



Effect of Zinc Sulfate and Organic Chromium Supplementation on the Performance, Meat Quality and Immune Response of Japanese Quails under Heat Stress Conditions

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Abstract

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This study was conducted to investigate the effects of different levels of zinc sulfate and chromium picolinate on the performance, immune response and meat quality of Japanese quails under heat stress condition. The birds (n = 540; 7-d-old) were randomly assigned to 9 treatment groups consisting of 3 replicates of 20 birds each in a 3 × 3 factorial arrangement of treatments [zinc (0, 40, 80 mg/Kg); chromium (0, 500, 1000 µg/Kg)]. Birds were kept in floor cages in a temperature controlled room and subjected to heat stress for 8 h/d (9.00 to 17.00) from 7 d to the end of the study. Zn was supplemented to the basal diet as ZnSO₄ and Cr as chromium picolinate. A linear decrease in feed intake and an improvement in feed conversion ratio were found by supplementing Zn and Cr to the quails diets. Chromium supplementation increased daily weight gain linearly. By increasing the level of dietary Zn and Cr in the diet, heterophil to lymphocyte ratio and meat quality parameters were improved linearly. The results of this study revealed positive effects of Zn and Cr supplementation on the performance, immune responses and meat quality of quails under heat stress condition. It seems that supplementation of the quail's diets with 80 mg/Kg Zn and 1000 µg/Kg Cr can be greatly helpful for improving the adverse effects of heat stress.

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Introduction

When birds are exposed to a high ambient temperature, a series of consecutive events appears in their body that finally damages their products (Niu *et al.*, 2009). Ward and Peterson (1973) perceived that plasma level of zinc was lower in broilers exposed to high ambient temperatures (33-35°C) rather than those reared under the thermoneutral condition (18-22°C). Since the production of free radicals escalates under heat stress conditions (Mujahid *et al.*, 2005), it seems to be beneficial to use elements in the poultry diets that somehow take part in clearance of the oxidant agents. One of the major functions of zinc is participation in the antioxidant defense system of the body (Prasad and Kucuk, 2002). The mechanism whereby zinc plays the role is not well recognized yet, but it is thought that Zn increases synthesis of metallothionein protein as the scavenger of free radicals (Oteiza *et al.*, 1996). In pancreas, Zn plays a protective role against oxidative damages and aids this organ to secrete digestive enzymes, which leads to the increased nutrients digestibility (Sahin *et al.*, 2009). Under the heat stress conditions, excretion of zinc from the body is intense (Belay and Teeter, 1996). Zinc influences utilization of feed via participating in the metabolism of carbohydrates, fats and proteins advantageously (McDonald, 2011). Zinc is an important element in all aspects of immunity (Chandra and Dayton, 1982; Sherman, 1992) and is essential for integrated cellular immunity response (Dardenne *et al.*, 1985). Zinc deficiency decreases the cellular immunity (Prasad and Kucuk, 2002) and adversely affects functions of the thymus (Fraker *et al.*, 1977) and spleen (Luecke *et al.*, 1978) and interleukin-2 production (Dowd *et al.*, 1986).

Conflicting reports have been published regarding the effect of zinc on growth performance of poultry under the heat stress conditions. Bartlett and Smith (2003) report that different dietary levels of zinc have no effects on the performance and plasma concentration of zinc in broilers under the heat stress conditions while Sahin *et al.* (2005) stated that the performance and carcass quality of quails fed a zinc supplemented diet were improved under the heat stress condition.

Insulin, a hormone that plays an important role in metabolism of nutrients requires chromium (Cr) to do its function properly. Chromium participates in the structure of an organometallic molecule called the glucose tolerance factor (GTF) that stimulates insulin receptors to pair with this hormone (Mertz, 1993). Many other functions have been reported for chromium such as participation in the metabolism of lipids, proteins and nucleic acids. It has been established that chromium shows anti-oxidative properties from itself in the body (Tezuka *et al.*, 1991). Furthermore, chromium improves health and subsequent performances via aiding the immune system (Mallard and Borgs, 1997). During the occurrence of environmental stresses, elevation of plasma level of corticosterone intensifies free radicals production that leads to suppression of the immune system (McIntosh and Sapolsky, 1996). It has been shown that supplementation of laying hens diet with Cr decreases plasma level of corticosterone under the cold stress conditions (Sahin

et al., 2001; Hanafy, 2011). Chromium is not a particularly toxic element in its trivalent form, and a wide margin of safety exists between the normal amounts ingested and those likely to produce deleterious effects (McDonald *et al.*, 2011). Stress depletes the body stores of chromium and increases the need for this element (Hayirli, 2005). Heterophile to lymphocyte ratio as a sensitive and important index for a variety of stresses (Gross and Sigel, 1983; Mashaly *et al.*, 2004) increases during heat stress (McFarlane and Curtis, 1989; Mashaly *et al.*, 2004). Adding chromium as a supplement to the diet of broilers that were reared under the heat stress condition decreased this ratio (Ebrahimzadeh *et al.*, 2012). Finally, some reports exist that confirm the positive effects of chromium on digestibility of nutrients in animals (Kim *et al.*, 1997; Sahin *et al.*, 2001; Onderci *et al.*, 2003; Emami *et al.*, 2012).

This experiment was conducted to investigate the effects of different levels of zinc sulfate and chromium picolinate on performance, immune response and meat quality of Japanese quails under the heat stress conditions.

Materials and Methods

Birds, treatments and management

Five hundred of forty 7-day-old Japanese quails (mixed sex) were housed in floor cages in a temperature controlled room and subjected to heat stress (to reach panting) for 8 h/d (0900 to 1700 h) from 7 d to the end of the study (35 d). Birds fed a corn-soybean meal basal diet (control) or the basal diet supplemented with either Zn as ZnSO₄ or Cr as chromium picolinate (CrPic).

The birds were randomly assigned to 9 treatment groups consisting of 3 replicates of 20 birds each in a 3 × 3 factorial arrangement of treatments [zinc (0, 40, 80 mg/Kg); chromium (0, 500, 1000 µg/Kg)]. The basal diet was formulated to meet the NRC requirements (NRC, 1994) of meat Japanese quails as shown in Table 1. Feed (in mash form) and fresh water were offered *ad libitum* throughout the experiment.

Sample and data collection

Growth performances (feed intake, body weight gain and feed conversion ratio) were recorded weekly. On days 35 of the experimental period, blood samples (via cervical cutting) of one male bird per cage (three birds per treatment) was collected and transferred to tubes containing EDTA as an anticoagulant. Blood films were made, air dried and then stained with Giemsa - Wright's stain. Differential white blood cell (WBC) counts were performed by using standard avian guidelines of Ritchie *et al.* (1994).

After slaughtering (on 35 d), breast and thigh muscles were obtained and kept frozen at -21°C for 40 days. Breast muscle lipid peroxidation was assessed as thiobarbituric acid-reactive substance concentrations in samples by the method of Tarladgis *et al.* (1960). Values were reported as the concentration of malondialdehyde

(MDA). Meat pH was measured by the method of Parizadian *et al.* (2011). The samples of breast muscles were used for MDA and pH analyses. Water holding capacity (WHC), driploss (DL) and cooking loss (CL) tests were done for thigh samples, using Grau and Hamm (1953) procedure.

Table 1. Ingredients and composition of the basal diet

Ingredients	Amount (g/Kg)
Corn	457.0
Soyabean meal (44% CP)	472.6
Soybean oil	40.0
Oyster shell	13.0
Dicalcium phosphate	7.5
Salt	3.6
DL-Methionine	1.3
Vitamin premix ¹	2.5
Mineral premix ²	2.5
<i>Chemical composition</i>	
ME (Kcal/Kg)	2937
Crude protein (g/Kg)	242.8
Lysine (g/Kg)	13.9
Methionine (g/Kg)	5.03
Methionine + Cystine (g/Kg)	8.97
Calcium (g/Kg)	8.07
Available Phosphorus (g/Kg)	3.06
Sodium (g/Kg)	1.54

¹provided the following per Kg of diet: retinol acetate 3.1 mg, thiamine 1.8 mg, riboflavin 6.6 mg, niacin 30 mg, pantothenic acid 10 mg, pyridoxine 3 mg, folic acid 1 mg, cyanocobalamine 15 µg, biotin 0.1 mg, cholecalciferol 0.05 mg, alpha-tocopherol acetate 18 mg, menadion 2 mg, choline chloride 0.4 g.

²provided the following per Kg of diet; Fe 50 mg, Mn 100 mg, Zn 85mg, Cu 10 mg, Se 0.2 mg and I 1 mg.

Statistical analyses

The data were analyzed using the GLM procedure of SAS software (SAS Institute, 2008). Duncan multiple range test was used to detect ($P<0.05$) differences among treatment means. Linear and quadratic polynomial contrasts were used to evaluate the effect of different levels of zinc and chromium.

Results

Performance parameters

The effects of supplemental Zn and Cr on growth performance of Japanese quails are shown in Table 2. Our results showed that Zn supplementation decreased feed intake (FI) quadratically and improved feed conversion ratio (FCR)

linearly but had no significant effect on body weight gain (BWG). Cr supplementation decreased FI and FCR while increased BWG linearly.

A significant interaction between Zn and Cr was observed for FI and FCR. Chicks fed diet contained 1000 µg Cr and 40 mg Zn per Kg, showed the lowest FI and FCR. In general, dietary combined supplementation of Zn and Cr decreased FI and FCR to a greater degree compared to supplementation with Zn and Cr separately.

White blood cells and immunity organs

The effect of Zn supplementation on heterophile, lymphocyte and heterophile/lymphocyte ratio was significant ($P<0.01$, Table 3). As the dietary level of Zn increased, the percentages of heterophile and heterophile/lymphocyte ratio were decreased and the percentage of lymphocyte increased, linearly. The percentages of monocyte, eosinophil and basophil were not affected by different levels of Zn. The relative weight of bursa, spleen and thymus did not show any significant responses to Zn supplementation.

In the present study, the percentages of heterophile, lymphocyte and heterophile/lymphocyte ratio were significantly affected by Cr ($P<0.01$). The relative weight of the thymus was also positively affected by Cr supplementation ($P<0.05$). The percentage of heterophile and heterophile/lymphocyte ratio decreased linearly but the percentage of lymphocyte increased linearly as the dietary level of Cr increased. The percentages of monocyte, eosinophil and basophil and relative weight of the spleen were not affected by Cr. Supplementation.

The interaction between Zn and Cr was significant for heterophile, eosinophil and basophil ($P<0.01$). The lowest percentage of heterophile was pertained to the birds that were supplemented with a combination of 80 mg/Kg Zn and 1000 µg/Kg Cr, whereas the highest rate was related to the control treatment. The highest percentage of eosinophil was observed in the birds that were supplemented with a combination of 40 mg/Kg Zn and 500 µg/Kg Cr, whereas the birds that were supplemented only by 500 µg/Kg Cr had the lowest percentage of eosinophil. Supplementation of quails with a combination of 80 mg/Kg Zn and 500 µg/Kg Cr or a combination of 40 mg/Kg Zn and 1000 µg/Kg Cr caused the highest percentage of basophil.

Meat quality

Some meat related quality parameters are shown in Table 4. The effect of Zn supplementation on drip loss (DL), cooking loss (CL), water holding capacity (WHC), pH and malondialdehyde value (MDAV) of meat were significant ($P<0.01$). As dietary levels of Zn increased, DL, CL and MDAV levels of the meat decreased and WHC and pH increased linearly.

The effect of Cr was significant on all of the meat quality parameters (DL, CL, WHC, pH and MDAV) ($P<0.01$). As the dietary level of Cr increased, DL, CL and MDAV level of the meat decreased and WHC and pH increased linearly. A significant interaction was observed for MDAV ($P<0.01$). The highest level of MDAV was related to the birds fed with control diet and the lowest level was related to birds that were supplemented with a combination of 80 mg/Kg Zn and 1000 µg/Kg Cr.

Table 2. Effects of zinc sulfate and chromium picolinate on the performance of Japanese quails reared under the heat stress condition

Treatments		FI ¹ (g/b/d)	BWG ² (g/b/d)	FCR ³
Chromium (µg/Kg)	Zinc (mg/Kg)			
0	0	21.83 ^a	6.80	3.21 ^a
	40	18.75 ^c	6.62	2.84 ^b
	80	19.67 ^b	6.93	2.84 ^b
500	0	19.35 ^b	6.89	2.81 ^{bc}
	40	18.39 ^{cd}	6.68	2.75 ^{bcd}
	80	18.32 ^d	6.78	2.70 ^{cde}
1000	0	18.30 ^d	6.94	2.64 ^{de}
	40	18.14 ^d	6.94	2.62 ^e
	80	18.54 ^{cd}	7.04	2.63 ^{de}
SEM		0.219	0.0363	0.0355
Main effect means				
Zinc:				
0		19.83 ^a	6.88	2.88 ^a
40		18.43 ^c	6.75	2.74 ^b
80		18.84 ^b	6.92	2.72 ^b
Chromium:				
0		20.08 ^a	6.79 ^b	2.96 ^a
500		18.69 ^b	6.78 ^b	2.75 ^b
1000		18.33 ^c	6.98 ^a	2.63 ^c
<i>Probabilities</i>				
Source of variation				
Zinc effect		<0.01	0.0963	<0.01
Chromium effect		<0.01	0.0338	<0.01
Zinc × Chromium effect		<0.01	0.5213	<0.01
Polynomial contrast				
Linear zinc		<0.01	0.5986	<0.01
Quadratic zinc		<0.01	0.0372	0.0216
Linear chromium		<0.01	0.0234	<0.01
Quadratic chromium		<0.01	0.1652	0.1558

¹Feed intake, ²Body weight gain, ³Feed conversion ratio.

^{a-c} Means with different superscripts in the same column differ significantly ($P<0.05$).

Table 3. Immune parameters of Japanese quails supplemented with different levels of zinc sulfate and chromium picolinate reared under the heat stress condition

Treatments		Hetero	Lymphoc	H/L ¹	Mono	Eosino	Basop	Bursa ²	Spleen ²	Thymus ²
Chromium ($\mu\text{g}/\text{Kg}$)	Zinc (mg/Kg)	phile (%)	yte (%)		cyte (%)	phil (%)	hil (%)			
0	0	43.00 ^a	48.00	0.900	3.67	3.67 ^{ab}	1.67 ^{ab}	0.0733	0.0563	0.112
	40	42.67 ^a	50.67	0.843	3.33	2.33 ^{bc}	1.00 ^{abc}	0.0830	0.1063	0.155
	80	42.00 ^a	51.33	0.817	4.00	2.33 ^{bc}	0.33 ^c	0.0887	0.0667	0.178
500	0	41.67 ^a	52.67	0.790	2.33	2.00 ^c	1.33 ^{abc}	0.0807	0.0663	0.154
	40	39.67 ^b	51.00	0.777	3.67	5.00 ^a	0.67 ^{bc}	0.1097	0.0877	0.190
	80	38.67 ^{bc}	54.00	0.720	3.00	2.33 ^{bc}	2.00 ^a	0.0957	0.0873	0.208
1000	0	37.67 ^c	55.67	0.677	3.67	2.33 ^{bc}	0.67 ^{bc}	0.0823	0.0883	0.202
	40	34.67 ^d	57.33	0.607	3.67	2.33 ^{bc}	2.00 ^a	0.1117	0.0933	0.235
	80	32.00 ^e	59.67	0.537	3.67	3.00 ^{bc}	1.67 ^{ab}	0.1313	0.0943	0.244
SEM		0.719	0.706	0.0220	0.163	0.220	0.147	0.00528	0.00576	0.0110
Main effect means										
Zinc:										
0		40.78 ^a	52.11 ^b	0.789 ^a	3.22	2.67	1.22	0.0788	0.0703	0.156
40		39.00 ^b	53.00 ^b	0.742 ^b	3.56	3.22	1.22	0.1014	0.0958	0.193
80		37.56 ^c	55.00 ^a	0.691 ^c	3.56	2.56	1.33	0.1052	0.0828	0.210
Chromium:										
0		42.56 ^a	50.00 ^c	0.853 ^a	3.67	2.78	1.00	0.0817	0.0764	0.148 ^b
500		40.00 ^b	52.56 ^b	0.762 ^b	3.00	3.11	1.33	0.0953	0.0804	0.184 ^{ab}
1000		34.78 ^c	57.56 ^a	0.607 ^c	3.67	2.56	1.44	0.1084	0.0920	0.227 ^a
<i>Probabilities</i>										
Source of variation:										
Zinc effect		<0.01	0.0004	<0.01	0.6302	0.2068	0.8954	0.0759	0.2401	0.0848
Chromium effect		<0.01	<0.01	<0.01	0.1792	0.3686	0.2619	0.1005	0.5474	0.0109
Zinc × Chromium effect		0.0086	0.0611	0.0945	0.4596	0.0015	0.0027	0.6380	0.6741	0.9956
Polynomial contrast:										
Linear zinc		<0.01	0.0001	<0.01	0.4103	0.7761	0.6879	0.0365	0.4011	0.0320
Quadratic zinc		0.6701	0.2896	0.8250	0.6324	0.0833	0.8163	0.3638	0.1425	0.6085
Linear chromium		<0.01	<0.01	<0.01	1.0000	0.5709	0.1198	0.0345	0.2966	0.0031
Quadratic chromium		0.0028	0.0274	0.0044	0.0674	0.1991	0.6430	0.9784	0.7665	0.8611

^{a-c} Means with different superscripts in the same column differ significantly ($P < 0.05$).

¹ Heterophile/Lymphocyte ratio; ² Values are expressed as a percentage of live body weight.

Table 4. Meat quality parameters of Japanese quails supplemented with different levels of zinc sulfate and chromium picolinate reared under the heat stress condition

Treatments		DL ¹ (%)	CL ² (%)	WHC ³ (%)	pH	MDAV ⁴ (mg/Kg)
Chromium (µg/Kg)	Zinc (mg/Kg)					
0	0	5.07	25.85	60.03	4.94	0.3447 ^a
	40	3.87	24.56	62.62	5.04	0.2880 ^b
	80	3.19	23.58	62.58	5.29	0.2007 ^c
500	0	3.22	23.63	62.60	5.29	0.2027 ^c
	40	2.98	22.91	64.29	5.35	0.1460 ^d
	80	2.68	22.32	65.40	5.41	0.1177 ^{de}
1000	0	2.68	22.40	66.66	5.54	0.1190 ^{de}
	40	2.22	21.88	67.28	5.70	0.1110 ^{de}
	80	1.88	21.45	68.83	5.80	0.0907 ^e
SEM		0.187	0.280	0.534	0.0543	0.0165
Main effect means						
Zinc:						
0		3.66 ^a	23.96 ^a	63.10 ^c	5.25 ^c	0.2221 ^a
40		3.02 ^b	23.11 ^b	64.73 ^b	5.36 ^b	0.1817 ^b
80		2.58 ^c	22.45 ^b	65.60 ^a	5.50 ^a	0.1363 ^c
Chromium:						
0		4.04 ^a	24.66 ^a	61.74 ^c	5.09 ^c	0.2778 ^a
500		2.96 ^b	22.95 ^b	64.10 ^b	5.35 ^b	0.1554 ^b
1000		2.26 ^c	21.91 ^c	67.59 ^a	5.68 ^a	0.1069 ^c
<i>Probabilities</i>						
Source of variation						
Zinc effect		0.0002	0.0008	<0.01	0.0002	<0.01
Chromium effect		<0.01	<0.01	<0.01	<0.01	<0.01
Zinc × Chromium effect		0.1056	0.5766	0.3004	0.2873	0.0019
Polynomial contrast						
Linear zinc		<0.01	0.0002	<0.01	<0.01	<0.01
Quadratic zinc		0.5789	0.7550	0.2678	0.7340	0.7724
Linear chromium		<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic chromium		0.2838	0.2494	0.1037	0.3886	0.0003

¹Drip loss; ²Cooking loss; ³Water holding capacity; ⁴Malondialdehyde.

^{a-c} Means with different superscripts in the same column differ significantly ($P < 0.05$).

Discussion

Performance parameters

Dietary Zn and Cr supplementation under the heat stress condition improved growth performance of Japanese quails. The proven role of Zn and Cr in metabolism of carbohydrates, lipids, proteins and nucleic acids is in accrediting of this argument. Although the exact biochemical mechanism of how high dietary Zn decreases feed intake and subsequent growth is not clear yet, however the concentration of the element in brain does not appear to be a factor (Sandoval *et al.*, 1998). Reduced feed intake could be associated with losing of appetite (Brink *et al.*, 1950) or reduced palatability of the diets containing high levels of Zn (Fox, 1989) as

well. Although there are no reports about the negative effect of high levels of chromium picolinate on the palatability of diet, but it can be considered in the investigation of the reasons for reduced FI. Zn plays a key role in cell division and proliferation (Rubin, 1972; Rubin and Koide, 1973) and activity of more than 300 enzymes in the body (Prasad and Kucuk, 2002). In the heat stress condition, the need for Zn intensifies, so, feed efficiency and growth performance will decline if this requirement is not to be supplied (Ensminger *et al.*, 1989). Furthermore, the proven role of Zn in increasing nutrients digestibility under the heat and cold stress (Onderci *et al.*, 2003; Sahin and Kucuk, 2003b) can play an important role in utilization of the consumed feed.

Improved performance parameters such as FCR in Cr supplemented quails can be related to Cr anti-stress properties and its booster effects on insulin, leading to more efficient utilization of nutrients and improved FCR. In addition, Cr increased nutrients digestibility by playing an antioxidant role in pancreas that results in decreased FCR. Cr also has an internal interplay with DNA templates which leads to stimulation of the RNA synthesis (Okada *et al.*, 1983).

The weight gain of the birds is mainly related to muscle tissue and most of this tissue is protein. Due to the proven role of RNAs in protein synthesis, the role of Cr in nucleic acids metabolism also can be another reason for the improved feed conversion ratio in Cr supplemented birds (Okada *et al.*, 1983). According to our findings, it seems that Cr has been more effective than Zn in improving FCR. Bartlett and Smith (2003) investigated the effects of diets with different levels (34, 68 and 181 mg/Kg) of Zn on the performance of broilers either in a thermoneutral or heat stress condition and observed no improvement in this parameters. Sahin *et al.* (2005) used different levels (30 and 60 mg/Kg) of zinc sulfate and zinc picolinate in the diet of quails reared under the heat stress condition. They found an improvement in FI, BWG and FCR in Zn supplemented quails. Uyanik *et al.* (2005) reported that supplementation of the diet with 20, 40, 80 or 100 mg/Kg CrCl₃ had no effect on performance of Japanese quails but in a newer study that was conducted by Sahin *et al.* (2010), supplementation of diet with 400 and 800 µg/Kg CrCl₃ and CrPic improved the performance of Japanese quails under the heat stress condition.

White blood cells and immunity organs

Namra *et al.* (2008) reported that dietary supplementation of quails with 50 mg/Kg Zn, in organic and inorganic forms, had no effect on the relative weights of spleen and bursa. Bartlett and Smith (2003) reported that supplementation of broiler chicks with Zn did not affect the relative weights of bursa, spleen and thymus but improved the primary and secondary antibody titers. Furthermore, the percentage of macrophages was increased by Zn supplementation. Another study that was conducted by Bun *et al.* (2011) showed that dietary supplementation of Zn to broilers infected with *Eimeria tenella* led to an improved immune response. Zn

supplementation of broilers increased lymphocyte and proliferation in visceral blood (Yang *et al.*, 2011).

Heterophile/lymphocyte ratio is considered as a sensitive and important index for many of the stresses such as heat stress in chicks (Gross and Sigel, 1983; Mashaly *et al.*, 2004). Under the heat stress condition H/L ratio increases that demonstrates the relationship between heat stress and non-specific immune cells (McFarlane and Curtis, 1989; Mashaly *et al.*, 2004). Zn contributes to non-covalent reactions of cytoplasmic components by tyrosine kinase that is an essential protein in early stages of lymphocytes activity (Walsh *et al.*, 1994). Moreover, Zn is an essential co-factor for thymulin that is a thymic hormone binds to surface receptors of lymphocyte T and leads to maturation and activation of these cells. Zn binds to thymulin by asparagine side chains and hydroxyl groups. It results in a structural change in thymulin and activates it (Dardenne *et al.*, 1982). Therefore, supplementation of the diet with Zn may increase thymuline activity that leads to a more appropriate maturation and activity of T lymphocytes. In humans and animals, in both laboratory and field conditions, thymuline activity depends on plasma level of Zn (Dardenne *et al.*, 1982; Prasad *et al.*, 1988). Thymuline is present in the serum of zinc-deficient animals but is not active (Prasad *et al.*, 1988). Thus, due to the increased T and B lymphocytes, the immune response of bird improves (Hudson *et al.*, 2005). Zn also participates in lymphocyte cycle. It has been shown that Zn is required for G₁ phase in thymidine kinase expression and for transitional stage into S-phase (Chesters *et al.*, 1993).

Ebrahimzadeh *et al.* (2012) used 200, 400 and 800 µg/Kg Cr in the diet of broiler chicks that were reared under the heat stress conditions (33±2°C) and studied the immune response of these birds. The antibody titers against Newcastle disease and infectious bronchitis viruses in Cr supplemented birds on days 21 and 42 were higher than those fed control diet with no added Cr. Cr supplementation also decreased plasma level of cortisol. The chicks supplemented with Cr at level of 800 µg/Kg, had a higher percentage of lymphocyte and a lower H/L ratio than the control group. Also, Bahrami *et al.* (2012) showed that Cr supplementation improved the immune response of broilers under the heat stress condition. Cr supplemented chicks had a higher antibody titer against Newcastle disease virus and a lower H/L ratio than those fed the control diet on days 18 and 30. No report is available about the effect of Cr supplementation on the immune response of quails.

The increase in the number of lymphocytes T and B in Cr supplemented birds can be attributed to Cr participation in RNA synthesis (Okada *et al.*, 1983), nuclear protein synthesis (Weser and Koolman, 1970) and DNA stability (Okada *et al.*, 1981). Another reason can be the increased glucose availability for lymphocytes as an energy source that is attributed to the improved insulin function (van Heugten and Spears, 1997). Gross and Sigel (1983) observed a greater number of

heterophiles in the blood of quails that were fed with corticosterone than those fed control diet. The increased percentage of lymphocyte and decreased H/L ratio in the heat stressed quails may be attributed to the decreased glucocorticoid secretion. The exact mechanism whereby Cr will strengthen the immune system is not yet known. Cortisol is the most important glucocorticoid that its role in suppression of immune system and prevention of white blood cells production and activity is proven (Roth and Kaeberle, 1982; Munck *et al.*, 1984).

Meat quality

Liu *et al.* (2011) reported a significant improvement in meat quality (redness value of breast muscle and pH of thigh muscle) in broiler chicks supplemented with different levels of Zn. Shear force of thigh muscle and drip loss of breast and thigh muscles were also decreased. The results of the study conducted by Yang *et al.* (2011) also show that supplementation of broiler chicks with Zn increases WHC of breast muscle.

Vakili and Rashidi (2011) used a combination of 50 mg Zn and 100 mg vitamin E per Kg of broiler diet under the heat stress condition and reported a decrease in thigh muscle level of MDAV. Sahin *et al.* (2005) used Zn supplementation in both inorganic (sulfate) and organic (picolinate) form at the levels of 30 and 60 mg/Kg in the diet of Japanese quails under the thermoneutral and heat stress conditions and reported that both forms of Zn supplementation decreased serum and liver levels of MDAV linearly, although ZnSO₄ did not have any effects on serum and liver MDAV under the thermoneutral condition. Zn positively affects meat quality by playing an antioxidant role. Heat stress causes free radicals production (Halliwell and Gutteridge, 1989) and decreases serum density of antioxidant vitamins (A, C and E) and minerals (Zn, Cr) (Feenster, 1985; Sahin and Kucuk, 2003a). Zn deficiency increases lipids peroxidation but this process can be inhibited by dietary Zn supplementation (Shaheen and Abd El-Fattah, 1995). In general, the antioxidant activity mechanism of Zn is divided to acute and chronic effects. The acute effects included long-term access of animal to Zn that induces synthesis of end antioxidants such as metallothionein. The interaction of Zn and vitamin E is one of the proposed mechanisms, so that in Zn-deficient animals, deficiency of vitamin E is also observed (Kim *et al.*, 1998; Salgueiro *et al.*, 2000). Zn deficiency causes oxidative damages through impressing the free radicals activity (Garfinkel, 1986; Powell *et al.*, 1994; Salgueiro *et al.*, 2000). Kim *et al.* (1998) reported that malabsorption of fat-soluble vitamins such as A and E, is probably due to the faulty construction of chylomicrons in the intestinal cells during the Zn deficiency period. Therefore, some of the oxidative damages in Zn-deficient animals may be related to the deficiency of vitamin E. In many cases, adding vitamin E has overcome some of these shortcomings. In the present study, Zn supplementation may be stimulating metallothionein production that leads to scavenging of free radicals. Improved WHC may be associated with the improved antioxidants

activity such as superoxide dismutase enzyme that benefits of Zn in its structure (Liu *et al.*, 2011).

Sahin *et al.* (2010) used Cr at the levels of 400 and 800 µg/Kg in the diet of Japanese quails under the heat stress condition and reported a decrease in MDA level of serum and liver of Cr supplemented birds. Hanafy (2011) reported that Cr supplementation of cocks at the level of 1500 µg/Kg decreased MDA level of semen in the winter season. It is proven that Cr reinforces the antioxidant defense system of the body (Preuss *et al.*, 1997). Decreased MDA level is probably due to the prevention of epinephrine production that occurs as the result of Cr effect on insulin and prevention of lipolysis process (Linder, 1991). Improvement of the other meat quality parameters is also probably due to the antioxidant effects of Cr, although researches conducted in this regard are extremely rare.

The results of this study revealed positive effects of Zn and Cr supplementation on the performance, immune responses and meat quality of quails under the heat stress condition. It seems that supplementation of the quails diets with 80 mg/Kg Zn and 1000 µg/Kg Cr can be greatly helpful for improving the adverse effects of heat stress.

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