

Effects of Dietary Supplemental Vitamin E and Chromium on Egg Production, Egg Quality and Blood Parameters of Laying Hens under Thermoneutral or Heat Stressed Conditions

Research Article
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Received on: 26 Aug 2017

Revised on: 3 Nov 2017

Accepted on: 15 Nov 2017

Online Published on: Jun 2018

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 Online version is available on: www.ijas.ir

ABSTRACT

The effects of dietary supplemental vitamin E (VE) and chromium (Cr) on egg production (EP), egg quality, serum concentrations of Cr and insulin and activity of glutathione peroxidase (GSH-Px) were evaluated in Lohmann LSL-Lite laying hens reared under thermoneutral or heat stress (HS) conditions. A total of 144 laying hens were distributed in 24 cages and assigned to feed four diets including two levels (0 and 200 mg/kg) of VE and two levels (0 and 1200 µg/kg) of Cr in a 2 × 2 factorial arrangement with six replicates. The daily temperature in the house was maintained at 17 °C for 10 weeks (30-40 wks of age) and afterward (41-45 wks of age) increased instantly to 32 °C to simulate HS. Decreased feed intake was observed in hens fed the Cr-supplemented diet during HS (P<0.05). Supplemental VE and Cr had no significant effect on egg weight, egg mass, albumen weight, specific gravity, egg shape index, yolk color and index (P>0.05). Increased feed conversion ratio (FCR) and decreased yolk weight and shell thickness were detected in hens fed the VE-supplemental diet before HS (P<0.05). A combination of VE and Cr increased Haugh unit, before and during HS, and also increased EP, yolk and shell weight during HS (P<0.05). No dietary effects (P>0.05) were observed on serum concentrations of Cr and the activity of GSH-Px at 45 wk of age (after exposure to heat stress), however, supplemental VE and Cr increased serum concentration of insulin (P<0.05). In conclusion, dietary supplemental VE in combined with Cr partly alleviated the detrimental effects of HS in laying hens, via improving EP and egg quality.

KEY WORDS chromium, heat stress, insulin, laying hens, performance, vitamin E.

INTRODUCTION

Since the range of thermoneutral temperature in chicken is narrow, they become easily exposed to heat or cold environmental stress. Heat stress in particular has a highly detrimental influence on laying hens, which depresses feed intake (FI), body weight, egg production (EP) and egg quality (Puthongsiriporn *et al.* 2001). It is shown that in poultry under HS situation, serum concentrations of vitamins, minerals, and insulin decrease (Siegel, 1995) and

minerals, and insulin decrease (Siegel, 1995) and mineral excretion increase (Gorman and Balnave, 1994).

In other words, increased requirements for these nutrients or increased marginal vitamin and mineral deficiency will occur during HS (Siegel, 1995; Sahin *et al.* 2002c). In addition, the increased blood levels of corticosterone and catecholamines have been detected in high ambient temperature, which initiated lipid peroxidation in cell membranes and disturbed oxidative stability in birds (Edens and Siegel, 1975; Siegel, 1995). Since the complete control of the poul-

try house condition is not always possible, there is a growing interest in strategies to alleviate the consequences of HS via nutritional manipulation in poultry (Sahin *et al.* 2002a; Rhoads *et al.* 2013). It has been reported that the harmful effects of HS in poultry may be alleviated when the effective vitamins and trace minerals such as VE (Puthongsiriporn *et al.* 2001; Ghazi *et al.* 2012a) and Cr (Onderci *et al.* 2002; Ghazi *et al.* 2012b; Torki *et al.* 2014) were supplemented to diets of laying hens. Diet supplementation with 65 IU of VE increased EP and egg quality of laying hens while decreased thiobarbituric acid values in egg yolk and plasma (Puthongsiriporn *et al.* 2001). The necessity of chromium (Cr³⁺) for animals was first confirmed when dietary supplemental Cr improved glucose tolerance in rats (Vincent and Stallings, 2007). The beneficial effect of Cr on performance of laying hens and egg quality, particularly in those reared under cold or HS conditions, has been shown in several studies (Onderci *et al.* 2002; Sahin *et al.* 2001; Torki *et al.* 2014). Chromium, as an insulin potentiator, is postulated as an antioxidant (Preuss *et al.* 1997); since increased oxidative stress appears to be a deleterious factor leading to insulin resistance and lipid peroxidation and affecting insulin metabolism (Gallaher *et al.* 1993). In previous studies, the combined effects of diet supplementation with Cr along with minerals and vitamins such as zinc (Onderci *et al.* 2002) and vitamin C (Torki *et al.* 2014) in poultry subjected to environmental stresses have been explored. In the study conducted by Sahin and Onderci (2002), diet supplementation with the combined form of vitamin C and Cr had the highest positive effect on the performance and egg quality of laying hens in low ambient temperature. The combined form of diet supplemental VE (250 mg/kg) and Cr (400 mg/kg), rather than the separate form, caused the highest bird performance, EP and serum concentrations of antioxidant vitamins, C and E, while decreased concentration of malondialdehyde (MDA) was detected in laying Japanese quails reared under cold stress condition (Sahin *et al.* 2003). The available information on the interactions between dietary supplemental VE and Cr on laying hens subjected to heat stress is rare. Therefore, the purpose of the present study was to evaluate the effects of dietary supplemental VE and Cr on egg production, egg quality and serum concentrations of Cr, insulin and the activity of GSH-Px in laying hens subjected to HS (32 °C).

MATERIALS AND METHODS

Care of laboratory animals

All experimental protocols adhered to the guidelines approved by the Animal Ethics Committee of Razi University (Kermanshah, Iran) and were in accordance with the guide-

lines on animal welfare (the number of approval letter: AD-197-2014).

Birds, dietary treatments and management

A total of 144 Lohmann LSL-Lite laying hens (30 weeks old) were purchased from a local farm and were randomly allocated in 24 cages (60×50 cm), which arranged in a well-ventilated windowless experimental room. The birds were assigned to receive one of the four dietary treatments with six replicates per treatment with six birds per cage. A corn-soybean meal based diet was formulated to meet nutritional requirements of laying hens as recommended by the Lohmann LSL-Lite catalogue.

The ingredient and calculated composition of the experimental diet are given in Table 1. Based on a 2 × 2 factorial arrangement of treatments, four iso-caloric and iso-nitrogenous diets including two levels (0 and 200 mg/kg) of VE, as DL- α -tocopherol acetate, and two levels (0 and 1200 μ g/kg) of Cr, as chromium methionine or CrMet, were formulated and used. All experimental diets were provided as mash form and all hens were given water *ad libitum* during the 15-week experimental period. The daily temperature in the house prior to the HS (30 to 40 weeks of age) was maintained at 17 °C, (10 weeks) and afterward (41 to 45 weeks of age) was increased to 32 °C, (5 weeks) to simulate HS. The relative humidity inside the house was kept at 45 to 55%, and lighting schedule was 16L:8D during the experimental period.

Production performance

Fresh eggs were collected, counted and weighed per cage during the experiment. Weekly feed consumption was also recorded. Hen-day egg production (HDEP), egg mass (EM, g egg/hen/day) and feed conversion ratio (FCR) (g feed/g egg) were determined according to the follows equations (Mohammadi and Ansari-Pirsarai, 2016):

HDEP= total number of eggs produced during the period / total number of hen-days in the same period × 100

EM= [egg production (%) × egg weight (g)] / 100

FCR= feed intake / egg mass

Egg quality parameters

Samples of 12 eggs were randomly collected from each treatment (3 eggs per each replicate cage) on 36 (before heat treatment) and 44 (after heat treatment) week of age to determine egg quality parameters. Different parts of eggs including yolk, albumen and shell were weighed after breaking the eggs gently on a flat surface. Shell weight was determined after cleaning eggshell from the adhering albumen and drying them to a constant weight at room temperature.

Table 1 Composition and calculated analysis of the experimental diet (as-fed basis)

Ingredients	Percentage
Maize	64.68
Soybean meal	22.91
Soybean oil	0.69
Oyster shell	8.78
Dicalcium phosphate	1.39
Common salt	0.34
Vitamin premix ¹	0.50
Mineral premix ²	0.50
DL-methionine	0.21
Nutrient composition	
Metabolizable energy (kcal/kg)	2720
Crude protein (%)	16.82
Lysine (%)	0.59
Methionine + cysteine (%)	0.46
Calcium (%)	3.73
Nonphytate phosphorus (%)	0.38
Chromium (mg/kg)	5.43
Zinc (mg/kg)	84.00

¹ Vitamin mixture per 2.5 kg of diet provides the following: vitamin A: 7700000 IU; vitamin D₃: 3300000 IU; vitamin E: 6600 mg; vitamin K₃: 550 mg; vitamin B₆: 4400 mg; vitamin B₁₂: 8.8 mg; Thiamine: 2200 mg; Riboflavin: 4400 mg; Ca pantothenate: 550 mg; Nicotinic acid: 200 mg; Folic acid: 110 mg; Choline chloride: 275000 mg; Biotin: 55 mg and.

² Mineral mixture per 2.5 kg of diet provides the following: Mn: 66000 mg; Zn: 66000 mg; Fe: 33000 mg; Cu: 8800 mg; Se: 300 mg and I: 900 mg.

The eggshell thickness was determined by using a micrometer (0.01 mm) on three pieces of eggshell (air cell, equator, and sharp end). The specific gravity of the sample eggs were measured using 11 gradient saline solutions ranging from 1.060 to 1.100 at 0.005-unit increments (Holder and Bradford, 1979). After determining the length and width of the eggs with a micrometer, egg shape index was calculated using the equation:

$$\text{Egg shape index (\%)} = 100 \times (\text{egg width/egg length})$$

The albumen heights were measured using a micrometer, and Haugh unit (HU) were calculated using the following equation described by Eisen *et al.* (1962):

$$\text{HU} = 100 \times \text{Log} (\text{H} - 1.7 \times \text{W}^{0.37} + 7.57)$$

Where:

H: albumen height.

W: egg weight.

The yolk color was evaluated visually by means of the usual La Roche scale, which ranges from pale yellow at score 1 to dark orange at score 15 (Vuilleumier, 1969) and the yolk index was determined as the ratio of yolk height to yolk width.

Serum biological parameters

Four birds from each treatment (one per each replicate cage) were randomly selected on week 36 (before heat stress) and 44 (after heat treatment) of age and blood sam-

posable syringe. Sera were collected after centrifugation at 3000 rpm (1008 g) for 10 min, and then stored at -20 °C until further analysis. The serum concentrations of insulin were measured using commercially available kits (Pars Azmoon®, Tehran, Iran), according to colorimetric enzymatic method. The activity of GSH-Px in serum was measured based on the method described by Paglia and Valentine (1967).

The serum concentration of Cr was determined using an atomic absorption spectrophotometer (Perkin Elmer HGA 500) with a graphite furnace atomizer in deuterium background correction method.

Statistical analysis

The data of 2 × 2 factorial arrangement of treatments based on a completely randomized design using the GLM procedure of SAS were analyzed (SAS, 2003). Significant differences among treatments were determined at P < 0.05 and the mean values were compared by Duncan's multiple range test. Analysis of variance was conducted according to the following model:

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \mu_{ijk}$$

Where:

Y_{ijk}: measured parameter.

μ: overall mean.

A_i: main effect of VE.

B_j: main effect of dietary Cr.

(AB)_{ij}: interaction between VE and Cr.

μ_{ijk}: effect of experimental error.

RESULTS AND DISCUSSION

Production performance

The effects of dietary supplemental VE (0 or 200 mg/kg) and Cr (0 or 1200 µg/kg) on FI, FCR and EP before (30 to 40 weeks of age) and during (40 to 45 weeks of age) HS exposure are presented in Table 2. As it is summarized in Table 4, since significant interactions between dietary VE and Cr on egg production, yolk weight, shell weight, HU and serum concentration of insulin were detected, the mean comparison of interactions has been done without considering the main effects of experimental factors, dietary supplemental VE and Cr.

Significant interaction ($P < 0.05$) between dietary supplemental VE and Cr on EP of laying hens was detected during HS (Table 4).

Based on the literature, diet supplementation with vitamins, such as VE (Puthongsiriporn *et al.* 2001; Ghazi *et al.* 2012a), and minerals, such as Cr (Ghazi *et al.* 2012b; Onderci *et al.* 2002; Torki *et al.* 2014), alleviated the adverse effects of HS on performance of laying hens. In the present study, the lowest and highest EP figures were observed in the control and the Cr-supplemented group, respectively. Ma W *et al.* (2014) indicated that Cr addition (400 µg/kg) significantly increased egg production in 60-weeks old laying hens.

Mirfendereski and Jahanian (2015) showed that CrMet adding significantly increased egg production. Attia *et al.* (2016) indicated that VE addition may increase laying rate in under chronic heat stress laying hens. The improving effect of dietary supplemental VE (Puthongsiriporn *et al.* 2001; Sahin *et al.* 2002e; Sahin *et al.* 2003) and Cr (Sahin *et al.* 2002b; Sahin *et al.* 2003; Torki *et al.* 2014) on EP of laying hens subjected to HS has been demonstrated.

Sahin *et al.* (2002b) reported that diet supplementation with 400 µg chromium picolinate and 250 mg vitamin C per kg diet increased egg weight and EP of laying hens subjected to cold stress (6 °C) compared to those reared at normal temperature (18 °C). No significant effect of dietary supplemental vitamin C (250 mg/kg) or chromium picolinate (400 µg/kg) on EP and EM was observed by Torki *et al.* (2014).

In the present experiment, there was no interaction between supplemental VE and Cr on FI of laying hens. Supplemental VE had no significant effect on FI of laying hens either before or after exposure to HS, which is in agreement with the study conducted by Puthongsiriporn *et al.* (2001). According to the Mirfendereski and Jahanian (2015) result, CrMet adding significantly increased FI in 26-week laying hens. The addition of Cr to the diet decreased FI of laying hens exposed to HS.

Sahin *et al.* (2002b) reported no significant effect of dietary supplemental Cr and vitamin C on FI of laying hens. It seems that the reduced FI as a result of supplemental Cr might be in part due to the increased digestibility of nutrients. Increased FI of HS-exposed laying hens fed diets supplemented with both Cr (400 µg/kg) and vitamin C (250 mg/kg) were reported by Torki *et al.* (2014). Sahin *et al.* (2003) demonstrated no significant effect of dietary supplemental VE and Cr on FI of laying Japanese quails exposed to cold stress. Dietary supplemental VE increased FCR of laying hens before HS ($P < 0.05$), but there was no significant effect of supplemental Cr on FCR of laying hens before or during HS. The insignificant effect of Cr on FCR in the present study is in harmony with the result of previous report (Mirfendereski and Jahanian, 2015). No significant interaction ($P > 0.05$) between dietary supplemental VE and Cr on FCR was found before or during HS. Supplemental Cr had no significant effect on FCR, which is in agreement with Torki *et al.* (2014), who indicated no effect of dietary supplemental Cr and vitamin C on FCR in heat-stressed laying hens. Improved digestibility of nutrients and feed efficiency has been reported in laying hens fed the Cr-supplemented diets (Onderci *et al.* 2002; Sahin and Sahin, 2002). Improved FCR in laying Japanese quails fed the diet supplemented with the combined form of VE and Cr was reported by Sahin *et al.* (2003). Ipek *et al.* (2007) found the positive effect of dietary supplemental VE (240 mg/kg) and vitamin C (240 mg/kg) on Japanese quails. Abbaszadeh Mobaraki and Shahryar (2015) did find no significant effect of 80 or 160 mg VE/kg plus 0.2 or 0.4 mg selenium/kg on FCR of breeding Japanese quail. No significant effect of dietary VE on FCR of laying hens was reported (Heydari *et al.* 2009). In the present study, laying hens fed on the diet supplemented with VE had higher FCR values compared to the control group before exposing to HS.

Egg quality parameters

The effects of dietary supplemental VE (0 or 200 mg/kg) and Cr (0 or 1200 µg/kg) on egg quality parameters before (30 to 40 weeks of age) and during (40 to 45 weeks of age) HS exposure are presented in Table 3. There was no significant effect of dietary supplemental VE and Cr on albumen weight, specific gravity, egg shape index and yolk index in laying hens before or during exposing to HS. Adding VE to diet decreased ($P < 0.05$) egg yolk weight of laying hens compared to other dietary groups prior to HS. During HS, there was no significant effect of dietary supplemental VE and Cr on egg yolk weight. Supplemental VE numerically decreased egg yolk weight. Significant interaction between VE and Cr on egg yolk weight was detected ($P < 0.05$).

Table 2 Effects of dietary supplemental vitamin E and chromium on production performance in laying hens before (36 week of age) and after (44 week of age) exposure to heat stress*

Items	Egg production (%)		Egg weight (g)		Egg mass (g/hen/day)		Feed intake (g)		Feed conversion ratio (g feed/g egg)	
	Before	During	Before	During	Before	During	Before	During	Before	During
Exposure to heat stress										
Vitamin E (mg/kg)										
0	98.0	95.4	59.4	59.2	58.2	56.5	109.8	108.5	1.88	1.92
200	97.6	96.6	59.2	58.7	57.5	56.9	109.9	109.5	1.90	1.92
Chromium (µg/kg)										
0	97.4	95.8	58.9	58.6	57.2	56.3	109.8	109.3	1.92	1.94
1200	98.2	96.2	59.9	59.3	58.5	57.0	109.9	108.9	1.86	1.90
Pooled SEM	0.23	0.4	0.28	0.28	0.37	0.39	0.06	0.29	0.011	0.013
Sources of variation	P-value									
Vitamin E	0.08	0.78	0.13	0.31	0.10	0.41	0.60	0.58	0.03	0.17
chromium	0.34	0.06	0.78	0.43	0.29	0.64	0.70	0.02	0.55	0.90
Vitamin E × chromium	0.28	0.03	0.68	0.71	0.76	0.05	0.49	0.89	0.91	0.08

* Duncan's multiple-range test were applied to compare means.
SEM: standard error of the means.

The hens received only VE or chromium (treatments 2 and 3) and the hens received the control diet significantly produced heavier egg yolks compared to the hens received VE and Cr simultaneously (treatment 4). Dietary Cr had no significant effect on egg yolk weight of laying hens (Piva *et al.* 2003). Sirirat *et al.* (2013) showed that laying hens fed diets supplemented with nano sized chromium picolinate (500 and 3000 µg/kg) had lower egg yolk weight than control group.

There was no significant effect of dietary treatments on albumen weight in laying hens before and during exposing to HS. Improving effect of dietary supplemental Cr on HU and albumen quality in laying hens is shown (Jensen *et al.* 1978). In the current study, both Cr and VE increased HU unit of the eggs in laying hens before or during HS. The lowest and the highest HU before exposing to HS were observed in hens fed the control and the VE-supplemented diet, respectively. Sahin *et al.* (2003) also observed the highest HU unit in the eggs of cold-stressed laying Japanese quails fed the diets supplemented with the combined form of VE and Cr. Reduced HU unit was detected in heat-exposed birds was partly due to lower protein synthesis and/or movement of water from the albumen to yolk (Ahmad *et al.* 1967). The results of the current study revealed synergetic interaction between dietary supplemental VE and Cr on the HU unit.

In the present study, no significant effect of diets on egg shell weight of the laying hens before exposing to HS was observed, but significant interaction between VE and Cr ($P < 0.05$) during HS was detected. The lowest and highest shell weight were observed in the treatments 3 (only 200 VE) and 4 (both VE and Cr), respectively.

The laying hens fed the VE-supplemented diets before HS had thinner ($P < 0.05$) egg shell compared to the hens fed the Cr-supplemented or control diets. There was no significant effect of diet on egg shell thickness of the laying hens during HS.

Torki *et al.* (2014) reported higher egg shell weight and thickness in the heat-stressed hens given either 250 mg/kg vitamin C or 400 µg/kg chromium picolinate compared to control birds.

Supplemental VE significantly decreased ($P < 0.05$) the color score of egg yolk in laying hens before exposing to HS. Yolk colors were not affected by the dietary treatments during HS, which is in contrast with the previous studies (Kirunda *et al.* 2001; Puthongsiriporn *et al.* 2001; Torki *et al.* 2014). Kirunda *et al.* (2001) reported the lighter-yellow egg yolk color in exposed hens to HS when fed high doses compared to a low dose of VE; but egg yolk color of those reared at normal temperature were not influenced by dietary supplemental VE. An inconsistency among various studies may be attributed to some other factors such as stress condition, quantity and type of cereal grain in basal diet, bioavailability of the supplemental VE, interaction with other fat soluble vitamins, and presence of synthetic antioxidant in the diet.

Serum parameters

The effects of dietary supplemental VE and Cr on the serum concentrations of Cr, insulin, and GSH-Px activity in laying hens subjected to HS (44 week of age) are presented in Table 5. Adding VE and Cr to diet had no significant effect on the serum concentration of Cr and GSH-Px activity in laying hens during HS.

Table 3 Effects of dietary supplemental vitamin E and chromium on egg quality parameters in laying hens before (36 week of age) and after (44 week of age) exposure to heat stress*

Items	Yolk weight (g)		Albumen weight (g)		Shell weight (%)		Shell thickness (mm×10 ⁻²)		Specific gravity		Egg shape index		Haugh unit		Yolk color (Roche)		Yolk index		
	Before	During	Before	During	Before	Before	Before	During	Before	During	Before	During	Before	During	Before	During	Before	During	
Exposure to heat stress																			
Vitamin E (mg/kg)																			
0	17.0	17.6	34.4	35.2	5.91	5.67	0.37	0.35	1.08	1.08	74.0	72.5	83.1	85.6	7.13	6.07	41.1	37.3	
200	16.4	17.6	34.1	34.3	5.78	5.72	0.36	0.35	1.08	1.08	73.6	73.8	98.7	100.3	7.00	6.10	41.3	38.2	
Chromium (µg/kg)																			
0	16.8	17.7	34.2	34.5	5.79	5.57	0.37	0.34	1.08	1.08	74.0	73.3	83.0	85.1	7.10	6.11	41.4	38.3	
1200	16.6	17.5	34.2	35.0	5.89	5.68	0.37	0.35	1.08	1.08	73.6	72.9	98.9	100.6	7.20	6.06	41.0	37.3	
Pooled SEM	0.10	0.12	0.34	0.50	0.041	0.060	0.002	0.002	0.001	0.001	0.23	0.32	3.15	3.03	0.09	0.07	0.20	0.266	
Sources of variation	P-value																		
Vitamin E	0.01	0.97	0.74	0.41	0.11	0.25	0.01	0.84	0.62	0.42	0.41	0.06	0.01	0.01	0.03	0.82	0.65	0.07	
Chromium	0.21	0.73	0.91	0.41	0.21	0.28	0.46	0.12	0.20	0.73	0.46	0.50	0.01	0.01	0.91	0.82	0.26	0.07	
Vitamin E × chromium	0.15	0.01	0.22	0.86	0.46	0.04	0.46	0.12	0.62	0.89	0.32	0.81	0.01	0.01	0.06	0.28	0.15	0.67	

* Duncan's multiple-range test were applied to compare means.
SEM: standard error of the means.

Table 4 Interaction effects of dietary supplementation of vitamin E and chromium on egg production (EP), yolk weight (YW), shell weight (SW), Haugh unit (HU) and serum concentration of insulin in laying hens before and / or after exposure to heat stress (HS)*

Factors	After HS		Before HS	After HS	After HS			
	Vitamin E (mg/kg)	Chromium (µg/kg)	EP (%)	YW (g)	SW (g)	HU	HU	Serum insulin (IU/mL)
0	0		94.32 ^c	17.29 ^a	5.52 ^b	67.24 ^b	70.16 ^b	9.25 ^c
0	1200		97.73 ^a	18.11 ^a	5.63 ^{ab}	98.79 ^a	100.10 ^a	27.79 ^a
200	0		96.66 ^{ab}	18.01 ^a	5.88 ^a	99.14 ^a	101.17 ^a	16.55 ^b
200	1200		95.74 ^b	17.18 ^b	5.51 ^b	98.79 ^a	100.15 ^a	8.72 ^c

* Duncan's multiple-range test were applied to compare means.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 5 Effects of dietary supplementation of vitamin E and chromium on serum concentrations of chromium, glutathione peroxidase activity and insulin in laying hens after exposure to heat stress (44 week of age)

Items	Chromium (µg/mL)	Insulin (IU/mL)	Glutathione peroxidase (U/g protein)
Vitamin E (mg/kg)			
0	1.04	12.3	912
200	1.15	16.9	830
Chromium (µg/kg)			
0	1.03	12.0	804
1200	1.16	17.2	938
Pooled SEM	0.102	2.24	92.9
Sources of variation	P-value		
Vitamin E	0.63	0.02	0.69
Chromium	0.56	0.01	0.51
Vitamin E × chromium	0.98	0.01	0.70

SEM: standard error of the means.

Based on the literature, dietary supplemental Cr resulted in increased serum concentration of Cr in laying hens (Sahin and Sahin, 2002; Sirirat *et al.* 2013; Torki *et al.* 2014), broilers (Ghazi *et al.* 2012b; Habibian *et al.* 2013) and laying Japanese quails (Sahin *et al.* 2003). Numerous factors such as supplementary level, duration of usage, bioavailability, interaction with the basal diet ingredients

and stress condition may influence the efficacy of a dietary supplement.

In the current study, significant interaction between VE and Cr on the serum concentration of insulin was observed (P<0.05). The relationship between Cr and insulin has been also reported in other studies (Sahin *et al.* 2002d; Sahin *et al.* 2002e).

Yardibi and Tuerkay (2008) showed that vitamin E may alleviate some of the metabolic consequences in heat stressed laying hens. The deficiency of vitamin E may promote susceptibility to dietary and environmental stresses (Tengerdy, 1989). Vitamin E is a potent biological chain-breaking antioxidant in the first line of defense against cellular damage caused by reactive oxygen species or free radical initiators; so it can protect cells and tissue against lipid oxidation (Halliwell and Gutteridge, 1989; McDowell, 1989). Vitamin E had no effect on insulin-stimulated glucose transport in cultured rat muscle cells, but in oxidative stressed cells, prevented oxidative stress-induced insulin resistance through improving the free radical defense system (Vinayaga Moorthi *et al.* 2006). Protective effect of plasma VE concentration against diabetes incidence among adults has been shown as evident by improved insulin sensitivity, which is associated with pancreatic hepatocellular function for insulin resistance (Doisy, 1978). It has been shown that birds are insulin responsive, however, there are probable alternative glucose transporters in avian species involving in these effects (Sweazea and Braun, 2006; Tokushima *et al.* 2005). Insulin increases the uptake of certain substances (most prominently glucose and amino acid) into muscle cells to regulate energy production, muscle tissue deposition, fat metabolism, and cholesterol utilization (Anderson *et al.* 1988). Body cells are unable to utilize free glucose, in such circumstance free glucose is converted into fat and deposit in fat cells. Moreover, inadequate amino acid entry into cells due to a low insulin level reduces muscle growth to a greater extent than fat growth (Anderson *et al.* 1988). In addition of the role of Cr in protein metabolism (NRC, 1997), it has been suggested that Cr is biologically active part of a Cr-binding protein biomolecule (chromodulin, part of insulin signaling pathway) which facilitates glucose uptake via increasing the sensitivity of cell receptors to insulin (Vincent, 2000). Disrupted carbohydrate and protein metabolism, reduced insulin sensitivity in peripheral tissues and impaired growth rate can also be caused by Cr deficiency (Doisy, 1978; Pagan *et al.* 1995). Urinary excretion of Cr increased during stressful or disease conditions and caused a marginal Cr deficiency in poultry (Anderson *et al.* 1988; Sahin *et al.* 2002c).

In the present study, dietary supplemental VE and Cr had no significant effect on GSH-Px activity in serum of the HS-exposed laying hens. The effect of VE and Cr on GSH-Px activity has not been reported earlier. The increased production of free radicals (Halliwell and Gutteridge, 1989) and decreased serum concentration of antioxidants, vitamin C, E and A, in stressful condition has been already reported (Klasing, 1998). Endogenous GSH-Px was recognized as the second defense line against cellular peroxidation damage due to inability of VE to destroy all metabolic perox-

ides (McDowell, 1992). GSH-Px activity was not affected by supplemental Cr; however antioxidant activity of Cr has been previously reported (Onderci *et al.* 2002; Preuss *et al.* 1997).

The results of the current study showed that supplemental VE and Cr had no significant effect on GSH-Px activity. This may show the mechanisms by which these supplements increase the antioxidant capacity of birds apart from GSH-Px activity.

CONCLUSION

Overall, based on the results of the current study, dietary supplemental VE and Cr, separately or together, increased egg production, shell weight, HU unit and serum concentration of insulin in laying hens after exposing to HS which has a potential ability to reduce the negative effects of HS in laying hens.

ACKNOWLEDGEMENT

This study was financed by Razi University.

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