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Synergistic Ameliorative Effects of Organic Chromium and Selenium Against Heat Stress in Japanese Quails: Performance, Immunological, Hematological, Biochemical And Antioxidant Studies

Doaa H. Abdelhady[1], Moshira A. El-Abasy[2], Mustafa S. Atta[3], Emad W. Ghazy[1,] Tarek K. Abuzed[4], Amira M. El-Moslemany[5]

[1]Department of Clinical Pathology, Faculty of Veterinary Medicine, Kafrelsheikh University. [2]Department of Poultry Diseases, Faculty of Veterinary Medicine, Kafrelsheikh University.[3] Department of physiology, Faculty of Veterinary Medicine, Kafrelsheikh University.[4] Department of Biochemistry,Faculty of Veterinary Medicine, Kafrelsheikh University. [5]Nutrition and Food Science Department, Faculty of Home Economics, Al-Azhar University, Egypt

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Correspondence to:
mostafa_shukry2002 @yahoo.com

ABSTRACT

This study conducted to clarify the effect of dietary supplementation of organic chromium (Cr), selenium (Se) and their combination on performance, immune responses, hematological and biochemical parameters as well as antioxidant capacity of Japanese quails *(Coturnix japonica)* under heat stress. A 90-day trial conducted using seventy-five, 10-day- old Japanese quails that randomly divided into 5 groups of 15 birds each. The 1st group received basal diet and kept in a temperature controlled room at 24°C. The other four groups reared under natural summer months and kept in a room temperature ranged from (25.3°C to 36.7 °C). The 2ndgroup kept under heat stress condition and fed basal diet only. The third, fourth and fifth groups fed basal diet supplemented by chromium (1400 µg/kg of diet), selenium (0.3 mg/kg of diet) and their combination respectively. In heat-stressed quail group, the data of growth performance revealed a significantly ($P<0.05$) reduced body weight gain (BWG), total feed intake (TFI) and a significantly ($P<0.05$) increased feed conversion ratio (FCR). A significantly ($P<0.05$) reduced phagocytic activity (PA), phagocytic index (PI) and the antibody titer against Newcastle disease virus as well as high mortality rate. a macrocytic hypochromic anemia and stress picture of leukogram. A significant ($P<0.05$) reduction in total proteins, albumin and thyroid hormones concentrations together with a significantly ($P<0.05$) elevated activity of serum AST, LDH, raised total cholesterol, triglycerides and glucose concentrations. Moreover, a significant ($P<0.05$) increase for lipid peroxidation marker (MDA) and a significant ($P<0.05$) reduction of glutathione peroxidase (GSH-Px), and total anti-oxidant capacity (TAC). However, simultaneous dietary supplementation with organic Cr and/or Se alleviated heat stress adverse effects. It could be concluded that combined dietary supplementation of organic Cr and Se can be considered to be a protective management in a quail diet, reducing the negative effects of heat stress.

1. INTRODUCTION

Global warming which can lead to heat stress (HS) considers one of the most important challenges facing the poultry industry affecting the performance of the birds (Gregory,2010).High ambient temperature not only affects performance parameters but various physiological (Star et al., 2008). Heat stress causes an increase in free radicals by starting lipid peroxidation in cell membranes and the release of stress hormones that directly influence glucose and lipid metabolisms as well as protein catabolism (Hosseini-Mansoub et al., 2010; Imik et al., 2013).Therefore, poultry production suffers significant economic losses every year because of heat stress such as high morbidity and mortality,

reduced growth rate and immune suppression (Utomo et al., 1994) and (Star et al., 2007).

Chromium is a trace mineral element, which has a positive effect in poultry nutrition. Recently, there has been considerable research interest in the utilization of organic chromium. Chromium is not a particularly toxic element, and a wide margin of safety exists between the normal amounts ingested and those likely to produce harmful effects (McDonald, 2002). Stress depletes the body stores of essential trace element and increases the need for them (Hayirli, 2005). It has been established that chromium shows anti oxidative properties and improves health and subsequent performances via aiding the immune system (Tezuka et al., 1991; Mallard and Borgs, 1997).

Selenium (Se) is an essential trace element, which serves a wide variety of functions. It is critical to the normal physiology of a wide range of species, including birds. It possesses critical roles in immune function, growth, productivity and anti-stress. It plays an important role in elevating production of immunoglobulin to Newcastle disease (ND)(Hegazy and Adachi, 2000; Hoffmann, 2007) . Se research has attracted tremendous interest because its main functions as an antioxidant action involved in protection against damage caused by free radicals and oxidative stress. Se considered an important antioxidant because it is critical to the formation of the glutathione peroxidase (GPx). Se exerts its physiological activity in the form of selenoproteins, such as GPx, superoxide dismutase (SOD), glutathione reductase (GR), selenoprotein P, and selenoprotein W (Jayaprakash and Marshall 2011).

Consequently, preventing and mitigating the heat stress (HS) against high summer temperature is becoming increasingly important in the poultry industry. Therefore, the current study conducted to assess whether chromium picolinate and/or organic selenium could attenuate the deleterious effect of heat stress on growth performance of Japanese quails with particular emphasis focused on hematological, biochemical and immunological changes associated with heat stress in Japanese quails.

2. MATERIALS AND METHODS

2.1. Trace elements:

Organic chromium (Cr) as (chromium picolinate) and organic selenium (Se) (Sel plex) purchased from a local commercial market, Cairo, Egypt.

2.2. Birds, treatments and management:

Seventy-five quails 10-day- old Japanese quails (*Coturnix coturnix japonica*) of similar body weight (150-200 gm). Birds were weighed and kept on floors pens covered with wood shavings and fed a balanced commercial ration (Council, 1994).The basal diet formulated according to nutrient requirements of poultry (NRC, 1994). Composition and chemical calculated analysis of the experimental diets are shown in Table (1). The control group was kept in a temperature controlled room at 24°C for 24 h/day, while the other quails were kept under local summer season condition of Kafrelsheikh Governorate for 12 weeks from July 1st to 30th of September. Quails randomly assigned into five groups of 15 birds each. The first group fed basal diet and kept as control normal. The second group kept as heat stressed control group. Third, fourth and fifth groups fed basal diet supplemented with 1400 µg /kg organic Cr, 0.3 mg organic Se and their combination respectively. All birds vaccinated against Newcastle disease on the 14th day of age. Ethics of Animal Use in Research committee in Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt, permitted the current study

2.3. Ambient temperature and relative humidity:

Daily ambient temperature (AT) and relative humidity (RH) were recorded inside the quails house using electronic digital thermo-hygrometer three times per day (at 8 a.m,12 PM and 4 PM) and twice at night (at 12 AM and 4 AM) during the experimental period. Additionally, means of maximum and minimum AT and RH recorded monthly (Table 2).

2.4. Body temperature:

Body temperature was measured (2 times per week) by using digital thermometer (± 0.1°C), after inserting the thermometer probe 3 cm into the cloacae and digital thermometer was stabilized 5 minutes before recording the reading (Deeb and Cahaner, 1999) (Table.6).

2.5. Samples collection and parameters measured:

Blood samples **were** collected from the wing vein of the birds at the end of the experiment and used for further biochemical analysis.

2.6. Evaluated Parameters

2.4.1. Performance parameters:

Birds **were** weighed at 10-day- old and after 12 weeks. Feed residues and average feed intake (FI) (g) **were** recorded weekly. The average weekly body weight gain (BWG) (g) and feed conversion ratio (FCR) **were** calculated over this period (12weeks). The body weight gain was determined as the difference between the weights of birds at the beginning and the end of experiment. Feed conversion rate (FCR) was determined as feed intake (FI) divided by body weight gain (BWG) (Brody and Lardy, 1946)

2.4.2. Immunological Parameters:

Including determination of phagocytic activity of heterophils and phagocytic index were performed according to the method described by (Wilkinson, 1981)**.**

AS well as estimation of humeral immunity by using HI test against ND by using the standard microplate system described by(King and Seal, 1998)

2.4.3. Hematological Parameters:

Packed cell volume (PCV), hemoglobin (Hb), red blood cell count (RBCs), total leucocyte count (TLC) and differential leukocyte count were assessed according to the routine avian hematological procedures(Jain, 1986)**.**

2.4.4. Blood Biochemical Parameters:

Serum samples were analyzed for enzymatic activities of aspartate amino transferase (AST) and lactate dehydrogenase (LDH), total cholesterol (TC), triglycerides (TG),glucose ,total proteins (TP) and albumin (Alb) while thyroid hormones (T3 and T4) concentrations were analyzed by using radioimmunoassay kits as described by (MIYAI et al., 1975). All these parameters were analyzed using spectrophotometer and commercial test kits of Randox (Antrim, UK) and following the manufacturer's instructions. Globulin concentration (Glob) in serum computed by subtracting albumin concentration from total protein and consequently albumin to globulin ratio (A/G) was calculated.

2.4.5. Oxidative stress and antioxidant biomarker:

Malonaldahyde (MDA) was determined according to the method adapted by (Esterbauer et al., 1982) , total anti-oxidants activity was determined according to (Koracevic et al., 2001) and glutathione peroxidase (GSH-Px) was assessed according to Paglia and Valentine (1967) . All these parameters were analyzed using spectrophotometer and commercial test kits of BOIDIAGNOSTICS and following the manufacturer's instructions.

2.7. Statistical Analysis:

The data were presented as mean ± standard error (SE) and were subjected to statistical analysis using one-way analysis of variance (ANOVA) according to (Snedecor, 1980). Differences at $p < 0.05$ considered significant.

3. RESULTS

3.1. Growth Performance:

The effects of heat stress and preventive effect of dietary supplementation of organic Cr and/or Se on growth performance and mortality of Japanese quails are shown in Table (3) .According to the result of this table ,heat stress group showed a significantly ($P<0.05$) reduced body weight gain (BWG), total feed intake (TFI) and a significantly ($P<0.05$) increased feed conversion ratio (FCR) and increased the mortality rate compared to control group .While Cr and/or Se supplementation significantly ($P<0.05$) increased the body weight gain (BWG),total feed intake (TFI) ,improved feed conversion ratio (FCR) and decreased the mortality rate, when compared with heat-stressed quails. The combined supplementation of Cr and Se revealed the greatest improvement than separate supplementation with Cr or Se.

3.3. Immune responses:

The deleterious effects of heat stress and the ameliorative effects of Cr and/or Se on immune responses are shown in (Figure.1) implicated that heat stressed quails showed immunosuppressive effect through a significantly ($P<0.05$) decreased phagocytic activity of heterophils (PA), phagocytic index (PI) and the antibody titer against NDV compared to control group . While Cr and/or Se simultaneous feeding resulted in a significantly ($P<0.05$) enhanced phagocytic activity, phagocytic index, the antibody titer against NDV, compared to heat stressed quail group. Furthermore, the best enhancement obtained in quails given combination of Cr plus Se more than when each of them was given separately.

3.3. Hematological parameters:

The effects of heat stress and preventive effects of Cr and/or Se on the hematological parameters are shown in (Table.4) in which, heat

stressed quail group showed a significant (*P<0.05*) decline in RBCs, PCV, Hb concentration reflecting a picture of macrocytic hypochromic anemia ,moreover, there were a significant (*P<0.05*) reduction in TLC , lymphocyte and eosinophil numbers with marked increase in heterophil and monocyte numbers reflecting stress picture of leukogram .Cr and/or Se feed addition were able to restore the altered hematological parameters compared to the heat stressed group .Notably, co-administration of Cr plus Se exhibited greater improvement than when each of them was used alone.

3.4. Biochemical parameters:

The effects of heat stress and alleviating effects of Cr and/or Se on the selected biochemical parameters presented in Table(5) showed that , heat stressed group compared to the control group caused a significant (P<0.05) decline in total proteins ,albumin and thyroid hormones (T3 and T4) concentrations together with a significantly (P<0.05) elevated serum enzyme activities of (AST and LDH) , total cholesterol (TC), triglycerides (TG), and glucose concentrations. Moreover, the dietary Cr and/or Se addition were able to reverse the altered biochemical parameters. Notably. The best results observed in quail group received diet supplemented with a combination of both organic Cr and Se.

3.5. Oxidative stress and antioxidant biomarkers:

The effects of heat stress and ameliorative effects of Cr and/or Se on the oxidative stress and antioxidant biomarkers shown in (Figure.2) illustrated that compared to control group, the heat stressed group revealed a significant (P<0.05) increase oxidative stress marker Malonaldialdehyde (MDA) with a significant (P<0.05) reduction in antioxidant biomarkers glutathione peroxidase (GSH-Px), and total anti-oxidant capacity (TAC). On the other hand these results were reversed by dietary simultaneous supplementation with organic Cr and/or Se as shown by a significant (P<0.05) reduction in oxidative stress marker MDA with a significant (P<0.05) enhancement in antioxidant biomarkers glutathione peroxidase (GSH-Px) and total anti-oxidant capacity (TAC) compared to heat stress group.

3.6. Body temperature and Respiratory rate:

The effects of heat stress and preventive effects of organic Cr and/or Se on the body temperature and respiratory rate shown in Table (6). Heat stressed group revealed a significant (P<0.05) increase body temperature and respiratory rate compared to control group. While there were a significantly (P<0.05) reduced body temperature and respiratory rate in quails fed diet supplemented with organic Cr and/or Se compared with heat stress quails.

Table (1): **Composition of the basal experimental ration**

Ingredients	%	Ingredients	%
Corn (yellow) Means within columns with different superscripts differ at P ≤0.05.	55.6	DL Methionin	0.15
Soy bean meal 48%	25.2	L Lysine	0.10
Wheat bran	5.50	Premix	0.3
D-Ca-P	1.50	Sodium chloride	0.25
Limestone	7.00	Mold guard	0.1
Calculated analysis			

DE Kcal/kg	2960	Crude fiber %	14.00	Lysine%	1.06
Crude protein %	20.35	Calsium %	3.07	Methionine %	0.49
C. Fat %	2.	Available phosphorus %	0.38	Methionine + cysteine %	0.8

Provided by per kg of diet: vitamin A, 12 500 IU; vitamin D3, 2500 IU; vitamin E, 30 mg; vitamin K3, 2 mg; vitamin B1, 2 mg; vitamin B2, 5 mg; niacin, 20 mg; vitamin B6, 3 mg;
vitamin B12, 0.015 mg; folic acid, 1 mg; biotin, 0.045 mg; ascorbic acid, 50 mg;
apocarotenoic acid ester, 0.5 mg; choline chloride, 125 mg; manganese, 80 mg; iron, 60 mg;
Zinc, 50 mg; copper, 5 mg; cobalt, 0.2 mg; iodine, 1 mg; selenium, 0.10 mg.

Table (2): Mean indoor ambient temperature, relative humidity within the quail's house during experimental period.

Months	Ambient Temperature (ºC)		Relative humidity (%)	
	minimum	maximum	minimum	Maximum
July	25.3 ± 0.08	35.25 ± 0.5	48.02 ± 2.27	79.05 ± 1.84
August	25.02 ± 0.47	36.66 ± 0.14	47.13 ± 0.19	79.9 ± 0.6
September	22.73 ± 0.59	33.28 ± 0.7	47.3 ± 0.97	77.87 ± 1.47

Table (3): Effect of dietary supplementation of organic chromium and selenium on performance and mortality of control and heat-stressed quails

Group No.	IBW	FBW	BWG	TFI	FCR	Mortality (%)
Gr1	48.8±2.2	244.2±4.3^{b}	195.4±3.3^{b}	864.9±5.07^{b}	4.43±0.08^{b}	2±0.00^{b}
Gr2	49 ± 3.11	209.6 ± 0.4^{c}	160.6 ± 3^{c}	782 ± 20.3^{a}	4.88 ± 0.14^{a}	12 ± 0.84^{a}
Gr3	49.2 ± 1.66	254 ± 2.66^{a}	204.8 ± 4^{a}	838.5 ± 15.4^{b}	4.1 ± 0.13^{c}	0.00 ± 0.00^{c}
Gr4	47.8 ± 2.76	251 ± 2.367^{a}	203.2 ± 3.4^{a}	832 ± 15.9^{b}	4.1 ± 0.11^{c}	0.00 ± 0.00^{c}
Gr5	51.2 ± 2.27	258.2 ± 2.06^{a}	207 ± 2.59^{a}	866 ± 16.8^{b}	4.18 ± 0.11^{c}	0.00 ± 0.00^{c}

a, b, c Means within columns with different superscripts are significantly different (P<0.05). IBW= initial body weight, FBW= final body weight, BWG= body weight gain, TFI= total feed intake, FCR = food conversion rate .Gr1 =control gr, Gr2 =Heat stress group, Gr3=heat stress +Chromium (1400 µg/kg of diet),Gr4= Selenium(0.3 mg/kg of diet) and Gr5 =Heat stress+ Chromium (1400µg) + Se (0.3 mg/kg of diet).

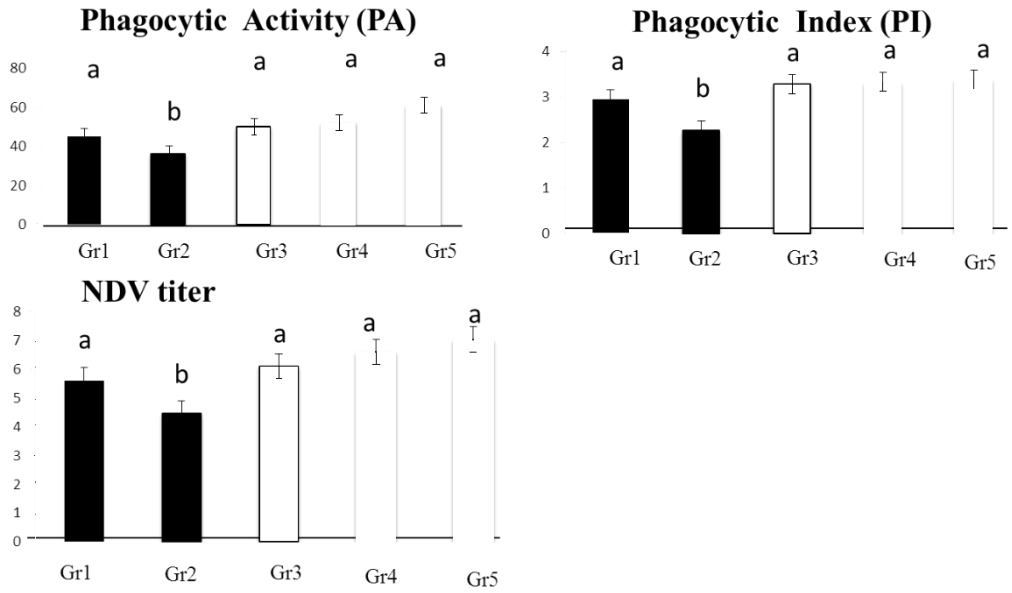

Figure 1 : Effect of dietary organic chromium and selenium on immune responses in normal and heat-stressed quails . a, b,.. Means different superscripts are significantly different (P<0.05). Values are means ± SE . PA = phagocytic activity, PI = phagocytic index and NDV= Newcastle disease virus titer. Gr1 =control gr, Gr2 =Heat stress group,Gr3=heat stress +Chromium (1400 µg/kg of diet),Gr4=Selenium (0.3 mg/kg of diet) and Gr5= Heat stress+ Chromium (1400µg) + Se (0.3 mg/kg of diet).

Table (4): Effect of dietary supplementation of organic chromium and selenium on hematological parameters of control and heat-stressed quails

Group No. Parameters	Gr1	Gr2	Gr3	Gr4	Gr5
RBCs (x10^6/µl)	2.9±0.3^a	2.4 ± 0.16^b	2.99±0.2^a	3.00±0.2^a	3.01a ±0.16^a
Hb (gm/dl)	9.2±0.37^a	7.6 ± 0.18^b	9.00±0.25^a	9.2 ±0.3^a	9.1± 0.06b^a
PCV (%)	31.2±0.73^a	29±0.48^b	33.6±1.5^a	31.2±1.5^a	31.5 ± 0.8^a
MCV (fl)	108±12.2^b	121 ± 6.4^a	112 ± 9.3^b	104 ± 7.6^b	105 ± 6.2^b
MCHC (%)	29.5±0.61^a	26.2 ± 0.6^b	27 ± 1.9^a	29.5 ± 5.8^a	29 ± 0.65^a
WBCs (x10^3/µl)	29.7±0.53^a	19.2±0.96^b	25.9±1.2^a	26.3±1.17^a	28.9 ± 0.84^a
Het. (x10^3/µl)	9.3±0.72^b	9.16 ± 0.63^a	8.8 ± 0.5^b	8.8 ± 0.63^b	9.8 ± 0.59^b
Lymph. (x10^3/µl)	16.8 ±0.32^a	6.1 ± 0.34^b	13.6±0.65^a	14.04±0.55^a	15.48±0.88^a
Mono.(x10^3/µl)	2.1 ±0.17^b	3.04 ± 0.05^a	2.2 ± 0.23^b	2.06 ±0.25^b	2.02 ± 0.12^b
Eosinophil(x10^3/µl)	1.5±0.16^a	0.9±0.08^b	1.3±0.24^a	1.4±0.24^a	1.6±0.24^a
H/L	0.55 ±0.65^b	1.50±0.64^a	0.65±0.14^b	0.63±0.43^b	0.63±0.06^b

Means within rows with different superscripts differ at P ≤0.05. Red blood cells (RBCs),Hemoglobin (Hb),Packed cell volum (PCV), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) ,Mean corpuscular hemoglobin concentration (MCHC) and White blood cells (WBCs). Gr1 =control gr, Gr2 =Heat stress group, Gr3=heat stress +Chromium (1400 µg/kg of diet), Gr4 =Selenium (0.3 mg/kg of diet) and Gr5 =Heat stress+ Chromium (1400µg) + Se (0.3 mg/kg of diet).

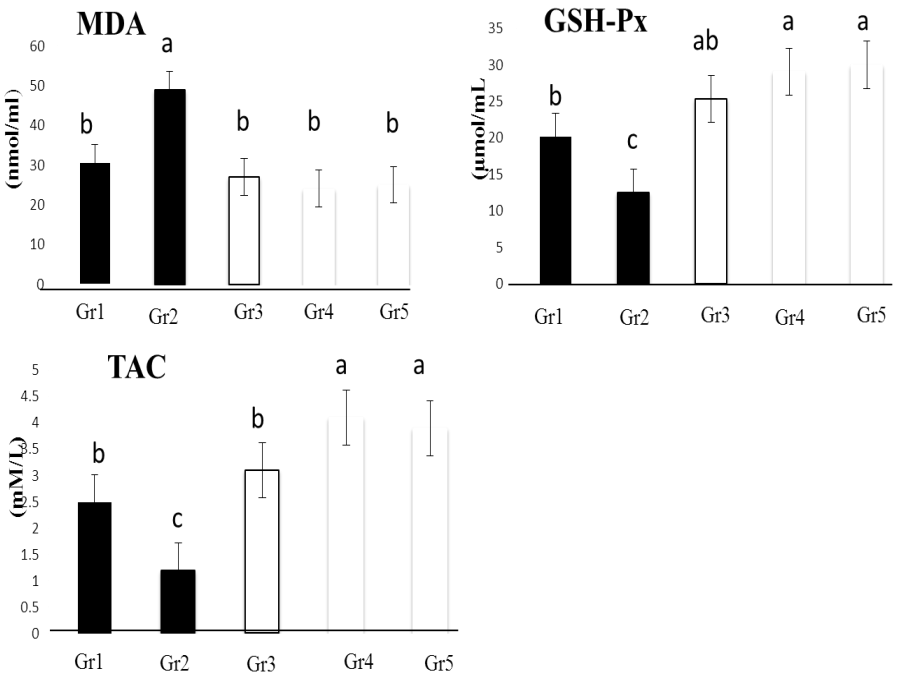

Figure2: Effect of dietary organic chromium and selenium on the oxidative stress and antioxidant biomarkers in normal and heat-stressed quails .. Values are means ± SE. a, b,.. Means different superscripts are significantly different (P<0.05) Malonaldialdehyde (MDA) , Glutathione peroxidase (GSH-Px) , Total anti-oxidant capacity (TAC). Gr1 =control gr, Gr2 =Heat stress group, Gr3=heat stress +Chromium (1400 µg/kg of diet),Gr4=Selenium (0.3 mg/kg of diet) and Gr5=Heat stress+ Chromium (1400µg) + Se (0.3 mg/kg of diet).

Table (5): Effect of dietary supplementation of organic chromium and selenium on Serum biochemical parameters of control and heat-stressed quails

Group No. Parameters	Gr1	Gr2	Gr3	Gr4	Gr5
TPs (gm/dl)	5.5±0.09^{a}	5.08 ± 0.12^{b}	5.58 ± 0.23^{a}	5.69 ± 0.19^{a}	5.7 ± 0.16^{a}
Alb (gm/dl)	2.2±0.04^{a}	1.86 ± 0.07^{b}	2.2 ± 0.1^{a}	2.2 ± 0.02^{a}	2.2 ± 0.05^{a}
Glob (gm/dl)	3.3 ±0.1^{a}	3.22 ± 0.16^{b}	3.38 ± 0.18^{a}	3.49 ± 0.18^{a}	3.5 ± 0.16^{a}
A/G	0.67±0.03^{a}	0.58 ± 0.04^{b}	0.65 ± 0.04^{a}	0.63 ± 0.04^{a}	0.63 ± 0.02^{a}
TC (mg/dl)	86±1.1^{b}	190 ± 0.44^{a}	87 ± 2.02^{b}	90 ± 1.1^{b}	88 ± 1.98^{b}
TG (mg/d)	96±1.6^{b}	114 ± 5.11^{a}	96 ± 1.9^{b}	88 ± 0.89^{b}	91 ± 3.3^{b}
Glu (mg/dl)	224±2.35^{b}	321 ± 24.9^{a}	223 ±10.1^{b}	245 ± 21.4^{b}	219 ± 5.15^{b}
AST (U/L)	43±1.46^{b}	85 ± 1.74^{a}	45 ± 1.36^{b}	44 ± 2.36^{b}	40 ± 0.6^{b}
LDH (U/L)	639±2.73^{b}	875 ± 4.13^{a}	645 ± 3.38^{b}	650 ± 1.57^{b}	629 ± 2.45^{b}
MDA(nmol/m)	30.4±0.24^{b}	49 ± 2.11^{a}	27 ± 1.6^{b}	24 ± 2.9^{b}	25 ± 1.9^{b}
TAC (mM/L)	2.5±0.18^{b}	1.2 ± 0.09^{c}	3.1 ± 0.07^{b}	4.1 ± 0.22^{a}	3.9 ± 0.09^{a}
GPx (µmol/mL)	20.11 ± 0.69^{a}	12.47 ± 0.41^{b}	25.32 ± 0.8^{a}	29.02 ± 0.8^{a}	29.99 ± 0.8^{a}
T3 (nM/L)	2.38±0.09^{a}	1.34 ± 0.08^{b}	1.99 ± 0.05^{a}	2.09 ±0.13^{a}	2.12 ± 0.3^{a}
T4 (nM/L)	22.9±0.35^{a}	12.92 ± 0.35^{b}	19 ± 0.51^{a}	22 ± 1.96^{a}	23 ± 2.33^{a}

Means within rows with different superscripts differ at P ≤0.05.Total proteins (TP),Albumin(alb),Globulin (glob), Albumin globulin ratio(A/G),Total cholesterol (TC),Triglycerides(TG),Glucose(Glu), Aspartate aminotransferase (AST) , Lactate dehydrogenase(LDH). T3= triiodothyronine and T4= thyroxine..Gr1 =control gr, Gr2 =Heat stress group, Gr3=heat stress +Chromium (1400 µg/kg of diet),Gr4=Selenium (0.3 mg/kg of diet) and Gr5 =Heat stress+ Chromium (1400µg) + Se (0.3 mg/kg of diet)

Table (6): Body temperature and Respiratory rate of quails during experimental period.

Group No.	Body temperature (oC)	Respiratory rate (r/min)
Gr1	40.50± 0.34^{b}	74.67 ± 1.45^{b}
Gr2	42.33 ± 0.33^{a}	81.67 ± 1.20^{a}
Gr3	41.00 ± 0.33^{b}	75.67 ± 1.45^{b}
Gr4	40.67 ± 0.34^{b}	73.63 ± 0.88^{b}
Gr5	40.33 ± 0.88^{b}	73.33 ± 0.89^{b}

Means within columns with different superscripts differ at P ≤0.05.

4. DISCUSSION

Acute and chronic induced heat stress linked to metabolic changes related to oxidative stress. According to Yang et al. (2010), birds subjected to stress by high temperatures may have reduced activity of the mitochondrial respiratory chain, followed by increased production of reactive oxygen species (ROS). Mujahid et al.(2005) concluded that oxidative damage associated with higher body temperature followed by decreased body weight gain .Many chemical and biochemical reaction rates increase with temperature via the accelerated metabolic reactions in the cells and tissues (Lin et al., 2006).

Regarding the effect of heat stress on growth performance as presented in (Table.3), heat stress group showed reduced body weight gain (BWG), total feed intake (TFI) and increased feed conversion ratio (FCR) as well as increased the mortality rate .Similar observations have been demonstrated by (Habibian et al. 2016).

(Melesse et al., 2011) provided an explanation for the reduced body weight during heat stress by reduced feed consumption, which lowered metabolic activity by minimizing excessive heat production, which consider as very essential step for the maintenance of body temperature during heat stress. Moreover, stress is documented to be related to neuro-chemical and hormonal changes, including alterations in adrenal and thyroid hormone levels (Hangalapura, 2006). Previuos studies showed that the environmental temperature could decrease the circulating levels of thyroid hormones (Melesse et al. 2011; Willemsen et al.2011). Furthermore, longer periods for heat stress

can result in some degree of thermotolerance and so, it can differently affect the proteins synthesis and degradation. High temperatures can reduce the T3 concentration and increase the plasma corticosterone concentration, factors known to reduce protein deposition through protein turnover in birds (Yunianto et al.1997).Similarly, Willemsen et al.(2011) who reported that when the birds are heat stressed, the insulin like growth factor-1(GF-I) levels are dropped, reducing rates of protein synthesis and increased rates of protein degradation, which provides an explanation for the reduced weight gain. In the current study, Cr and/or Se administration significantly improved the growth performance variables by increased body weight gain (BWG), feed intake, improved feed conversion ratio (FCR) and similarly decreased mortality rate. In consistent with our results ,some reports have indicated that dietary addition with Cr alone or in combination to broilers subjected to heat stress increased feed intake (Sahin et al., 2002b), improved weight gain (Lien et al., 1999)and feed efficiency (Jahanian and Rasouli, 2015). This improvement in growth performance might be due to the mediated activity Cr by the anabolic action of insulin (SM and Gh, 2014).Similarly.(Fan et al., 2009) reported that supplementation of dietary selenium improved feed efficiency, furthermore, it elevated antioxidant activities and decreased lipid peroxidation products in stressed broilers. The antioxidant activity of Se as well as the enhance natural antioxidant body system has been documented by(El-Demerdash and Nasr, 2014).

Effect of heat stress on immunological parameters illustrated by (Figure.1) and (Table.4), heat stress suppress immune response as indicated by reduced phagocytic activity of heterophils, phagocytic index ,antibody titer against Newcastle disease virus side by side with reduced total leukocyte count and lymphocyte numbers .The present study revealed an improvement in immune responses through the significant (*P<0.01*) increased phagocytic activity of heterophils, phagocytic index and antibody titer against Newcastle disease virus as well as relief stress picture of leukogram in quails that were reared under the heat stress conditions (34±2°C) and simultaneously supplemented with Cr and/or Se in their diets . Several studies demonstrated that Cr dietary incorporation results in improved the immune response and immune functions of broilers (Bahrami et al., 2012).Enhancement of the cell-mediated immune responses in chickens (Rajalekshmi *et al.,* 2014). Improved the proliferation of peripheral blood lymphocytes in birds (Uyanik *et al.,* 2002) .Higher antibody titers against Newcastle disease and infectious bronchitis viruses (Ebrahimzadeh et al., 2012). Enhanced the vaccination response against Newcastle disease and Influenza viruses in broiler chickens (Naghieh et al., 2010) . Jan *et al.* (2017) showed a stimulatory effect of high dose of inorganic chromium enriched soya meal on number of lymphocytes in caecal tonsil of laying hens on adaptive immune system by almost two times higher number of $CD4^+$ cells suggested enhancement of the response against potentially dangerous antigens . The increase in the number of T and B lymphocytes in Cr supplemented birds can be attributed to either Cr participation in RNA synthesis or may be due to the increased glucose availability for lymphocytes as an energy source that is attributed to the improved insulin function (Van Heugten and Spears, 1997). Moreover, (Huang et al., 2012) investigated that, supplementing diets with adequate levels of Se improved the immune cell functions.

Regarding the effect of heat stress on hematological parameters as shown in (Table.4), heat stress, significantly alter erythrogram by decreasing RBCs counts, PCV and Hb concentration producing anemia of macrocytic hypochromic nature. Macrocytic hypochromic anemia developed during the period of heat stress due to red cell destruction or hemorrhagic anemia induced by ROS production and decreased antioxidant levels resulting in various deleterious effects such as enhanced membrane rigidity, osmotic fragility, membrane fluidity, reduced erythrocyte survival, and structural damage to RBC membranes, ultimately resulting in hemolytic anemia (Del Vesco et al., 2014).

Alteration in leukogram was detected by significant reduction in TLC, lymphocyte and eosinophil numbers as well a significant increase in heterophil and monocyte numbers reflecting stress picture of leukogram in heat stress group compared with control quail group. Our results are in line with that of Ajakaiye et al. (2010) who detected a significant reduction in total leukocyte count (TLC),

lymphocyte and eosinophil numbers. However, there is a slight increase in heterophil released by bone marrow, thus increasing their number in circulation, but their bactericidal and phagocytic activity decreased. Increase in heterophil and reduction in lymphocyte has been used as sensitive and reliable measures of stress (Maxwell and Robertson, 1998) . Smilarly, Altan et al. (2000) reported that after 2 hours of heat stress, broiler chickens exhibited significantly reduced lymphocyte and raised heterophil ratios. Additionally,(Gross, 1992) recorded that, exposure of birds to high temperature resulted in an increase in the plasma corticosterone which subsequently depressed the activity of the total leucocytes count and lymphoid organs. lymphopenia induced by the release of glucocorticoids resulting in sequestration of lymphocytes in lymphoid tissues or bone marrow rather than entering efferent lymph and blood furthermore, long-term effect of corticosteroids may cause lysis of thymic cortical lymphocytes in the lymph nodes(Saag and Furst, 2015).The biochemical effects of heat stress as shown in (Table.5) reduced plasma total proteins and albumin concentrations compared to control group. This result is agreement with the observation of Tawfeek et al. (2014) who reported that heat stress significantly decreased the serum total proteins concentrations of broiler compared with that of control broilers. Our study demonstrated that dietary Cr and/or Se feeding improved serum albumin concentration explained by reducing the synthesis and secretion of corticoid hormones. Which may have reduced protein catabolism and increased serum albumin concentration (Seyrek et al., 2004).

Our data detected that, heat stress raised serum total cholesterol and triglycerides levels compared to that of control group .Our results are in agreement with the findings of Kataria etal.(2008), Hosseini Mansoub et al. (2010) and Tawfeek etal. (2014) they reported that heat stress enhances lipid metabolism associated with the increase in serum total cholesterol (TC) and triglyceride (TG). This raise in blood lipids under heat stress was explained by Rashidi et al. (2010) who pointed out that heat stress reduced feed intake of broilers compensate their energy requirement by lipolysis. Additionally, Hajati et al. (2016) reported that the higher levels of stress hormones due to heat stress stimulate lipolysis and increase circulating cholesterol and triglyceride levels. On the other hand, the declined concentrations of serum lipid content in heat stressed quails dietary supplemented with organic chromium and /or selenium are in line with Wang et al. (2006) who detected that chromium dietary supplementation improve the lipid metabolism especially in obesity or stress condition through the ability to convert chromium into a form that potentiates insulin action. This result emphasizes the findings of (Abraham et al., 1982) who recorded that Cr is essential for lipids metabolism.

Results of the present study revealed that heat stress increased serum glucose level in quails compared to control quails. Previous studies demonstrated that heat stress increased plasma glucose level (Kutlu and Forbes ,1993) and (Rashidi et al.,2010) . Rudich et al. (1998) reported that insulin signaling is impaired under conditions of oxidative stress. Similarly, Ajakaiye et al. (2010) and (Kondo et al., 2012) pointed out the increasing level of serum glucose may be derived from an increase in free radicals and the release of ACTH and cortisol hormones that prevent insulin release by β cells and stimulate gluconeogenesis due to heat stress .Supplementation organic Cr in quail diets decreased glucose level in blood in agreement with (Sahin et al., 2002b; El-Hommosany, 2008) who stated that chromium is essential for normal glucose metabolism and it is a component of glucose tolerance factor, which works with insulin to move glucose into cells and increased glucose utilization, thus resulting in an improvement of live weight gain, feed efficiency, and carcass qualities.

This study showed as illustrated in (Figure.2) that heat stress mediated an oxidative stress as evidenced by elevated serum MDA and depleted enzymatic and nonenzymatic antioxidant, including glutathione peroxidase (GSH-Px) and total antioxidant capacity (TAC) compared to control group. These results implied that disturbance in the balance between the oxidative and antioxidant systems in quails occurred during heat stress these finding was in consistence with that reported by Wang et al. (2009) who demonstrated that broiler chickens exposed to heat stress exhibited more than a twofold increase of malondialdehyde (MDA) as a secondary lipid oxidation product, in the breast meat.

The oxidative stress created by heat stress enhances free radical production, which induces oxidative damage to cellular membranes and lipid peroxidation which led to hepatocellular injury and release of intracellular enzymes including AST and LDH (Table.5).Our results are in line with (Mujahid et al., 2007; Tan et al., 2010)**.**In the present study, dietary supplementation with organic Cr and/or Se tended to normalize the increased serum hepatic biomarkers (AST, LDH, cholesterol, triglycerides) and increased the serum total proteins, albumin and thyroid hormones (T3 and T4) concentrations compared to heat stress group. This improvement may be partly due to the antioxidant activity of Cr and Se, high concentration of antioxidants (Se and Cr) can decrease lipid peroxidation and keep cellular integrity and therefore reduce the serum concentration of AST, LDH, triglycerides and cholesterol (Sahin et al., 2002a; Sadeghi et al., 2016) .Similar to our findings for selenium (Fan et al., 2009) reported that supplementation of dietary selenium improved feed efficiency, promoted conversion of thyroxine (T4) to triiodothyronine (T3), minimized the changes of blood biochemical parameters; furthermore, it elevated antioxidant activities and decreased lipid peroxidation products in stressed broilers. The antioxidant activity of Se and the enhancement of natural antioxidant body system have been documented by (El-Demerdash and Nasr, 2014) .

The obtained data from this study showed that heat stress group exhibited higher body temperature, higher oxidative stress marker MDA, lower growth rate and reduced thyroid hormones (T3 and T4). Heat stressed birds showed a pronounced decrease in thyroid hormones, which are usually correlated with whole-body energy expense and heat production (Baumgard and Rhoads Jr, 2013)**.** The mechanism by which heat stress reduces thyroid parameters are not fully understood. Although chronic heat stress obviously depressed the activity of the thyrotrophic axis in layer birds as reflected by reduced plasma T3 concentration inducing functional hypothyroidism (Mitchell and Carlisle, 1992).Our results are in line with Yunianto et al. (1997) who reported that high temperatures can reduce the T3 concentration and increase the plasma corticosterone concentration, which are factors known to reduce protein deposition through protein turnover in birds . The hormone T3 appears to be involved in regulating growth rate. Circulating T3 levels negatively correlated with temperature and positively correlated with feed ingestion in chickens (Yahya, 2000).

Conclusion

Based on the results obtained from this study under heat stress conditions and elevated body temperature, change in corticosterone levels occurred together with metabolic changes such as oxidative stress which damage cellular membranes inducing lipid peroxidation which led to hepatocellular injury and disrupt carbohydrates, lipids and protein metabolism. On the other hands, dietary chromium and/or selenium supplementation are effective in alleviating the deteriorating effects of heat stress on performance, immunological, hematological and biochemical parameters via their effective antioxidant, free radical-scavenging capacities and potent antioxidant activity.

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