
Before trans	0.205±0. 037^{b,c}	0.220±0.041^{b,c}	0.203±0. 022^{c}	0.572±0.141^{b,d}	0.02
After trans	0.078±0.032^{a}	0.072±0.015^{a}	0.209±0.106	0.157±0.090^{a}	0.40
P value	0.03	0.004	0.29	0.03	

Means±SEM (n=6)

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

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

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

transport. However, the insulin level was significantly higher in the Vit C+Cr group when compared with those in the control and Cr groups after the transport. The G/I ratio significantly increased due to the transport process in the groups. This ratio was not significantly different among treatments before the transport. However, this ratio was significantly lower in the Vit C+Cr group compared with those in the other groups after the transport. Transport significantly decreased T_3 concentration in the groups except the Vit C+Cr group. Plasma T_3 concentration was not significantly affected by treatments at any sampling time. Transport significantly increased T_4 level in the Vit C+Cr group. The Vit C+Cr group had lower T_4 concentration compared with the Vit C and Cr groups before the transport. Transport significantly decreased T_3/T_4 ratio in the groups except the Cr group. Before the transport, T_3/T_4 ratio was greater in the Vit C+Cr group than those in the other groups. After the transport, this ratio was not significantly different among the treatments (Table 3).

The level of ALP was significantly decreased in the Vit C+Cr group by transport. This parameter was not significantly different among treatments at both sampling times. The ALT, AST, CK, and phosphorus levels were not significantly affected by any of the factors studied (Table 4).

Neither transport nor treatments had significant effects on plasma protein, albumin, and globulin concentrations, and on albumin/globulin (A/G) ratio. Plasma uric acid concentration was not significantly changed by any of the factors studied (Table 5).

Transport process significantly decreased triglyceride concentrations. The LDL-C level was significantly decreased in the Vit-C-supplemented groups by transport. Treatments had no significant effects on these parameters. No significant effects were observed in this study on cholesterol and HDL-C concentrations and on LDL-C/HDL-C ratio, neither due to transport nor to treatments (Table 6).

Antioxidant Status

Transport significantly increased MDA concentration in the control and Vit C groups. This plasma parameter was not significantly different among treatments at any sampling time. The GPx activity was significantly increased in the Cr-supplemented broilers by transport. The GPx activity was not significantly different among treatments before the transport. However, the GPx activity was significantly higher in the Vit C+Cr group compared with those in the control and Cr

Table 4 Effects of supplemental vitamin C and chromium on enzyme activities and phosphorus concentration

	Control	Vit C	Cr	Vit C+Cr	*P* value
ALP (U/l)					
Before trans	373±105	394±59	791±216	710±170[b]	0.16
After trans	241±65	588±174	336±74	205±35[a]	0.12
P value	0.31	0.52	0.07	0.02	
ALT (U/l)					
Before trans	13.5±0.8	15.3±1.9	11.8±2.4	14.8±2.0	0.54
After trans	15.7±1.3	14.5±1.5	15.5±1.1	15.7±2.0	0.96
P value	0.20	0.76	0.17	0.77	
AST (U/l)					
Before trans	234.0±6.8	261.8±24.4	237.2±23.1	241.7±21.7	0.78
After trans	268.0±13.3	242.6±11.4	266.0±17.6	261.4±20.5	0.69
P value	0.06	0.52	0.34	0.53	
CK (U/l)					
Before trans	8,951±1,676	6,848±1,092	8,686±1,557	6,406±1,451	0.53
After trans	6,830±1,199	8,005±925	8,138±1,556	7,419±932	0.87
P value	0.36	0.44	0.81	0.60	
Phosphorus (mg/dl)					
Before trans	6.65±0.23	6.59±0.30	6.68±0.14	7.28±0.35	0.27
After trans	6.76±0.26	6.40±0.23	6.30±0.21	6.42±0.13	0.49
P value	0.77	0.62	0.19	0.06	

Means±SEM (n=6)

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

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

Table 5 Effects of supplemental vitamin C and chromium on some blood metabolites

	Control	Vit C	Cr	Cr+Vit C	*P* value
Total protein (g/l)					
Before trans	32.8±0.98	33.2±0.98	36.5±1.85	34.5±1.23	0.22
After trans	32.8±2.33	31.7±1.43	31.7±1.52	32.5±2.92	0.97
P value	1.00	0.41	0.08	1.00	
Albumin (g/l)					
Before trans	18.0±0.86	17.2±0.48	17.4±1.57	17.2±0.87	0.91
After trans	18.8±1.89	16.4±0.81	16.0±0.45	17.0±1.81	0.52
P value	0.63	0.42	0.37	0.51	
Globulin (g/l)					
Before trans	14.8±0.60	16.0±0.58	16.8±1.20	17.3±0.56	0.12
After trans	14.0±0.52	15.4±1.12	15.7±1.33	15.5±1.15	0.67
P value	0.32	0.63	0.55	0.25	
A/G ratio					
Before trans	1.15±0.04	1.08±0.03	1.03±0.04	1.03±0.02	0.11
After trans	1.28±0.10	1.08±0.06	1.06±0.09	1.08±0.06	0.20
P value	0.40	0.98	0.78	0.46	
Uric acid (mg/dl)					
Before trans	5.29±0.28	5.24±0.20	5.65±0.44	5.53±0.12	0.62
After trans	4.32±1.05	4.31±0.72	5.06±0.70	4.88±0.81	0.88
P value	0.36	0.71	0.59	0.75	

Means±SEM (n=6)

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

groups after the transport. The FRAP value was significantly decreased in the Vit C and Cr groups by transport, and supplementation of Cr significantly increased FRAP value before the transport. Treatments had no significant effect on this parameter after the transport (Table 7).

Tonic Immobility

Neither transport nor treatments had significant effects on the duration of TI and number of inductions to induce TI (Table 8).

Discussion

The performance parameters were similar among treatments. It indicates that the supplementation of Cr or Vit C independently or in combination was not able to affect the performance of broilers fed with corn–soybean-based diet from 42 to 49 days of age. These results are in agreement with those of Kutlu and Forbes [31], who reported that supplemental Vit C had no effects on body mass gain, feed intake, and feed efficiency of broilers reared at thermoneutral conditions. Data on the effect of Cr on performance parameters have been inconsistent. Similar to the results of this study, Suksombat and Kanchanatawee [32] reported that the addition of Cr to broiler diets in the form of organic or inorganic had no effects on body mass gain, feed intake, and feed conversion ratio. These results are not in accordance with Lien et al. [13], who found that supplemental Cr improved broiler performance. This varying response to Cr supplementation could be due to the Cr status of animals, bioavailability of Cr from common dietary ingredients, bioavailability of supplemental Cr, stress condition, degree of stress, supplemented level, dietary composition, duration of usage of supplemented Cr, and age of animal [8, 9, 33, 34].

According to the results of the present study, plasma glucose level was significantly increased in the Vit C+Cr group by transport. In other dietary treatments, this effect was not present. Similarly, Nijdam et al. [4] and Yalçin et al. [35] found no effect of transport on the blood glucose concentration in broilers. Transport has previously been shown to elicit gluconeogenesis and therefore increased plasma glucose concentration [36]. Treatments did not have any significant effect on glucose concentration in this study and is in agreement with that of Kegley et al. [37], who reported no significant effect of supplemental Cr on the glucose concentration in transported steers. The findings of the present study indicated that transport decreased the insulin level and therefore increased the G/I ratio. On the other hand, Zhang et al. [38]

Table 6 Effects of supplemental vitamin C and chromium on plasma lipid profile

	Control	Vit C	Cr	Cr+Vit C	*P* value
Triglyceride (mg/dl)					
Before trans	125.5±9.7[b]	129.3±10.2[b]	154.5±16.5[b]	131.3±12.1[b]	0.41
After trans	31.5±3.4[a]	42.5±5.3[a]	39.8±4.1[a]	39.8±9.1[a]	0.52
P value	0.0001	0.0001	0.01	0.0001	
Cholesterol (mg/dl)					
Before trans	130.3±7.1	126.8±6.2	132.8±12.7	129.2±7.3	0.97
After trans	125.7±7.4	126.2±5.3	121.7±3.1	127.3±9.3	0.94
P value	0.66	0.94	0.36	0.88	
HDL-C (mg/dl)					
Before trans	32.0±5.3	32.3±8.3	39.8±5.0	35.0±5.3	0.74
After trans	32.7±5.3	39.8±5.3	31.3±3.9	31.3±5.4	0.59
P value	0.93	0.39	0.21	0.47	
LDL-C (mg/dl)					
Before trans	26.3±2.5	25.8±1.5[b]	27.2±4.9	31.7±1.9[b]	0.43
After trans	22.3±1.4	18.8±1.0[a]	20.7±2.0	22.8±2.8[a]	0.42
P value	0.19	0.003	0.22	0.02	
LDL-C/HDL-C ratio					
Before trans	0.91±0.13	0.97±0.19	0.87±0.29	1.01±0.16	0.95
After trans	0.77±0.11	0.52±0.07	0.72±0.10	0.92±0.18	0.14
P value	0.43	0.08	1.00	0.73	

Means±SEM (n=6)

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

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

Table 7 Effects of supplemental vitamin C and chromium on the antioxidant parameters

	Control	Vit C	Cr	Vit C+Cr	*P* value
MDA (nmol/ml)					
Before trans	0.355±0.034[a]	0.322±0.026[a]	0.334±0.034	0.388±0.037	0.54
After trans	0.522±0.090[b]	0.432±0.034[b]	0.390±0.037	0.355±0.013	0.07
P value	0.05	0.03	0.31	0.75	
GPx (U/l)					
Before trans	326±21	348±24	287±28[a]	380±30[a]	0.11
After trans	300±36[c]	438±49[d,e]	381±31b[b,c,d]	540±49[b,e]	0.005
P value	0.56	0.13	0.05	0.02	
FRAP (μmol/l)					
Before trans	314±36[c]	408±41[b,c,d]	471±35[b,d]	425±23[d]	0.03
After trans	273±47	287±23[a]	341±44[a]	363±60	0.46
P value	0.50	0.03	0.04	0.35	

Means±SEM (n=6)

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

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

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

Table 8 Effects of transport and supplemental vitamin C and Chromium on tonic immobility reaction

	Tonic immobility	
Group	Duration	Induction
Transported		
Control	269±46.8	1.65±0.17
Vit C	225±47.8	1.76±0.15
Cr	194±45.4	1.60±0.17
Vit C+Cr	167±26.7	1.79±0.17
Nontransported control	288±46.5	1.45±0.11
P value	0.28	0.64

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

reported that the level of insulin was not significantly affected by transport. The insulin concentration was significantly higher in the Vit C+Cr group than those in the control and Cr groups after the transport, confirming previous results [39]. The G/I ratio is an indirect index of insulin sensitivity. In this study, this ratio was found to be lowest in the Vit C+Cr group after the transport. This suggests that the combination of Vit C and Cr potentiates the action of insulin and results in an increase in glucose uptake. Following the results, transport decreased T_3 concentration in the groups and only increased T_4 concentration in the Vit C+Cr group. The T_3/T_4 ratio was significantly decreased in all dietary groups except the Cr group by transport. In a previous study, Nijdam et al. [40] found that transport decreased the levels of T_3 and T_4 in broilers. However, Nijdam et al. [4] found no significant difference in T_3 concentration of transported and nontransported broilers. In this experiment, treatments did not change the T_3 concentration. Compared with the Vit C and Cr groups, the broilers supplemented with Vit C+Cr had the lower concentration of T_4 before the transport. The Vit C+Cr group had the greatest T_3/T_4 ratio before the transport.

In the current study, the ALP activity was significantly decreased in the Vit C+Cr group by transport. This result is inconsistent with those of Ajakaiye et al. [41], who reported that transport decreased serum ALP level in control and Vit-C-treated layer hens. The present research showed that treatments had no significant effect on ALP activity. The ALT, AST, CK, and phosphorus levels were not significantly changed by any of the factors studied. These results are not in accordance with those of Huff et al. [1], who found that transport stress significantly decreased the level of phosphorus and increased the activities of ALT, AST, and CK in turkeys.

The results obtained in this study suggested that neither transport nor treatments had significant effects on plasma protein, albumin, and globulin concentrations, and on A/G ratio. In a previous study, Huff et al. [1] found that transportation of turkeys for a period of 3 h decreased the albumin concentration without changing the protein concentration. In contrast, Vosmerova et al. [2] reported that total protein concentration was decreased after long transportation distances. Dietary Cr picolinate (CrPic) supplementation had no effects on the total protein and albumin contents of growing turkeys [42]. Samanta et al. [43] reported that the supplementation of Cr from CrPic decreased albumin concentration without affecting the serum total protein and globulin concentrations. In the present study, the concentration of uric acid was not significantly affected either by transport or dietary treatments. Similarly, Nijdam et al. [4] observed no significant effect of transport on plasma uric acid level in broilers. In addition, as in the present experiment, Samanta et al. [43] found no significant effect of supplemental Cr on serum uric acid concentration.

Results from this study showed that triglyceride concentrations decreased due to transport and agree with the results of Vosmerova et al. [2] and Nijdam et al. [40] in broilers. However, Nijdam et al. [4] found no significant effect of transport on triglyceride concentrations in broilers. Transport also decreased the LDL-C levels in the Vit C-treated groups. Treatments had no effects on triglyceride and LDL-C concentrations. The results of the present study indicate that neither transport nor treatments had significant effects on plasma cholesterol and HDL-C concentrations and on LDL-C/HDL-C ratio. These results are in agreement with Samanta et al. [10], who found no effects of supplemental Cr chloride on serum triglyceride and cholesterol levels in broilers. Contrary to the results of this study, Lien et al. [13] found that HDL content was increased, and LDL and VLDL contents were decreased by supplementing CrPic to broiler diet.

The data obtained in this study suggested that transport resulted in a significant increase in lipid peroxidation in the control and Vit C groups. The results also confirmed the previous report that road transportation increased the level of MDA in a 6-month-old sheep [44]. The dietary treatments did not significantly affect the concentration of MDA in this study. However, the supplemented broilers had lower MDA concentration than their control counterparts after the transport ($P<0.07$). In this study, the GPx activity and total antioxidant power were used to determine the responses in enzymatic and nonenzymatic antioxidant systems, respectively. According to the results of this study, GPx activity was significantly increased in the Cr-fed broilers by transport. The Vit C+Cr group had higher GPx activity compared with the control group or Cr group after the transport. The GPx activity was also higher in the Vit C group compared with that in the control group. The FRAP value was decreased in the Vit C and Cr groups by transport in this study. Either in combination or alone, Cr increased the FRAP value before the transport.

The prolonged or severe fear can noticeably reduce welfare. Duration of TI is used as an indicator of fearfulness of birds. The duration of TI was not significantly different between transported chicks without any supplements and non-transported chicks. This result is in accordance with the results of Akşit et al. [45] and Prieto and Campo [46], who found that heat stress did not affect TI duration in broiler chickens. In the present study, dietary supplements had no significant effect on TI duration. However, dietary additives numerically decreased TI duration. In contrast, Zulkifli [23] and Zulkifli et al. [24] found that transport and handling stresses caused an increase in TI duration, and supplemental Vit C markedly reduced TI duration in stressed broilers. Neither transport nor treatments had a significant effect on the number of inductions to induce TI in this experiment.

Conclusion

The results of the present study revealed that dietary supplements especially Vit C+Cr had positive effects on the broiler's stress responses to transport. Further studies are needed to investigate the effects of these supplements on stress responses and meat quality of transported broilers.

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