ALLEVIATING ADVERSE EFFECTS OF HEAT STRESS BY USING ORGANIC SELENIUM AND CHROMIUM FOR LOCAL LAYING HENS.

El-Samra H.A. Abo-Egla*; Z.M. Kalaba*; A.A.H. Tolba** and M.A.I. El-Deeb**

* Poult. Prod. Dep., Fac. Agric., Mansoura Univ.

** Anim. Prod. Res. Inst., Agric. Res. Center, Ministry of Agric. Dokki, Giza.

ABSTRACT

This experiment was conducted to evaluate the effect of selenium (Sel-Plex) and chromium picolinate on some blood parameter of local laying hens reared under high environmental temperatures. Two hundred and seventy laying hens (24 weekold) were divided into nine groups, 30 hens per group using two levels of selenium (0.3 and 0.5 mg/kg diet) and two levels of chromium picolinate (1200 and 1400 µg/kg diet). The control group was fed a basal diet. The obtained results can be summarized as follows: Laying hens fed the Se and Cr-supplemented diets achieved significantly higher total counts of blood erythrocytes and leukocytes, and lymphocytes, hemoglobin, packed cell volume as compared to their control counterparts. However, there were significant decreases (P≤0.05) in total monocytes but total heterophil were not affected compared with the un-supplemented control group. Dietary supplementation with Se and Cr caused significant (P≤0.05) increases in blood plasma levels of total protein, globulin, chloride, calcium, phosphorus, triiodothyronine, and thyroxine, while levels of albumin, albumin: globulin ratio, creatinine and urea were decreased as compared to those of the control group. Hens fed the Se and Crsupplemented diets attained significantly lower (P≤0.05) levels of plasma concentrations of cholesterol, glucose, total lipids and triglycerides as well as activity of transaminases (ALT and AST) as compared to their control counterparts. From the present results, it is concluded that enhancing hen's diets with selenium (as Sel- Plex) and chromium picolinate singly or in combination at levels of 0.3 mg Se plus 1400 µg Cr /kg diet can consider as an effective management practice for reducing the adverse effects of heat stress for laying hens.

Keywords: heat stress, local laying hens, blood parameters.

INTRODUCTION

High environmental temperature is a major problem faced by poultry farmers particularly laying hens, during summer months. High ambient temperature reduces feed intake, live weight gain, egg production, egg quality and feed efficiency (Donkoh, 1989; Siegel,1995), thus negatively influencing the performance of poultry. Plasma corticosterone concentration also increases during heat stress (Hurwitz *et al.* 1980). In addition, Donkoh (1989) reported that reduced plasma protein and markedly increased blood glucose concentrations during heat stress. Such ambient temperatures decrease serum vitamin and mineral concentrations in poultry as well as humans (Ensminger *et al.*, 1990; Anderson, 1994). Heat stress has also been shown to increase mineral excretion (Siegel, 1995). Several methods are available to alleviate the effect of high environmental temperature on poultry

performance. Because it is expensive to cool animal buildings, such methods focus mainly on manipulating the diet.

Chromium is used in the poultry diet because of the reported benefits of Chromium supplementation to laying hens (Sahin *et al.*, 2001; Sahin *et al.*,2002) during cold and heat stress and because chromium is reduced during environmental stress. The primary role of Chromium in metabolism is to potentiate the action of insulin through its presence in an organometallic molecule, the glucose tolerance factor (GTF) (Anderson, 1994; Sahin *et al.*, 2001). As Insulin metabolism influences lipid peroxidation (Gallaher *et al.*, 1993), chromium, as an insulin potentiator, is therefore postulated to function as an antioxidant (Preuss, *et al.*, 1997). Moreover, Chromium is thought to be essential for activating certain enzymes and for stabilizing proteins and nucleic acids (Anderson; 1994; Linder, 1991).

Selenium supplementation as a single agent or in combination has been suggested to improve performance and immune response to diseases and also to decrease the economic losses related to high temperature (Smart *et al.,* 1995). Sodium selenite is considered the traditional source of supplementation (Leeson and Summers, 1991). Recently, organic selenium from yeast (Sel-Plex, Alltech Inc.) is more active form of selenium in chickens than selenite (Collins *et al.,* 1993). Leeson and Summers (1991) have shown that selenium is required for maximum performance of chickens. Mahmoud and Edens (2003) found that chickens fed organic selenium as Sel-Plex, a selenized yeast, had elevated GPX activity in both blood and liver in a thermoneutral environment and after heat distress.Therefore, the present study was undertaken to evaluate of the effect of dietary supplementation with organic selenium and chromium on some blood parameters of heat-stressed laying hens.

MATERIALS AND METHODS

Birds and experimental design

The present experiment was carried at Sakha Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Kafr El-Sheikh Governorate, Egypt. A total of 270 local 24-week-old laying hens (Inshas) were used in this study. Hens were randomly distributed into nine groups, each containing 30 hens in 3 replicates of 10 hens each. Laying houses were provided with 17 h light per day. The hens were randomly assigned according to initial body weights. Feed and water were given *ad libitum*. The hens were vaccinated against Marek and Newcastle diseases. Similar management conditions were maintained for all groups. The experiment was carried out from 1st July to 30th September, 2012.

Dietary treatments

The laying hens were fed a basal diet supplemented with two levels of each Selenium (Sel- Plex) and chromium picolinate. Birds were assigned to each of the following diet treatments:

1 - Basal diet (without any additives and served as control).

2 - Basal diet + 0.3 mg Se /kg diet.

- 3 Basal diet + 0.5 mg Se /kg diet.
- 4 Basal diet + 1200 µg Cr /kg diet.
- 5 Basal diet + 1400 µg Cr /kg diet.
- 6 Basal diet + 0.3 mg Se + 1200 μ g Cr /kg diet.
- 7 Basal diet + 0.5 mg Se + 1200 μ g Cr /kg diet.
- 8 Basal diet + 0.3 mg Se + 1400 μ g Cr /kg diet.
- 9 Basal diet + 0.5 mg Se + 1400 μ g Cr /kg diet

Ambient temperature and relative humidity

Daily ambient temperature (AT) and relative humidity (RH) were recorded inside the laying houses three times per day (at 8 a.m., 12 p.m. and 4 p.m.) and twice at night (at 12 a.m. and 4 a.m.) during the experimental period. Also, means of maximum and minimum AT and RH were recorded monthly (Table 2). Indoor climatic conditions were recorded using electronic digital thermo-hygrometer. Temperature-humidity index (THI) was calculated according to Marai *et al.* (2001). They established several stages of thermal comfort values such as: absence of heat stress (<27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9-29.9) and very severe heat stress (>30.0), based on variations in the bird's body temperature, using the following mathematical model:

THI = db $^{\circ}$ C - [(0.31 – 0.31 x RH) x (db $^{\circ}$ C – 14.4)]

Where: THI = Temperature-humidity index.

 $db^{\circ}C$ = dry bulb temperature in centigrades.

RH = relative humidity %.

Temperature-humidity index

During the experimental period (Summer 2012), birds exposed to very severe heat stress as the estimated THI values were 32.97, 33.39 and 31.06 during July, August and September, respectively (Table 2). In this study, the ambient temperature was higher than the recommended normothermia zone of 18-28°C (Donkoh, 1989; Holik, 2009) established for poultry in the tropical regions. This is a clear indication that the experimental layers, used in the present study, were subjected to a severe heat stress.

Blood constituents of laying hens

At 36 weeks of age, three hens per treatment were slaughtered within one to two hours post-oviposition in order to take some measurements. Blood samples of individual hens were collected from the jugular veins during slaughter into two heparinized tubes. The blood sample in the first tube was used for the measurement of some hematological characteristics. Improved Neubauer hemocytometer (Brand,Wertheim, Germany) was used for counting RBC_S and WBC_S according to Natt and Herrick (1952). The hemoglobin (Hb) content was determined using the Drabkin's technique according to Dein (1984). Packed cell volume (PCV) was determined by using the micro hematocrit capillary tubes and the micro hematocrit centrifuge at 12,000 rpm for 5 min. according to Bara (1988). Blood films were stained by Giemsa stain and the differential leucocytic count was performed according to Jain (1986).

The second half of blood sample was centrifuged at 4000 r.p.m. for 15 minutes to separate blood plasma, then stored at -20 °C for determining the concentrations of plasma total protein (Gornall *et al.*, 1949), albumin (Dumas

et al., 1971), creatinine (Bartles et al., 1972), urea (Fawcett and Socct, 1960), Cl (Schales and Schales, 1941), Ca⁺⁺ (Moorehead and Biggs, 1974), inorganic P (Goldenberg and Fernandez, 1966), cholesterol (Richmond, 1973), glucose (Trinder, 1969), total lipids (Zollner and Kirsch, 1962), triglycerides (Fassati and Prencipe, 1982), and activities of plasma tansaminases: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Reitman and Frankel, 1957). Globulin concentration was obtained by subtracting the values of albumin from the corresponding value of total protein. At the same time, plasma concentrations of triiodothyronine (T3) and thyroxine (T4) were assayed according to methods described by Darras et al. (1992).

Statistical analysis

Data were subjected to statistical analysis by one-way analysis of variance using SPSS @ statistical software **(SPSS, 1999, version 10.0)**. Significant difference among treatment means were detected by Duncan's Multiple Range Test **(Duncan, 1955)** at 5% level of significancet at (P≤0.05). The following model was used to study the effect of Selenium (Sel- Plex) and chromium picolinate on blood parameters of laying hens as follows:

 $Y_{ij} = M + T_i + e_{ij}.$

Where,

 Y_{ii} = observation for each dependent variable.

M = over all mean.

 T_i = Treatment effects (i = 1, 2,..... and 9).

e_{ii} = Random error.

RESULTS AND DISCUSION

Blood hematological variables

Effects of dietary supplementation with Se and Cr and their combination on some blood hematological variables of 36-week-old laying hens reared under the summer hot climate are presented in (Table 3).

There were significant increases (P≤0.05) in total counts of blood leukocytes (WBCs), hemoglobin (Hb), packed cell volume (PCV) and total counts of blood erythrocytes (RBCs) as affected by dietary supplementations comparing to their control counterparts. When 0.5 mg Se plus 1200 μ g/kg Cr was used total counts of blood leukocytes were comparable to that of the control. Also, treatments 1200 μ g/kg Cr, 0.5 mg Se plus 1200 μ g/kg Cr and 0.5 mg Se plus 1400 μ g/kg Cr were similar with control in packed cell volume. Also, treatments 1200 μ g/kg Cr and 0.5 mg Se separately or in combination with both levels of Cr in total counts of blood erythrocytes were similar with control value. However, there were significant decreases (P≤0.05) in monocytes cells compared to its counterpart in control group. Furthermore, lymphocytes cells were not affected by the addition of dietary supplementation, but lymphocytes were affected for hens given 1400 μ g/kg Cr in their diet. Also, no significant differences were detected in heterophil cell due to the dietary supplementation.

It is well known that heat stress is one of the major stressors on poultry production which produces a wide range of physiological changes. The

inhibiting effect of heat stress on blood concentrations of RBCs and Hb (Donkoh, 1989) and WBCs (Lin *et al.*, 2003; Mashaly *et al.*, 2004) is well documented in both broiler chicks and laying hens. Kutlu and Forbes (1993) found elevations in plasma concentrations of protein, glucose, heterophil, lymphocyte and heterophil/lymphocyte ratio and decreases in potassium ion concentration in response to thermal stress. On other hand Hanafy *et al.* (2009) reported that organic selenium significantly ($P \le 0.05$) increased blood Hb, RBC's and WBC's. Dietary selenium and Cr and their different combination inhibit leukocyte migration in broilers (Swain and Johri, 2000).

Blood biochemical variables

The effects of inclusion of Se and Cr and their combination on some blood biochemical variables of 36-week-old laying hens reared under the summer hot climate are presented in Tables 4a and 4b.

In general, the results of dietary supplementation with Se and Cr and their combinations in (Table 4), exhibited no significant differences (P≤0.05) in plasma concentrations of total protein, albumin, globulin, albumin: globulin ratio, creatinine, urea and chloride in response to dietary supplementation. But when the diets were supplemented with 0.3 mg Se plus 1200 µg/kg Cr or 0.3 mg Se plus 1400 µg/kg Cr plasma levels of total protein and globulin were significantly higher (P≤0.05) than those of the control group. However, albumin: globulin ratio was significantly lower (P≤0.05) for the hens fed the diet supplemented with 0.3 mg Se plus 1400 µg/kg Cr as compared to the control birds. Similarly, blood plasma urea levels were significantly decreased (P \leq 0.05) when hens fed diets supplemented with 1200 µg/kg Cr, 0.3 mg Se plus 1200 µg/kg Cr and 0.3 mg Se plus 1400 µg/kg Cr compared with their control counterparts. Feeding Cr-supplemented diets at levels of 1200 or 1400 µg/kg to laying hens under hot environment conditions led to significantly higher (P≤0.05) levels of plasma chloride compared with that of the control group. Finally, no significant differences were detected in plasma levels of albumin and creatinine due to the dietary supplementation. Dietary treatments, applied in the present study, significantly improved (P≤0.05) plasma calcium but did not significantly affect, while insignificantly increased phosphorus except the combination of 0.3 mg Se by 1400 µg/kg Cr witch achieved the highest value of plasma calcium and also significantly increased phosphorus.

Our observation in this study is in agreement with that reported by Ozbey *et al.* (2004) and Seyrek *et al.* (2004) who reported that concentrations of blood serum glucose, triglyceride and cholesterol were significantly higher whereas, levels of total protein and albumin were significantly lower in laying Japanese quails exposed to heat stress. Also, Nalini *et al.* (2008) reported that at 42-45 °C, serum, growth hormone, creatinine, urea, glucose, cholesterol, triglycerides, AST and ALT increased significantly (P<0.05) from respective control mean values. The mechanism of this alteration has been reported to be through increased generation of free radicals at the cell level. Several authors have documented that free radical generation affects blood serum metabolites of plasma total protein, cholesterol and glucose which is manifested in bird's adaptation response through decreased production performance (Lin *et al.* 2005; Imik *et al.* 2009). In contrary, Koelkebeck and

Odom (1995) found that acute heat stress had no effect on blood plasma levels of glucose, total protein and creatinine in laying hens.

Organic selenium had more pronounced effect on total protein, Kim and Mahan (2003) indicated that selenium is biochemically similar to sulphur, selenium replaces the sulphur molecule in the normal biosynthetic pathways of the yeast cell and is absorbed actively across the intestine by the same amino acid carrier. Combs and Combs (1986) reported that supplemented organic Se to broiler breeders and layers was actively absorbed and can be directly incorporated into protein. In harmony with our results, Attia et al. (2010) found that vitamin E and/or Se supplementation significantly decreased triglycerides, albumin, globulin and albumin / globulin ratio on layer, however Hanafy et al. (2009) found that organic selenium increase albumin. The increase in plasma total protein concentration by Cr supplementation is in agreement with previous results obtained for laying Japanese guails (Sahin et al., 2001; Abdel-Mageed and Hassan, 2012). The positive effect of Cr on plasma protein and its fractions may be due to the anabolic action of insulin mediated through increasing the amino acids synthesis by liver, enhancement the incorporation of several amino acids into protein (Uyanik et al., 2002).

Results presented in Table 4b exhibited that laying hens fed diets supplemented with Se and Cr and their combinations had significantly lower (P≤0.05 plasma levels of cholesterol, glucose, total lipids and triglycerides as well as activity of ALT and AST while plasma concentrations of triiodothyronine (T₃), and thyroxine (T₄) were significantly higher (P≤0.05) compared with their control counterparts.

In agreement with the present results, Kalaba (2007) and Ibrahiem (2008) reported that stressful factors can increase serum levels of cholesterol, glucose, total lipids and triglycerides, and activity of transaminases (ALT and AST), but may decrease the plasma levels of the hormones: T_3 and $T_{4..}$ According to Bhatti and Dil (2005), alteration in serum enzymes activity under stress conditions occur due to malfunctioning of liver, as degenerating and necrotic cells leak enzymes from cytoplasm.

According to the current results added dietary selenium alleviated the adverse effect of heat stress on the activity of AST and ALT in the blood plasma. In this direction, El-Mallah et al. (2011) observed a beneficial effect for selenium yeast on ALT and AST. The use of organic selenium (Sel-plex) as a source of supplemental dietary Se provides a highly efficient form of organic Se and facilitates a greater antioxidant enzymes which can readily reduce peroxides and other free radicals thereby compromising the cell membranes (Edens and Gowdy, 2005). On the other hand, Abaza (2002) observed that plasma cholesterol concentration was significantly increased by supplemental Se and/or vitamin E. However, Ljubic et al. (2006) suggested that the organic Se supplementation influences cholesterol metabolism in adipose tissue by decreasing the total cholesterol concentration during the fattening period and increasing the free cholesterol concentration after 48 h feed deprivation. Changes in enzymes responsible for regulating cholesterol synthesis, oxidation or elimination may be responsible for lowering the cholesterol synthesis in mature as well as

immature chickens (Konjufca *et al.*, 1997). Also, Saito *et al.* (2007) demonstrated that the oxidative stress induced by selenium deficiency can enhance lipid and cholesterol peroxidation in cultured cells.

In agreement with the present findings, several studies have shown that dietary Cr supplementation decreased blood glucose and cholesterol concentrations in laying hens (Lien *et al.*, 1996; Uyanik *et al.*, 2002). Also, Abdallah *et al.* (2013) found that Cr picolinate administration significantly decreased serum cholesterol and glucose, and activity of ALT and AST. In addition, Ibrahiem (2008) found the same results for laying hens reared under heat stress. According to Prasada and Gowda (2005) Cr act as glucose tolerance factor which can increase the uptake of glucose by cells and thus attenuating the insulin action. The reducing effect of Cr on plasma glucose in the present study may support this suggestion.

experimental diets.	
Ingredients	%
Yellow corn	66.00
Soybean meal (44%)	24.00
Limestone	7.59
Di-calcium phosphate	1.71
Sodium chloride	0.30
Vit.& Min. Mixture*	0.30
DL.Methionine	0.10
Total	100
Calculated analysis	
Metabolizable energy (kcal/kg)	2750
Crude Protein, %	16.43
Crude fiber, %	3.20
Ether extract, %	2.70
Calcium, %	3.33
Available phosphate, %	0.45
Total phosphate , %	0.66
Lysine, %	0.86
Methionine, %	0.39

Table 1: The composition and chemical calculated analysis of the experimental diets.

*Supplied per kg of diet: vit.A, 10000 IU; D₃, 2000 IU; Vit.E, 10mg; Vit.K₃,1mg; vit.B₁, 1mg; vit.B₂, 5mg; vit.B₆, 1.5mg; vit. B₁₂, 10mcg; Niacin, 30mg; Pantothenic acid, 10mg; Folic acid, 1mg; Biotin, 50µg; Choline, 260mg; Copper, 4mg; Iron; 30mg; Manganese, 60mg; Zinc, 50mg; Iodine, 1.3mg; Selenium, 0.1mg; Cobalt, 0.1mg.

Several researchers reported reduced blood plasma concentrations of T_3 and T_4 in heat-stressed chickens (Kalaba, 2007). The inverse relationship between plasma concentration of T_3 and environmental temperature has been well known (Yahav, 1999). In this regard, Beckett *et al.* (1987) found that T_3 is produced by 5'-deiodination of T_4 particularly in the thyroid, liver and kidney. The activity of the selenoenzyme catalyzing 5'-deiodination (5'-ID) in rats is affected by Se deficiency (Kohrle *et al.*, 1992). It has been reported that hepatic 5'-ID activity in the Se deficient rats is 10-fold lower but plasma

El-Samra H.A. Abo-Egla et al.

 T_3 concentration is significantly lower in Se supplemented rats than in normal rats (Beckett *et al.*, 1992) which is in accordance with the present results. Greater plasma concentrations of T3 and T4, reported in the present study, with higher dietary chromium could support a greater performance of laying hens, as T3 and T4 are considered as important growth promoters in animals (McNabb and King, 1993). Also, Sahin *et al.* (2001) reported similar results on Japanese quails.

From the present results, it is concluded that enhancing hen's diets with selenium (as Sel- Plex) and chromium picolinate singly or in combination at levels of 0.3 mg Se plus 1400 μ g Cr /kg diet can consider as an effective management practice for reducing the adverse effects of heat stress for laying hens.

Table (2): Means of indoor ambient temperature, relative humidity and temperature-Humidity Index measured within the open-sided laying house, during experimental period (from July 2012 to September 2012).

Manufia		emperature	Relative h		Temperature-Humidity Index			
Months	(°C)	(%					
	minimum	maximum	minimum	maximum	minimum	maximum		
lukz	25.30	34.25	48.02	79.05	23.54	32.97		
July	±0.08	±0.50	±2.27	±1.84	±.05	±0.56		
August	25.02	34.66	47.13	79.90	23.28	33.39		
Augusi	±0.47	±0.14	±0.19	±0.60	±.40	±0.11		
September	22.73	32.28	47.30	77.87	21.37	31.06		
	±0.59	±0.70	±0.97	±1.47	±0.51	±0.72		

Table 3: Blood hematological variables of laying hens fed diets supplemented with Se, Cr or their combinations at 36 wks old.

Items	WBCs	Lymph	Heter	Mono	PCV	Hb	RBCs			
nems	10 ³ /mm ³	%	%	%	%	gm/dl	10 ^{6/} mm ³			
control	25.17 ^e	58.34 ^b	27.82	13.85 ^a	26.33°	8.17 ^b	2.03 ^c			
CONTION	±0.20	±2.09	±1.79	±0.31	±1.20	±0.12	±0.19			
0.3mg mg Se	28.57 ^{cd}	64.33 ^{ab}	25.52	10.14 ^b	30.67 ^{ab}	10.47 ^a	2.87 ^{ab}			
0.5mg mg Se	±0.82	±5.36	±4.75	±0.94	±2.19	±.43	±0.29			
0.5mg mg Se	30.30 ^b	69.26 ^{ab}	30.74	10.00 ^b	30.67 ^{ab}	10.00 ^a	2.47 ^{abc}			
0.5mg mg Se	±0.72	±4.68	±5.48	±1.15	±1.20	±0.60	±0.03			
1200 µg/kg mg Cr	28.10 ^d	62.53 ^{ab}	26.45	11.02 ^b	29.00 ^{bc}	9.67 ^a	2.57 ^{abc}			
1200 µg/kg mg ci	±0.36	±4.22	±4.26	±0.07	±0.58	±0.18	±0.12			
1400 µg/kg mg Cr	30.03 ^{bc}	65.40 ^a	24.10	10.50 ^b	32.33 ^{ab}	10.33 ^a	2.80 ^{ab}			
1400 µg/kg mg Ci	±0.33	±6.98	±6.28	±2.20	±1.45	±0.17	±0.06			
0.3mg Se + 1200	35.10 ^ª	69.59 ^{ab}	20.55	9.86 ^b	31.00 ^{ab}	9.77 ^a	2.70 ^{ab}			
µg/kg Cr	±0.49	±2.38	±2.29	±0.14	±1.00	±0.33	±0.26			
0.5mg Se + 1200	26.43 ^e	69.56 ^{ab}	29.88	10.53 ^b	30.00 ^{abc}	10.00 ^a	2.40 ^{abc}			
µg/kg Cr	±0.30	±7.82	±8.11	±0.30	±1.15	±0.51	±0.25			
0.3mg Se + 1400	35.43 ^ª	72.47 ^{ab}	20.81	6.72 ^c	30.33ª	10.95 ^a	2.90 ^a			
µg/kg Cr	±0.38	±0.76	±0.55	±0.24	±0.88	±0.26	±0.06			
0.5mg Se + 1400	28.20 ^d	71.12 ^{ab}	20.51	8.37 ^{bc}	30.00 ^{abc}	10.03 ^a	2.30 ^{bc}			
µg/kg Cr	±0.61	±1.14	±1.40	±0.26	±0.58	±0.60	±0.15			
Significant	*	*	Ns	*	*	*	*			

^{b.c.đ} means in the same column having different superscripts differ significantly at (P≤ 0.05).

NS (Not significant), * (Significant at P≤ 0.05).

	010.								
items	Protein	Albumin	Globulin	A/G	Creatine	Urea	Chloride	Ca	Р
items	g/dl	g/dl	g/dl	ratio	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl
a a m f m a l	4.93 ^b	2.43	2.50 ^b	0.98	1.35	5.57 ^a	0.08 ^b	18.52 ^d	3.02 ^c
control	±0.05	±0.04	±0.08	±0.04	±0.27	±0.52	±0.01	±0.39	±0.29
0.3 mg Se	5.85 ^{ab}	2.35	3.50 ^{ab}	0.70	0.79	4.12 ^{ab}	0.08 ^b	27.84 ^{bc}	3.83 ^{abc}
0.3 mg 3e	±0.37	±0.26	±0.43	±0.14	±0.28	±0.80	±0.01	±0.51	±0.67
0.5 mg Se	4.94 ^b	2.29	2.65 ^b	0.97	0.94	4.23 ^{ab}	0.08 ^b	25.54 ^c	3.77 ^{abc}
0.5 mg Se	±0.49	±0.06	±0.54	±0.26	±0.27	±0.50	±0.01	±0.91	±0.48
1200 µg Cr	5.13 ^{ab}	2.18	2.95 ^{ab}	0.80	1.24	3.94 ^b	0.11 ^a	25.55 [°]	3.04 ^{abc}
1200 µg Ci	±0.37	±0.11	±0.48	±0.17	±0.21	±0.34	±0.01	±0.40	±0.02
1400 µg Cr	5.67 ^{ab}	2.40	3.26 ^{ab}	0.79	1.17	4.13 ^{ab}	0.11 ^a	27.88 ^{bc}	4.13 ^{abc}
1400 µg Ci	±0.35	±0.20	±0.52	±0.17	±0.32	±0.35	±0.003	±0.63	±0.06
0.3 mg Se +	6.08 ^{ab}	2.54	3.54 ^{ab}	0.73	0.77	3.79 ^b	0.08 ^b	29.49 ^{ab}	4.46 ^{ab}
1200 µg Cr	±0.33	±0.11	±0.34	±0.08	±0.21	±0.39	±0.01	±0.38	±0.23
0.5 mg Se +	5.31 ^{ab}	2.46	2.87 ^{ab}	0.85	1.31	4.54 ^{ab}	0.08 ^b	25.40 [°]	3.39 ^{bc}
1200 µg Cr	±0.36	±0.22	±0.20	±0.08	±0.25	±0.31	±0.01	±0.35	±0.20
0.3 mg Se +	6.26 ^a	2.27	3.99 ^a	0.58	1.18	3.63 ^b	0.09 ^b	30.51 ^ª	4.73 ^a
1400 µg Cr	±0.38	±0.04	±0.34	±0.04	±0.11	±0.35	±0.01	±0.38	±0.32
0.5 mg Se +	5.02 ^b	2.42	2.60 ^b	0.93	1.24	4.14 ^{ab}	0.08 ^b	26.17 ^c	3.53 ^{bc}
1400 µg Cr	±0.30	±0.15	±0.17	±0.03	±0.15	±0.17	±0.01	±1.81	±0.26
Significant	*	Ns	*	Ns	Ns	*	*	*	*

Table (4a): Blood biochemical of laying hens fed diets supplemented with organic forms of Se, Cr or their combinations at 36 wks old

a,b,c means in the same column having different superscripts differ significantly at (P≤ 0.05).

NS (Not significant), * (Significant at P≤ 0.05).

Table (4b): Blood biochemical of laying hens fed diets supplemented with organic forms of Se, Cr or their combinations at 36 wks old.

	Ulu.							
Items	cholesterol mg/dl	glucose mg/dl	total lipids mg/dl	triglycerides mg/dl	ALT u/ml	AST u/ml	T₃ nmol/l	T₄ nmol/l
control	203.43 ^a	242.37 ^a	22.07 ^a	391.64 ^a	52.67 ^a	65.33 ^a	1.18 [°]	12.70 ^c
CONTO	±1.26	±0.24	±2.03	±34.27	±5.33	±0.67	±0.10	±1.22
0.3 mg Se	189.11 ^{ab}	229.67 ^b	17.59 ^{bc}	232.81°	27.00 ^d	41.67 ^b	1.62 ^a	20.04 ^{ab}
0.5 mg Se	±3.88	±0.33	±0.82	±11.96	±8.14	±4.10	±0.08	±0.95
0.5 mg Se	174.58 ^{bcd}	230.30 ^b	17.13 [∞]	238.89 ^c	30.67 [°]	37.67 ^{bc}	1.32 ^{bc}	17.99 [⊳]
0.5 mg Se	±8.79	±0.812	±0.52	±17.94	±5.67	±3.28	±0.03	±1.19
1200 ug Cr	176.02 ^{bcd}	229.23 ^b	18.48 ^b	322.86 ^b	25.00 ^{bc}	38.67 ^b	1.52 ^{ab}	18.63 ^{ab}
1200 µg Cr	±1.23	±0.32	±0.82	±10.37	±0.00	±5.21	±0.04	±.42
1400 ug Cr	158.69 ^{de}	224.17 [°]	17.39 ^{bc}	216.43 [°]	25.00 [°]	43.33 ^b	1.53 ^{ab}	18.89 ^b
1400 µg Cr	±2.31	±3.35	±1.13	±17.38	±0.00	±1.76	±0.08	±0.58
0.3 mg Se +	163.19 ^{cde}	219.50 ^d	16.46 ^{bcd}	191.17 ^c	27.0 ^d	37.33 ^{bc}	1.68 ^a	20.72 ^a
1200 µg Cr		±0.35	±2.01	±20.42	±8.14	±3.18	±0.03	±0.25
0.5 mg Se +	177.14 ^{bc}	228.30 ^b	15.46 ^{bcd}	221.34 [°]	36.33 [⊳]	36.00 ^{bc}	1.32 ^{bc}	17.55 [⊳]
1200 µg Cr	±9.86	±0.45	±0.31	±26.57	±5.67	±0.00	±0.06	±0.58
0.3 mg Se +	152.42 ^e	218.37 ^d	13.17 ^ª	212.92 ^c	21.33 ^d	27.67 [°]	1.75 ^a	20.96 ^a
1400 µg Cr	±2.79	±0.41	±0.03	±21.72	±3.67	±3.18	±0.10	±0.96
0.5 mg Se +	184.49 ^b	226.97 ^{bc}	14.20 ^{cd}	254.09 ^c	30.67 [°]	40.00 ^b	1.58 ^a	20.00 ^{ab}
1400 µg Cr	±2.33	±1.72	±1.31	±16.01	±5.67	±3.46	±0.11	±0.51
Significant		*	*	*	*	*	*	*
a,b,c moone	in the come	aalumn	having	lifforont supp	reerinte	diffor al	anificant	by at /Dr

^{a,b,c} means in the same column having different superscripts differ significantly at (P≤ 0.05).

NS (Not significant), * (Significant at P≤ 0.05).

REFERENCES

- Abaza, M. (2002): Immune system and some physiological aspects in Japanese quail affected by antioxidants. Egyptian Poultry Science Journal, 22, 259–276.
- Abdallah, E.A., M.H. abdel Samad and A.M. Abdel latif (2013): Effect of supplementation diet with chromium picolinate on productive, reproductive, physiological performance and immune response of Golden Montazah chickens. Egypt. Poult. Sci., 33 (1V): 751-767.
- Abdel-Mageed, M. A. A. and H. A. Hassan (2012): Effect of Chromium-Methionine chelate on performance and some plasma constituents of laying Japanese quail during summer months. Egypt. Poult. Sci., 32 (1V): 883-894.
- Anderson, R.A. (1994): Stress effects on chromium nutrition of humans and farm animals. In: Proceedings of Alltech's 10th Annual Symposium, Biotechnology in the Feed Industry, Lyons P., Jacques K. A. (eds.), Nottingham University Press, UK, 267–274.
- Attia, Y.A., A.A. Abdalah, H.S. Zeweil, F. Bovera, A.A. Tag El-Din and M.A. Araft (2010): Effect of inorganic or organic selenium supplementation on productive performance, egg quality and some physiological traits of dual-purpose breeding hens. Czech J. Anim. Sci., 55 (11): 505–519.
- Bara, A.B. (1988): Hematology principles and procedures, 5th ed. Lee and Fedbiger, Philadelphia, USA, p. 81-83.
- Bartles, H., M. Bohmer and C. Heirli (1972): Colorimetric kinetic method for creatinine determination in serum and urine. Clin. Chem. Acta, 37: 193-197.
- Beckett, G.J., S.E. Beddows, P.C. Morrice, F. Nicol, and J.R. Arthur (1987): Inhibition of hepatic deiodination of thyroxine is caused by selenium deficiency in rat. Biochem. J., 248: 443-447.
- Beckett, G.J., A. Russel, F. Nicol, P. Sahu, C.R. Wolf and A.R. Arthur (1992): Effect of selenium deficiency on hepatic type I 5'- iodothyronine deiodinase activity and hepatic thyroid hormone levels in rat. Biochem. J., 282: 483-487.
- Bhatti, B.M. and S. Dil (2005): Effect of vitamin C on immune response in Desi chicken against Newcastle disease. Pakistan J Vet Res., 2 (1):48-9.
- Collins, V.C., A.H. Cantor, M.J. Ford and M.L. Straw (1993): Bioavailability of selenium in selenized yeast for broiler chickens.Poultry Science, 72(suppl. 1): 85.
- Combs, Jr, G.F. and S.B. Combs (1986): The role of selenium in nutrition. Academic Press, Inc New York.
- Darras, V.M., I.R. Berghaman, A. Vanderpooton and E.R. Kuhn (1962): Growth hormone acutely decrease type 111 iodothyronine deiodenase in chicken liver. FEBS. Lett., 310: 5-8.
- Dein, D.F.J. (1984): Avian hematology: the basis. Vet. Clin. North Am.: Small Anirn. Pract. 14:223- 248. Diagn Invest, 20:656– 660.

- Donkoh, A. (1989): Ambient temperature: A factor affecting performance and physiological response of broiler chickens. Int. J. Biometeorol. 33: 259-265.
- Dumas, B.T. W.A. Watson and H.G. Biggs (1971): Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chim. Acta, 31: 87-96.
- Duncan, D. B. (1955): Multiple range and multiple F tests. Biometrics, 11:1– 42.
- Edens, F.W. and K.M. Gowdy (2005): Improvement of the thioredoxinreductase system in the maintenance of cellular redox status. In: T.P. Lyons and K. A. Jacques (Eds.). Nutritional Biotechnology in the Food and Feed industry. Nottingham university Press, Nottingham, United Kingdom. Proc. 20th.Alltech Ann. Sympos., 20: 369-382.
- El-Mallah G.M., S.A. Yassein, M. M. Abdel-Fattah, A.A. El-Ghamry (2011): Improving performance and some metabolic response by using some antioxidants in laying diets during summer season. J. Am. Sci., 7: 217-224.
- Ensminger, M.E., J.E. Oldfield and W.W. Heinemann (1990): Feeds and Nutrition (Heinemann, W.W., ed.), pp. 108–110. The Ensminger Publishing Company, Clovis, CA.
- Fassati, P. and L. Prencipe (1982): Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical Chemistry, 28: 2077-2080.
- Fawcett, J.K. and J.E. Socct (1960): A rapid and precise method for the determination of urea. J. Clin Pathol. Mar 1960, 13(2): 156–159.
- Gallaher, D.D., A.S. Csallany, D.W. Shoeman and J.M. Olson (1993): Diabetes increases excretion of urinary malondehyde conjugates in rats. Lipids, 28: 663-666.
- Goldenberg, H. and A. Fernandez (1966): Simplified method for estimation of inorganic phosphorus in body fluids. Clinical chemistry, 12: 871-882.
- Gornall, A.G., C.J. Bardawill, and M.M. David (1949): Determination of serum protein by means of the biuret reaction. J. Biol. Chem., 177: 751-766.
- Hanafy, M.M., A.M.H. El-Sheikh and E.A. Abdella (2009): The effect of organic selenium supplementation on productive and physiological performance in a local strain of chicken. 1- The effect of organic selenium (sel-plex) on productive and physiological traits of Bandarah strain. Egypt. Poult. Sci., 29: 1061 1084.
- Holik, V. (2009): Management of laying hens to minimize heat stress World Poultry. 44 (1): P.29.
- Hurwitz, S., Weiselberg, M., Eisner, U., Bartov, I., Riesenfeld, G., Sharvit, M., Niv, A. & Bornstein, S. (1980) The energy requirements and performance of growing chickens and turkeys, as affected by environmental temperature. Poult. Sci., 59: 2290–2299.
- Imik, H., S. Ozkanlar, O. Kaynar and M. Koc (2009): Effects of vitamin E, C, and α -lipoic acid supplementation on the serum glucose, lipid profile, and proteins in quails under heat stress. Bull Vet Inst Pulawy, 53: 521-6.

- Ibrahiem, H.A.B. (2008): Studies on some nutritional aspects on laying hens under hjeat stress conditions. M. Sc. thesis, Fac. Agric., Mansoura University.
- Jain, N.C. (1986): Schalm's, veterinary hematology 4th ed. Loe and Febiger Philadelphia, U.S.A.
- Kalaba, Z. M. (2007): Applied nutritional and physiological studies on poultry. Ph. D. thesis, Fac. Agric., Mansoura University.
- Kim, Y.Y. and D.C. Mahan (2003): Biological aspects of selenium in farm animals. Asian-Aust. J. Anim. Sci., 16: 435-444.
- Koelkebeck, K.W. and T.W. Odom (1995): Laying hen responses to acute heat stress and carbon dioxide supplementation: II. Changes in plasma enzymes, metabolites and electrolytes. Comp. Biochem. Physiol., 112A (1): 119-122.
- Kohrle, J., M. Oertel, and M. Gross (1992): Selenium supply regulates thyroid function, thyroid hormones synthesis and metabolism by altering expression of the selenoenzymes type 5'-deiodinase and glutathione peroxidase. Thyroidology, 4: 17-21.
- Konjufca V.H., Pesti G.M. and R.I. Bakalli (1997): Modulation of cholesterol levels in broiler meat by dietary garlic and copper. Poultry Science, 76: 1264–1274.
- Kutlu, H.R. and J.M. Forbes (1993): Changes in growth and blood parameters in heat-stressed broiler chicks in response to dietary ascorbic acid. Livestock Prod. Sci., 36: 335-350.
- Leeson, S. and J. D. Summers (1991): Commercial Poultry Nutrition. University Books. Guelph, Ontario, Canada.
- Lien, T.F., S. Chen, S. Shiau, D. Froman and C.Y. Hu (1996): Chromium picolinate reduces laying hen serum and egg yolk cholesterol. The Professional Animal Scientist, 12: 77–80.
- Lin, H., J. Buyse, Q. Sheng, Y. Xie and J. Song (2003): Effects of ascorbic acid supplementation on the immune function and laying performance of heat-stressed laying hens. Feed Agric. Envir., 1(2): 103-107.
- Lin, H., Jiao, H.C., E. Decuypere and J. Buyse (2005): Physiological responses to heat stress in poultry. Journal of Thermal Biology (submitted).
- Linder, M.C. (1991): Nutrition and metabolism of the trace elements. In: Nutritional Biochemistry and Metabolism with Clinical Applications (Linder, M. C., ed.), 215–276. Elsevier, New York, NY.
- Ljubic B., Milinkovic-Tur S., Pirsljin J., Zdelar-tuk M. and N. Filipovic (2006): Effect of organic selenium food supplementation and fasting on adipose tissue lipid concentration and lipoprotein lipase activity in broiler chickens. In: Proceeding of European Poultry Conference. Verona, Italy.
- Mahmoud, K.Z. and F.W. Edens (2003): Influence of selenium sources on age related and mild heat stress-related changes of blood and liver glutathione redox cycle in broiler chickens (*Gallus domesticus*). Comp. Biochem. Physiol., 136: 921–934.

- Marai, I.F.M., M. S. Ayyat and U.M. Abdel-Monem (2001): growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation under Egyptian conditions. J. Top. Animal health Pod., 33:1-12.
- Mashaly, M.M., G.L. Hendricks, M.A. Kalama, A.E. Gehad, A.O. Abbas and P.H. Patterson (2004): Effect of Heat Stress on Production Parameters and Immune Responses of Commercial Laying Hens. Poultry Sci., 83:889-894.
- McNabb, F.M.A. and D.B. King (1993): Thyroid hormones effect on growth development and metabolism, in The Endocrinology of Growth Development and Metabolism in Vertebrates, T. Schreibman et al., eds., Academic Press, NY, Zoological Science, 10: pp. 873–885.
- Moorehead, W.R. and H.G. Biggs (1974): 2-amino-2-methyl-1-propanol as the alkalizing agent in an improved continuous flow cresolphthalein complexone procedure for calcium in serum. Clinical Chemistry, 20: 1458-1460.
- Nalini, K., A.K. Kataria and A.K. Gahlot (2008): Ambient temperature associated variations in serum hormones and interrelated analytes of broiler chickens in arid tract. Slov. Vet. Res., 45 (4), 127-34.
- Natt, M.P., and C.A. Herrick (1952): A new diluent for counting the erythrocytes and leukocytes of the chicken. Poult. Sci., 31:735–737.
- Ozbey, O., N. Yildiz, M.H. Aysöndü and Ö. Özmen (2004): Effects of high temperature on blood serum parameters and the egg productivity characteristics of Japanese quails (*Coturnix coturnix japonica*). Int. J. Poultry Sci., 3(7): 485-489.
- Prasada, C.S. and N.K.S. Gowda (2005): Importance of trace minerals and relevance of their supplementation in tropical animal feeding system: A review. Nd. J. Anim. Sci., 75:92-100.
- Preuss, H.G., P.L. Grojec, S. Lieberman and R.A. Anderson (1997): Effects of different chromium compounds on blood pressure and lipid peroxidation in spontaneously hypertensive rats. Clin. Nephrol., 47: 325–330.
- Reitman, S. and S. Frankel (1957): A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Amer. J. Clin. Pathol., 28: 56-63.
- Richmond, W. (1973): preparation and proporation of cholesterol oxidase and it applicaytion to enzymatic assay of total cgolesterol in blood. Clin., Chem., 19:1350.
- Sahin K., O. Kucuk, N. Sahin and O. Özbey (2001): Effects of dietary Chromium picolinate supplementation on egg production, egg quality, and serum concentrations of insulin, corticostrerone and some metabolites of Japanese quails. Nutr. Res., 21: 1315–1321.
- Sahin N., Onderci M. and K. Sahin (2002): Effects of dietary chromium and zinc on egg production, egg quality, and some blood metabolites of laying hens reared under low ambient temperature. Biol. Trace Elem. Res. (in press, MMI-059).

- Saito, Y., Y. Yoshida and E. Niki (2007): Cholesterol is more susceptible to oxidation than linoleates in cultured cells under oxidative stress induced by selenium deficiency and free radicals. Fed. Europ. Biochem. Soc., 581: 4349-4354.
- Schales, O. and S.S. Schales (1941): A simple and accurate method for the determination of chloride in biological fluids. J. Biol. Chem., 1941 140: 879-884..
- Seyrek, K., C. Yensey, M. Serter, F.K. Kral, P.A. Ulutas, and H.E. Bardakcogl (2004): Effects of dietary vitamin C supplementation on some serum biochemical parameters of laying Japanese quails exposed to heat stress (34.8 degrees C). Revue-de-Medecine -Veterinarire., 155(6): 339-342.
- Siegel, H.S. (1995): Stress, strains and resistance. British Poultry Sci., 36: 3-20.
- Smart, I.J., D.H. Embury, D.A. Barr, A.J. Sinclair, H. Karunajeewa; I. Ewing, R.L.Reece, W.M. Forsty and P.T. Hooper (1995): Absenceof a role selenium deficiency in the runting syndrome of broiler chickens in Australia. Avian Diseases, 29: 1201-1211.
- SPSS (1999): SPSS[®] statistical software package for the sicial science. Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL 60606-6307.
- Swain, B.K. and T.S. Johri (2000): Effect of supplementation of different combinations of different levels of selenium and vitamin E on relative weight of some organs and serum enzyme level in broilers. Indian J. Poult. Sci., 35: 66–69.
- Trinder, P. (1969): Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem, 6: 24-27.
- Uyanik, F., S. Kaya, A.H. Kolsuz, M. Eren and N. Sahin, (2002): The effects of chromium supplementation on egg production, egg quality, and serum parameters in laying hens. Taurk. J. Vet. Anim. Sci., 26:379-387.
- Yahav, S. (1999): The effect of constant and diurnal cyclic temperatures on performance and blood system of young turkeys. J Thermal Biol, 24:71–8.
- Zollner, N. and K. Kirsch (1962): Col-orimetric method for determination of total lipids". Z. Ges. Exp. Med., 135: 545 -550.

تخفيف التأثيرات العكسية للإجهاد الحراري على الدجاج البياض المحلى باستخدام السلينيوم والكروم العضويين. السمرة حسن على ابو عجلة*، زياد محمد العوضى قلبة*، أحمد عباس حسين طلبة**و ماجد عبد النبى اسماعيل الديب** * قسم انتاج الدواجن-كلية الزراعة- جامعة المنصورة.

** قسم تربية الدواجن- معهد بحوث الانتاج الحيوانى- مركز البحوث الزراعية –الدقى- الجيزة.

أجريت هذه الدراسة في محطة بحوث الإنتاج الحيواني بسخا- كفر الشيخ خلال موسم صيف ٢٠١٢ (يوليو - أغسطس - سبتمبر) لبحث تأثير إضافة السلينيوم والكروم العضويين لعليقة الدجاج البياض المحلى على بعض خصائص الدم أثناء ارتفاع درجات الحرارة خلال فصل الصيف في مصر. استخدم في هذه الدراسة ٢٠٢ دجاجة من سلالة الإنشاص المستنبطة محليا عند عمر ٢٤ أسبوع وقد وزعت الطير عشوائيا في أقفاص جماعية إلى تسع مجمو عات (كل مجموعة ٣٠ دجاجة) قسمت المجموعة إلى ثلاث مكررات (كل مكررة ١٠ دجاجات) وتكون الغذاء التجريبي من إضافة الغذاء الأساسي مع مستويان من كل من السلينيوم سلبلكس (٢٠ و ٥. مللجم/كجم) وبيكولينات الكروم (١٢٠٠ و ١٤٠٠ جزء في المليون/كجم). وأستمرت التجربة من عمر ٢٢ وحتى ٢٢ أسبوع وكانت أهم النتائج كالآتي:

- أدت الاضافات لزيادة معنوية في عد كرات الدم البيضاء بانواعها وكذلك الهيموجلوبين وحجم الخلايا وعدد كرات الدم الحمراء مقارنة بالمعاملة الكنترول.
- كان هناك تحسن أيضا فى محتوى الدم من البروتين الكلى والليبيدات والجلسريدات الثلاثية واليوريا والكلوريد والالبيومين والجلوبيولين والكرياتين وكذلك الكالسيوم والفوسفور حيث كانت أفضل النتائج عند اضافة ٣.٠ مللجم سلينيوم مع ١٤٠٠ جزء فى المليون كروم الى العليقة.
- كان هذاك نقص فى محتوى الدم من ALT وAST نتيجة أضافة الكروم والسيلينيوم الى العليقة منفردين أو مجتمعين عن المعاملة الكنترول بينما كان تركيز هرمونى الغدة الدرقية (T₃ and T₄) أعلى نتيجة اضافة الكروم والسيلينيوم للعليقة.

يستنتج من الدراسة أن إضافة بيكولينات الكروم والسلينيوم سلبلكس خاصة بمعدل ٢.٣ مللجم سلينيوم مع ١٤٠٠ جزء في المليون كروم يمكن ان يستخدما في مقاومة التثيرات السلبية للحرارة العالية خلال فصل الصيف من خلال تحسين بعض خصائص الدم الدجاج البياض.

El-Samra H.A. Abo-Egla et al.