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INFLUENCE OF ADDING ORGANIC CHROMIUM IN DIET ON PRODUCTIVE TRAITS, SERUM CONSTITUENTS AND IMMUNE STATUS OF BANDARAH LAYING HENS AND SEMEN PHYSICAL PROPERTIES FOR COCKS IN WINTER SEASON

By

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Abstract: *This study was to assess the effects of organic chromium supplementation on productive traits, some serum constituents and immune status of Bandarah laying hens and semen physical properties for cocks in winter season. The mean value of temperature and relative humidity during that period in the house was 12.3± 2.2°C and 67.4 ± 6.5%, respectively. One hundred and fifty laying hens and forty cocks of Bandarah local strain at 32 weeks of age were used during winter season and distributed randomly in individual cages to five treatment groups (30 hens and 8 cocks in each group). The basal diet was supplemented with 0 (Control), 250, 500, 1000 and 1500 ppb chromium (Cr) which produced from chromium yeast. The experiment was terminated at 44 weeks. The results indicated that Cr had no significant effect on overall mean of body weight and feed consumption for hens and cocks, while, significantly (P≤0.05) improved egg production, egg weight, albumen percentage, egg shell percentage, egg shell thickness, Haugh unit and yolk index. The interaction between Cr and age was significant for egg production, where, the highest egg production was observed at 44 age of weeks for 1500 ppb Cr. Also, overall means of egg weight was significantly (P≤0.05) increased with the increase of hens age especially at 44 weeks of age. Moreover, Cr had no significant effect on egg shape index, while, yolk weight percentage was decreased compared with the control group. Increased supplemental Cr linearly significantly (P≤0.05) increased the primary or secondary antibody titers against SRBCs, serum total protein, globulin, calcium and insulin concentrations, whereas, total lipids, cholesterol, glucose and corticosterone levels significantly decreased. Serum albumin values were not significantly affected in all Cr supplementation groups compared with control. In addition, supplementation of Cr significantly (P≤0.05) improved semen physical properties and seminal malondialdehyde concentration with one exception the lowest level of Cr (250 ppb) did not change ejaculate volume significantly compared with those for control group. Regardless of Cr supplementation, overall means of semen physical properties and seminal malondialdehyde were significantly (P≤0.05) affected by cock's age. Generally, fertility and hatchability percentages significantly (P≤0.05) increased with the increase of dietary Cr levels. In conclusion, supplementation the chicken diet with 1500 ppb Cr is recommended especially in winter season for improving most of the previous mentioned productive traits, immune response, semen quality, fertility and hatchability.*

INTRODUCTION

Stress is a state of threatened homeostasis provoked by psychological, physiological or environmental stress **(Ramnath and Rekha, 2009)**. Cold conditions cause some adverse effects including increased feed intake, decreased egg production, egg quality, nutrient digestibility, and feed efficiency in laying hens **(Sagher, 1975; Spinu and Degen, 1993; Sari, 1993)**. Attempts to improve productivity under cold climatic conditions were mostly focused on the ways of enhancing energy intake **(Sari, 1993)**. It was reported that the negative effects of environmental stress could be prevented by the use of some minerals and vitamin supplements such as vitamin C and chromium **(Mowat, 1994; Sands and Smith, 1999; Sahin *et al.*, 2001a, 2002)**. Chromium (Cr) is used in poultry diets because of the reported benefits of Cr supplementation to laying hens **(Anderson, 1987; Sahin *et al.*, 2001a, 2002)** during cold and heat stress because Cr is reduced during environmental stress. Supplemental dietary Cr is also recommended by **NRC (1997)** for animals undergoing environmental stress. The primary role of Cr in metabolism is to potentiate the action of insulin through its presence in an organometallic molecule (the glucose tolerance factor) **(Anderson, 1994)**. A stressful condition leads to the excessive production of free radicals, which results in oxidative stress and an imbalance in the oxidant / antioxidant system **(Maier and Watkins, 1998)**. During environmental stress, elevation in the corticosterone level accelerates the generation of free radicals and suppresses the immune function **(McIntosh and Sapolsky, 1996)**. Chromium is an insulin potentiator, therefore, postulated to function as an antioxidant **(Preuss *et al.*, 1997)**. Antioxidants are effective in stressful conditions, as they readily give up their own electrons to free radicals. Moreover, Cr is thought to be essential for activating certain enzymes and for stabilizing proteins and nucleic acids **(Anderson, 1994; Linder, 1991)**.

Research on animals has confirmed that chromium from organic complex such as chromium picolinate, nicotinate and high chromium yeast is absorbed more efficiently, about 25-30 % more than inorganic compounds like chromium chloride, which are poorly absorbed (1-3 %) regardless of does or dietary chromium status **(Underwood and Suttle, 1999; Mowat, 1994)**. Also, previous studies reported that Cr supplementation resulted in higher egg production, egg weight, mass and albumin quality **(Kim *et al.*, 1997; Uyanık *et al.*, 2002 and Yıldız *et al.*, 2004)**. Organic Cr supplementation did not affect body weight and feed consumption for laying hens **(Eseceli *et al.*, 2010)** or laying quails **(Yıldız *et al.*, 2004)**. Moreover, It decreased serum cholesterol **(Yıldız *et al.*, 2004)**, whereas, in other studies Cr had no effect on serum cholesterol concentrations **(Uyanık *et al.*, 2002)**. **Sahin *et al*, (2001b)** reported that organic Cr supplementation, particularly at 1200 ppb, increased the performance criteria, egg quality and serum insulin concentration of Japanese quails. **Jensen *et al.* (1978)** reported that Cr^{+3} had a favourable effect on albumen quality (Haugh unit score) and suggested that this element may be necessary to maintain the physical state of albumen. On the other hand, Cr supplementation supports the immune function by enhancement the cell mediated and humoral immune responses **(Lee *et al.*, 2003; Li *et al.*, 2004 and Lien *et al.*, 2005)**.

Lipids are one of the main components of sperm membrane providing them energy and participate in numerous biochemical processes. The lipid and fatty acid composition (saturated: unsaturated) play an important role in sperm function. The high concentration of polyunsaturated fatty acids within sperm membrane determines peroxidative susceptibility of spermatozoa

(**Surai *et al.*, 1998; Douard *et al.*, 2003**). Pervious studies indicate that lipid peroxidation occurs during ejaculation (**Blesbois *et al.*, 2005**) and even considerably earlier in the male reproductive tract (**Long and Kramer, 2003**). Malondialdehyde (MDA) production is a result of lipid peroxidation. Its level determines the degree of sperm membranes integrity and their fertilizing ability (**Aitken *et al.*, 1989; Long and Kramer, 2003**). Significant correlation between increase in MDA level and decrease in fertility has been shown by **Douard *et al.* (2003)**. Therefore, the objective of this study was to assess the effects of organic Cr supplementation on productive traits, some serum constituents and immune status of Bandarah laying hens and semen physical properties for cocks in winter season.

MATERIALS AND METHODS

This experiment was conducted at El-Sabahia Poultry Research Station (Alexandria), Animal production Research Institute, Agricultural Research Center, Ministry of Agriculture. One hundred and fifty laying hens and forty cocks of Bandarah local strain at 32 weeks of age were housed individually in single cages in an open system house and distributed randomly in five treatment groups (30 females and 8 males in each group). Birds were fed a basal diet of layers (Table 1). Dietary treatments consisted of the basal diet supplemented with 0 (control), 250, 500, 1000 and 1500 ppb Cr produced from Cr yeast. The trial was terminated at 44 weeks of age. The mean value of daily temperature and relative humidity during that period in the house was 12.3± 2.2°C and 67.4 ± 6.5%, respectively The experiment was carried out between December and February. All birds received feed and water *ad libitum*.

Body weights were recorded at the beginning of the experiment then every four weeks, while, feed intake was measured weekly. Egg production and egg weights were recorded daily. At 44 weeks of age, twenty eggs were randomly taken from each treatment for egg quality measurements [egg shape index, egg yolk %, albumen %, shell %, egg shell thickness (mm), Haugh units and yolk index].

Every four weeks, all cocks in each group were massaged and semen was collected individually to determine some semen physical properties such as ejaculate volume (ml), advanced motility (%), alive sperm (%) and sperm concentration (10^6 / mm^3). Malondialdehyde (MDA) in seminal plasma was measured in the form of thiobarbituric acid reactive substance (TBARS) as described by **Richard *et al.* (1992)**. From 40 to 44 wks of age, laying hens were inseminated twice a week with 0.05 mL undiluted semen from cocks that received the same treated diets. Eggs were daily collected from each group every week during the last three weeks and incubated to determine fertility and hatchability percentages.

Antibody response against SRBC was measured from 6 hens in each treatment at 36 and 40 wks of age. Hens were injected with 0.2 mL of 9% SRBC in 0.9% saline. Serum samples were collected at 7 day after each injection to determine anti-SRBC primary or secondary antibody titers, respectively. Antibody production was measured by an agglutination test using the microtiter technique (**Trout *et al.*, 1996**).

On the last day of the trial, 6 hens randomly chosen from each treatment and blood samples were obtained from the brachial vein for serum total protein, albumin, calcium, total lipids, cholesterol, glucose, insulin and corticosterone determination. Blood serum was separated by centrifugation of blood at 3000 rpm for 15 min and was then frozen at -20°C for analysis. Serum total protein, albumin,

calcium, total lipids, cholesterol and glucose concentrations were measured by spectrophotometer using available commercial Kits produced by Bio-diagnostic, Egypt. Also, globulin was calculated in serum of the same samples. Insulin and corticosterone were determined in serum by using radioimmunoassay Kits.

Data were subjected to analysis of variance using the general linear model of SAS software program (**SAS, 1996**). All data percentages were transformed to their arcsine values before analysis and significant differences among means were determined by Duncan multiple rang test (**Duncan, 1955**) at 5 % level of significant. Data of body weight, feed consumption, egg production, egg weight, semen physical properties and seminal malondialdehyde concentration were analyzed by two way method using the following model: $X_{ijk} = \mu + T_i + A_j + (TA)_{ij} + e_{ijk}$ Where : X_{ijk} = an observation, μ =Overall mean, Ti = Cr levels (i=5; 0, 250, 500, 1000 and 1500 ppb), A_j = age (j= 3; 36, 40 and 44), (TF)ij= interaction between Cr levels and age and e_{ijk} = experimental error

Data of egg quality, antibody response against SRBC, blood constituents, fertility and hatchability were analyzed by one way method using the following model: $Y_{ij} = \mu + T_i + e_{ij}$ Where: Y_{ij} = an observation, μ = overall mean, T_i = treatment (i=5; 0, 250, 500, 1000 and 1500 ppb) and e_{ij} = experimental error

RESULTS AND DISCUSSION

Body Weight and Feed Consumption

No any concentration of Cr supplementation had a significant effect on body weight and feed consumption among all ages for both sexes of experimented birds (Table 2). Moreover, the interaction between Cr and age was not significant for body weight and feed consumption for both sexes. Supporting to these results in the current study, **Eseceli *et al.* (2010)** reported that chromium yeast supplementation did not affect body weight in laying hens (40- 47 wks). Also, **Yıldız *et al.* (2004)** reported that supplementation of Cr from Cr picolinate did not affect body weight change and feed intake in laying Japanese quail.

Egg Production and Egg Weight

The results of the current study indicate that supplementation Cr significantly ($P \leq 0.05$) increased egg production and egg weight in winter season (Table 3). Regardless, of Cr supplementation overall means of egg production percentage had not significantly influenced by the laying hen age, whereas, egg production percentage increased numerically at 44 wks compared with those at 36 and 40 wks of age. The interaction between Cr and age was significant for egg production, where, the highest egg production was observed at 44 age of weeks for 1500 ppb Cr. Irrespective, of Cr supplementation, overall means of egg weight was significantly ($P \leq 0.05$) increased with the increase of hens age especially at 44 weeks of age. Besides, Regardless of laying age, supplementation the diet with 1000 or 1500 ppb concentrations significantly ($P \leq 0.05$) increased egg weight being 48.47 and 48.96, respectively compared with other experimented concentrations and control. The results of increasing egg production and egg weight with Cr supplementation are consistent with earlier reports. **Kim *et al.* (1997)** reported that feeding 800 ppb Cr from Cr picolinate to laying hen diets resulted in higher egg production and egg weight compared with the negative control group. Also, **Liu *et al.* (1999)** reported that Cr fed at 10 ppm level enhanced egg production and egg weight of layer. **Sahin *et al.* (2001a)** reported that supplementation of 400 ppb chromium to the diet of laying hens reared

under a low ambient temperature increased egg production. **Sahin *et al.* (2002)** stated that higher doses of supplemental Cr increased egg production and improved egg weight in laying hens kept under low temperature. In addition, adding of 400 ppb Cr to diet of laying hens increased egg production **(Piva *et al.*, 2003)**.

Egg Quality

Effect of Cr supplementation on egg quality at 44 wks of age is shown in Table 4. No significant differences in egg shape index were observed between control and the treatment groups. On the other hand, albumen percentage, egg shell percentage, egg shell thickness, Haugh unit and yolk index were significantly increased as dietary Cr levels increased. Conversely, Cr supplementation to the diet showed a decrease in yolk weight percentage compared with the control group. The data obtained in this study are in agreement partially with the results of **Sahin *et al.* (2001b)** who reported that an increment in egg weight, eggshell weight, eggshell thickness, albumen index, albumen weight, yolk index and yolk weight are affected by supplementing chromium picolinate in laying Japanese quails. **Jensen *et al.* (1978)** reported that Cr^{+3} had a favorable effect on albumen quality (Haugh unit score) and suggested that this element may be necessary to maintain the physical state of albumen. **Hossain (1998)** suggested that the possible mechanisms by which Cr could work to maintain egg quality are: (1) as a structural component of egg albumen or in the cross linking of proteins, (2) Cr is necessary for the synthesis of ovomucin which is responsible for gel structure of albumen, and (3) facilitate transfer of cations (possibly magnesium) into the albumen of eggs during the plumping process in the uterus.. In Lohman White laying hens chromium yeast supplementation increased albumen and yolk index **(Eseceli *et al.*, 2010)**. **Uyanık *et al.* (2002)** indicated that supplementation of 20 ppm Cr from chromium chloride supplementation to the diet of laying hens increased albumen and egg yolk index values. **Kucukersan *et al.* (2005)** found that 20 ppm organic chromium and 250 ppm Vitamin E supplementation increased egg shell thickness in 40 weeks old laying hens. **Yıldız *et al.* (2004)** reported that egg, yolk and albumen weights increased linearly as dietary Cr level increased ($P \leq 0.05$), but did not affect eggshell weight and eggshell thickness. These findings strongly suggest that Cr is involved in maintenance of the normal physical state of egg albumen. The decrease in egg yolk percentage may due to the decrease in egg yolk cholesterol where **Nichols *et al.* (1963)** reported an inverse relationship between yolk weight and yolk content. Increasing egg shell thickness may due to Cr stimulates and regulates the action of insulin **(Anderson, 1994; Mowat, 1994)**; thus increasing the effectiveness of insulin, Cr also indirectly empowers the ascorbic acid transportation **(Kapeghian and Verlangieri, 1984; Seaborn *et al.*, 1994)** which has an important role in egg shell formation **(Dorr and Balloun, 1976)**.

Antibody Response and Blood Constituents

With respect to humoral immune responses, the results in Table 5 indicated that primary and secondary antibody titers against SRBCs were significantly ($P \leq 0.05$) increased in Bandarah laying hens receiving different level of Cr as compared to the control group. Similarly to our results, **Lee *et al.* (2003) and Cao *et al.* (2004)** investigated that total antibody tended to be higher ($P \leq 0.05$) in Cr added groups. **El-Hommosany (2008)** demonstrated that total antibody and IgG titers against SRBCs were significantly higher in quail chicks received Cr at low (125 µg/kg feed) and middle (250 µg/kg feed) doses compared with those of control at secondary immune responses. Also, it has been reported that chromium modulates the immune response through its effect on cytokine release **(Wang *et al.*, 1996)**. Cytokines are small proteins or

glycoproteins messenger molecules transporting information among cells. Cytokines, together with their receptors are playing a role as central regulators of immune system by affecting the activity of other cells **(Callard *et al.*, 1999; Davison, 2003).**

With regard to blood constituents (Table 5) serum total protein, globulin, calcium and insulin concentration significantly ($P \leq 0.05$) increased linearly as dietary Cr levels increased. However, serum albumin value did not significantly affected in all Cr groups compared with the control group. While, serum total lipids, cholesterol, glucose and corticosterone concentrations significantly ($P \leq 0.05$) decreased. Like in our study, **Sahin *et al.* (2001b)** found that Cr supplementation markedly decreased blood glucose, corticosterone and cholesterol concentrations while serum insulin and protein concentrations increased linearly ($P = 0.001$) with higher dietary chromium supplementation in Japanese quail. Besides, **Yıldız *et al.* (2004)** reported that Cr supplementation from Cr picolinate decreased serum glucose and cholesterol concentrations, while insulin and total protein concentrations increased linearly as dietary Cr level increased ($P \leq 0.05$). **Uyanik *et al.* (2002)** obtained controversial results. They found that Cr did not affect serum cholesterol, but reduced serum glucose and increased serum protein, albumin and globulin. **Lee *et al.* (2003)** reported that serum Ca was higher as affected by Cr supplement. **Sahin and Onderci (2002)** found that adding Cr to broiler diet increased serum Ca. A possible explanation for the effect of Cr supplementation on Ca metabolism may be due to that this mineral (Cr) compete for the same binding sites, so increased Cr concentration causing a decrease in freeing of binding sites on the transferring, competed by the individual minerals. According to **Prasad and Gowda (2005)** Cr act as glucose tolerance factor increased the uptake of glucose by cells and potentiated insulin action. The reduction effect of Cr on plasma glucose in the present study may support this suggestion. On the other hand, **Uyanik *et al.* (2002)** attributed the positive effects of Cr on plasma protein and its fractions to the anabolic action of insulin mediated through increasing the amino acids synthesis by liver, enhancement the incorporation of several amino acids into protein. However, **Mc Namara and Valdez (2005)** suggested that effect of Cr on lipid metabolism may be due to that Cr increased the synthesis of fat in the adipose tissue and decreased the release of it. This might be acting through increased glucose flux into the adipocytes.

Semen Physical Properties and Seminal Malondialdehyde

Significant improvements in semen physical properties and seminal malondialdehyde concentration were observed as affected by supplementing Cr from Cr yeast with one exception the lowest level of Cr (250 ppb) did not change ejaculate volume significantly compared with those for control group (Table 6). Regardless of Cr supplementation, overall means of semen physical properties and seminal malondialdehyde were significantly ($P \leq 0.05$) affected by cock's age. The improvement in semen physical properties might be due to the action of antioxidants of chromium which reduced the oxidants damage and maintained the integrity of cell membrane. This result agrees with previous studies which showed that chromium is an antioxidant and influences lipid peroxidation by fighting free radical damage in the body **(Gallaher *et al.*, 1993; Preuss *et al.*, 1997).** The reduced in seminal malondialdehyde might also be due to the ability of Cr antioxidants in the supplementations to resist the lipid peroxidation damage in the spermatozoa. Moreover, there is a significant correlation between increase in MDA level and decrease in fertility has been shown by **Douard *et al.* (2003)**. The depression in

malondialdehyde level is an indicator the degree of sperm membranes integrity and their fertilizing ability (**Aitken *et al.*, 1989; Long and Kramer, 2003**).

Fertility and Hatchability Percentages

All the concentrations of dietary Cr supplementations significantly ($P \leq 0.05$) increased the fertility and hatchability percentages compared with those for control. Moreover, the best significant result of of fertility (92.87%) was recorded for eggs produced from birds fed diet supplemented with 1500 ppb Cr compared with those for other egg groups produced from birds fed the other experimented concentrations of Cr (Table 7). The same concentration of Cr (1500 ppb) realized the best significant result of fertility (95.67%) compared with other groups, while, the difference with those for 1000 ppb Cr was not significant. This result agrees with those reported by **Contreras and Barajas (2001)** who reported that a supplement 400 ppb of Cr from Cr Methionine improved hatchability compared with the control (74 and 64.8 % respectively) of Japanese quail in winter season. Moreover, **Contreras *et al.* (2000)** showed that 200 ppb chromium Methionine supplementation improves hatchability in Japanese quail under weather controlled conditions in dry tropic areas (25°C).

Table 1: Composition and calculated analysis of basal diet.

Ingredients	%
Yellow corn	64.00
Soybean meal 44%	24.78
Wheat bran	1.00
Di-calcium phosphate	1.61
Limestone	7.91
DL-Methionine	0.10
Sodium chloride	0.30
Vit. & Min. Mixture*	0.30
Total	100.00
Calculated analysis:	
Metabolizable energy (Kcal/Kg)	2718.00
Crude protein %	16.02
Crude fiber %	3.46
Crude fat %	2.96
Calcium %	3.34
Available phosphorous %	0.42
Lysine %	0.89
Methionine %	0.39
Met+cystine %	0.66

*Supplied per kg diet: Vit A, 10000IU; Vit D_3, 2000 IU; Vit E, 10 mg; Vit K_3, 1 mg; Vit B_1, 1 mg; Vit B_2, 5mg; Vit B_6, 1.5 mg; Vit B_{12}, 10 mcg; Niacin, 30 mg; Pantothenic acid, 10 mg; Folic acid, 1 mg; Biotin, 50mcg; Choline, 260 mg; Copper,4 mg; Iron, 30 mg; manganese, 60 mg;Zinc, 50 mg; Iodine, 1.3 mg; Selenium, 0.1mg; Cobalt,0.1mg.

Table 2: Effect of chromium yeast supplementation on body weight and feed consumption of Bandarah laying hens and cocks at different ages (Means ± SE).

sex	Age (wks)	Chromium levels (ppb)					Overall mean
		0	250	500	1000	1500	
Hens	Body weight (kg)						
	36	1.260±0.07	1.265±0.08	1.275±0.03	1.280±0.09	1.281±0.08	1.272±0.05
	40	1.300±0.08	1.310±0.05	1.310±0.09	1.316±0.05	1.325±0.08	1.310±0.07
	44	1.320±0.07	1.322±0.04	1.373±0.10	1.345±0.06	1.359±0.03	1.336±0.06
	Overall mean	1.293±0.06	1.296±0.03	1.319±0.07	1.314±0.05	1.322±0.06	
	Feed consumption (g/ bird /day)						
	36	113.12±0.055	114.51±0.75	114.30±0.42	114.63±0.51	114.83±0.77	114.28±0.50
	40	114.20±0.68	114.62±0.80	114.71±0.59	114.85±0.67	114.91±0.84	114.66±0.61
	44	114.30±0.39	114.97±0.45	114.90±0.77	114.87±0.76	115.02±0.90	114.81±0.42
	Overall mean	113.87±0.67	114.7±0.51	114.64±0.60	114.78±0.45	114.92±0.70	
Cocks	Body weight (kg)						
	36	1.767±0.10	1.783±0.08	1.800±0.07	1.833±0.09	1.843±0.09	1.805±0.06
	40	1.773±0.10	1.800±0.10	1.826±0.10	1.848±0.10	1.863±0.07	1.822±0.08
	44	1.820±0.09	1.827±0.08	1.835±0.06	1.870±0.06	1.881±0.10	1.847±0.05
	Overall mean	1.787±0.08	1.803±0.06	1.820±0.08	1.850±0.06	1.862±0.08	
	Feed consumption (g/ bird /day)						
	36	117.47±0.74	117.57±0.77	117.75±0.45	117.80±0.59	117.83±0.69	117.68±0.33
	40	117.80±0.75	117.77±0.85	117.85±0.47	117.97±0.60	118.01±0.73	117.88±0.55
	44	118.10±0.95	118.20±0.66	118.15±0.56	118.45±0.43	118.53±0.50	118.29±0.25
	Overall mean	117.79±0..65	117.85±0.51	117.92±0.35	118.07±0.34	118.12±048	

Table 3: Effect of chromium yeast supplementation on egg production and egg weight of Bandarah laying hens at different ages (Means ± SE).

Age (wks)	Chromium levels (ppb)					Overall mean
	0	250	500	1000	1500	
Egg production (%)						
36	54.11 ± 0.91^{g}	59.95 ± 0.82^{defg}	61.80 ± 0.99^{defg}	62.91 ± 0.79^{defg}	63.05 ± 0.86^{defg}	60.29±0.78
40	55.78 ± 1.01^{fg}	60.81 ± 0.74^{defg}	61.88 ± 0.75^{defg}	62.99 ± 0.90^{defg}	63.40 ± 0.91^{defg}	60.97±0.85
44	56.78 ± 1.10^{defg}	65.06 ± 0.62^{def}	66.62 ± 0.85^{def}	67.40 ± 0.99^{def}	67.95 ± 1.03^{d}	64.76±0.77
Overall mean	55.550 ± 0.87^{b}	61.82 ± 0.55^{a}	63.43 ± 0.65^{a}	64.43 ± 0.81^{a}	64.80 ± 0.89^{a}	
Egg weight (g)						
36	46.61±0.32	47.28±0.36	47.57±0.34	48.11±0.33	48.56±0.29	47.62 ± 0.16^{B}
40	46.96±0.22	47.22±0.15	47.58±0.11	48.18±0.29	48.78±0.32	47.74 ± 0.20^{B}
44	47.05±0.23	48.08±0.36	48.60±0.39	49.13±0.40	49.55±0.37	48.48 ± 0.19^{A}
Overall mean	46.87 ± 0.16^{c}	47.52 ± 0.25^{b}	47.92 ± 0.30^{b}	48.47 ± 0.21^{a}	48.96 ± 0.19^{a}	

a,b,c = Means having different letters exponents within each row are significantly different at $P \leq 0.05$.
d,e,f,g = Means within age of hens by chromium yeast supplementation interaction effect within no common superscript differ significantly ($P \leq 0.05$).
A, B = Means having different letters exponents within each column are significantly different at $P \leq 0.05$.

Table 4: Effect of chromium yeast supplementation on egg quality at 44 weeks of age (Means ± SE).

Items	Chromium levels (ppb)				
	0	250	500	1000	1500
Egg shape index	75.62±0.85	75.80±1.01	75.93±0.95	76.11±1.00	76.32±0.96
Egg yolk (%)	31.70±0.35^{a}	29.44±0.18^{b}	29.68±0.20^{b}	29.23±0.33^{b}	28.80±0.36^{c}
Egg albumen (%)	55.13±0.20^{b}	56.53±0.21^{a}	56.26±0.25^{a}	56.05±0.13^{a}	56.39±0.21^{a}
Egg shell (%)	13.17±0.10^{c}	14.03±0.13^{b}	14.07±0.14^{b}	14.72±0.13^{a}	14.81±0.12^{a}
Shell thickness (mm)	0.332±0.007^{b}	0.392±0.002^{a}	0.406±0.001^{a}	0.400±0.005^{a}	0.424±0.007^{a}
Haugh unit	75.15±0.42^{b}	80.83±0.49^{a}	81.13±0.65^{a}	81.44±0.28^{a}	82.00±0.52^{a}
Yolk index	45.21±0.53^{b}	52.81±0.94^{a}	53.16±0.66^{a}	55.68±0.72^{a}	57.69±0.32^{a}

a,b,c = Means having different letters exponents within each row are significantly different at $P \leq 0.05$.

Table 5: Effect of chromium yeast supplementation on antibody titers against sheep red blood cells and some blood constituents of Bandarah laying hens (Means ± SE).

Items	Chromium levels (ppb)				
	0	250	500	1000	1500
Primary antibody titers against SRBCs	3.14±0.07^{d}	3.47±0.08^{c}	3.84±0.18^{b}	4.17±0.08^{a}	4.32±0.12^{a}
Secondary antibody titers against SRBCs	4.00±0.09^{c}	4.70±0.16^{b}	5.07±0.07^{a}	5.12±0.09^{a}	5.31±0.09^{a}
Total protein (g/dl)	3.98±0.25^{c}	4.85±0.21^{b}	4.90±0.25^{b}	5.34±0.34^{ab}	5.98±0.30^{a}
Albumin (g/dl)	1.95±0.18	1.77±0.19	1.79±0.20	1.88±0.12	1.67±0.06
Globulin (g/dl)	2.02±0.35^{c}	3.08±0.13^{b}	3.81±0.29^{b}	3.46±0.30^{b}	4.31±0.25^{a}
Calcium (mg/dl)	8.33±0.21^{b}	9.70±0.36^{a}	10.01±0.24^{a}	10.22±0.30^{a}	10.74±0.71^{a}
Total lipids (g/dl)	5.91±0.37^{a}	4.27±0.36^{b}	3.87±0.19^{bc}	3.61±0.23^{bc}	3.02±0.27^{c}
Cholesterol (mg/dl)	190.83±3.95^{a}	137.53±6.55^{b}	121.71±9.92^{b}	120.86±9.14^{b}	119.15±7.19^{b}
Glucose (mg/dl)	4.95±0.15^{a}	4.03±0.19^{b}	3.48±0.28^{bc}	3.34±0.24^{bc}	3.15±0.31^{c}
Insulin (ng/ml)	0.142±0.009^{e}	0.166±0.007^{d}	0.185±0.005^{c}	0.207±0.009^{b}	0.234±0.006^{a}
Corticosterone (ng/ml	3607.6±193.8^{a}	2346.1±168.1^{b}	1479.1±155.6^{c}	1459±145.3^{c}	1294.8±140.6^{c}

a,b,c,d,e = Means having different letters exponents within each row are significantly different at $P \leq 0.05$.

Table 6: Effect of chromium yeast supplementation on semen physical properties and seminal malondialdehyde level of Bandarah cocks at different ages (Means ± SE).

Age (wks)	Chromium levels (ppb)					Overall mean
	0	250	500	1000	1500	
Ejaculate volume (ml)						
36	0.24±0.032	0.30±0.019	0.38±0.040	0.41±0.052	0.43±0.056	$0.35±0.023^{B}$
40	0..25±0.027	0.31±0.023	0.43±0.037	0.46±0.037	0.50±0.070	$0.39±0.031^{B}$
44	0.28±0.025	0.34±0.026	0.55±0.057	0.59±0.064	0.61±0.079	$0.47±0.037^{A}$
Overall mean	$0.25±0.020^{b}$	$0.32±0.015^{b}$	$0.45±0.031^{a}$	$0.49±0.035^{a}$	$0.51±0.041^{a}$	
Advanced motility (%)						
36	83.75±1.25	87.50±0.94	89.38±1.48	90.63±1.48	91.25±1.57	$85.50±0.98^{B}$
40	86.25±1.26	88.75±1.29	90.00±1.34	91.88±0.95	93.75±0.82	$90.13±1.00^{A}$
44	87.50±0.94	90.63±1.48	92.50±1.34	93.13±0.91	94.38±0.62	$91.63±0.89^{A}$
Overall mean	$85.83±0.90^{d}$	$88.96±0.88^{c}$	$90.63±1.01^{bc}$	$91.88±0.93^{ab}$	$93.13±0.72^{a}$	
Alive sperm (%)						
36	76.50±1.76	79.25±1.31	82.50±1.67	86.63±1.68	87.25±1.71	$82.43±1.21^{B}$
40	77.25±1.74	81.0±1.54	84.25±1.16	88.25±0.96	89.50±2.04	$84.05±1.37^{AB}$
44	78.25±1.50	81.88±1.90	85.38±1.78	89.88±0.79	91.88±0.87	$85.45±1.13^{A}$
Overall mean	$77.33±1.11^{d}$	$80.71±1.06^{c}$	$84.04±0.94^{b}$	$88.25±0.87^{a}$	$89.54±1.18^{a}$	
Sperm concentration (106/mm3)						
36	1.80±0.09	2.41±0.13	2.50±0.16	2.63±0.13	2.71±0.11	$2.41±0.10^{C}$
40	2.20±0.19	2.43±0.14	2.68±0.13	2.83±0.19	2.96±0.18	$2.62±0.12^{B}$
44	2.53±0.20	2.74±0.10	2.78±0.11	2.98±0.15	3.16±0.19	$2.84±0.13^{A}$
Overall mean	$2.18±0.10^{d}$	$2.53±0.07^{c}$	$2.05±0.05^{bc}$	$2.81±0.11^{ab}$	$2.94±0.09^{a}$	
Malondialdehyde (nmol/ml)						
36	0.56±0.04	0.58±0.03	0.63±0.04	0.66±0.05	0.67±0.05	$0.62±0.04^{B}$
40	0.57±0.04	0.65±0.02	0.67±0.02	0.69±0.04	0.74±0.06	$0.66±0.03^{B}$
44	0.57±0.04	0.69±0.03	0.72±0.03	0.75±0.03	0.84±0.03	$0.71±0.02^{A}$
Overall mean	$0.57±0.03^{d}$	$0.64±0.01^{c}$	$0.67±0.03^{bc}$	$0.70±0.04^{ab}$	$0.75±0.05^{a}$	

a,b,c,d = Means having different letters exponents within each row are significantly different at $P \leq 0.05$.
A,B,C = Means having different letters exponents within each column are significantly different at $P \leq 0.05$.

Table 7: Effect of chromium yeast supplementation on fertility and hatchability percentages of Bandarah chickens (Means ± SE).

traits	Chromium levels (ppb)				
	0	250	500	1000	1500
Fertility (%)	$86.83±0.62^{d}$	$91.00±0.58^{c}$	$93.00±0.59^{bc}$	$94.67±0.57^{ab}$	$95.67±0.26^{a}$
Hatchability (%)	$83.67±0.33^{d}$	$88.33±0.34^{c}$	$91.00±0.58^{b}$	$91.67±0.88^{b}$	$92.87±0.36^{a}$

a,b,c,d = Means having different letters exponents within each row are significantly different at $P \leq 0.05$.

REFERENCES

Aitken, J. R.; Clarkson, J. S. and Fishel, S. (1989). *Generation of reactive oxygen species, lipid peroxidation, and human sperm function. Biol. Reprod. 40: 183-197.*

Anderson, R. A. (1987). *Chromium. Trace Elements in Human and Animal Nutrition, pp. 225–244. Academic Press, New York, NY.*

Anderson, R. A. (1994). *Stress effects on chromium nutrition of humans and farm animals. In: Biotechnology in the Feed Industry (Lyons, T. P. & Jacques, K. A., eds.), pp. 267–274. University Press, Nottingham, UK.*

Blesbois, E.; Grasseau, I. and Seigneurin, F. (2005). *Membrane fluidity and the ability of domestic bird spermatozoa to survive cryopreservation. Reproduction 129: 371-378.*

Callard, R.; George, A. J. and Stark, J. (1999). *Cytokines, chaos, and complexity. Immunity 11:507-513.*

Cao, J. Y.; Li, K.; Lu, X. C. and Zhao,Y. X. (2004). *Effect of florfenical and chromium (III) on humoral immune response in chicks. Asian Austr. J. Anim. Sci. 17: 366-370.*

Contreras, G. and Barajas, R. (2001). *Effect of chromium-methionine level in diet on hatchability of Japanese quail in dry tropic weather: II. response under temperature-controlled in winter season. Poult. Sci. 80:323 (abstract).*

Contreras, G.; Barajas, R. and Montoya, A. (2000). *Effect of chromium methionine supplementation on egg hatching response of Japanese quail under controlled temperature condition in dry tropic weather. J. Anim. Sci. 78:241(abstract).*

Davison, T.F. (2003). *The immunologists' debt to the chicken. Br. Poult. Sci. 44: 6 – 21.*

Dorr, P. and Balloun, S. L. (1976). *Effect of dietary vitamin A, ascorbic acid and their interaction on turkey bone mineralization. Br. Poult. Sci. 17: 581–599.*

Douard, W.; Hermier, D.; Magistrini, M. and Blesbois, E. (2003). *Reproductive period affects lipid composition and quality of fresh and stored spermatozoa in turkeys. Theriogenology 59: 753-764.*

Duncan, D. B. (1955). *Multiple range and multiple F- test, Biometrics 11:1-42.*

El-Hommosany, Y. M. (2008). *Study of the physiological changes in blood chemistry, humoral immune response and performance of quail chicks fed supplemental chromium. Int. J. Poult. Sci. 7: 40-44.*

Eseceli, H.; Degirmencioglu, N. and Bilgic, M. (2010).*The effect of inclusion of chromium yeast (Co- Factor II, Alltech Inc.) and folic acid to the rations of laying hens on performance, egg quality, egg yolk cholesterol, folic acid and chromium levels. J. Anim. Vet. Advan. 9:384-391.*

Gallaher, D. D.; Csallany, A. S.; Shoeman, D. W. and Olson, J. M. (1993). *Diabetes increases excretion of urinary malondehyde conjugates in rats. Lipids 28: 663-666.*

Hossain, S. M. (1998). *Organic chromium in poultry: Metabolic responses, effects on broiler carcass composition, nutrient composition of eggs. In: Lyons, T.P. and K.A. Jacques (Eds.). Biotechnology in the Feed Industry. Nottingham University Press, Nottingham, UK 203-216.*

Jensen, L. S.; Chang, C. H. and Wilson, S. P. (1978). *Interior egg quality: improvement by distillers' feeds and traces elements. Poult. Sci. 57: 648-654.*

Kapeghian, J. C. and Verlangieri, A. J. (1984). *The effects of glucose on ascorbic acid uptake in heart endothelial cells: possible pathogenesis of diabetic angiopathies. Life Sci. 34: 577–584.*

Kim, J. D.; Han, I. K.; Chae, B. J.; Lee, J. H.; Park, J. H. and Yang, C. J. (1997). *Effects of dietary chromium picolinate on performance, egg quality, serum traits and mortality rate of brown layers. Asian Austr. J. Anim. Sci. 10: 1-7.*

Kucukersan, S.; Yesilbag, D.; Kucukersan, K. and Goncuoglu, E. (2005). *The effects of combination of organic and inorganic chromium with*

vitamin E supplemented to diet on performance, egg production and egg quality in laying hens. III. National Congress of Animal Nutrition. 7-10 September, Adana-Turkey.

Lee, D. N.; Wu, F. Y.; Cheng, Y. H.; Lin, R. S. and Wu, P. C. (2003). *Effect of dietary chromium picolinate supplementation on growth performance and immune responses of broilers. Asian Austr. J. Anim. Sci. 16: 227-233.*

Li, S. H.; Wang, J.; Zheng, Y. M.; Xu, W. X.; Jin, G. M. and Dong, H. (2004). *Effect of chromium on the organization and structure of spleen of heat stressed layer. Chin. J. Vet. Sci. 24: 71-74.*

Lien, T. F.; Yang, K. H. and Lin, K. J. (2005). *Effect of chromium propionate supplementation on growth performance, serum traits and immune response in weaned pigs. Asian Austr. J. Anim. Sci. 18: 403- 408.*

Linder, M. C. (1991). *Nutrition and metabolism of the trace elements. In: Nutritional Biochemistry and Metabolism with Clinical Applications (Linder, M. C., ed.), pp. 215–276. Elsevier, New York, NY.*

Liu, P. X.; Chen, L. J.; Xie, D. B. and Xiong, X. M. (1999). *Effects of dietary chromium on the productivity of laying hens and the distribution of chromium. Acta Agric. Univ. Jangxiensis 21: 564-568.*

Long, J. A. and Kramer, M. (2003). *Effect of vitamin E on lipid peroxidation and fertility after artificial insemination with liquid-stored turkey semen. Poult. Sci. 82: 1802-1807.*

Maier, S. F. and Watkins, L. R. (1998). *Cytokines for psychologists, Implications for bidirectional immune to brain communication for understanding behaviour, mood and cognition. Psychol. Rev. 105:83–107.*

McIntosh, L. J. and Sapolsky, R. M. (1996). *Glucocorticoids increase the accumulation of reactive oxygen species and enhance adriamycin induced toxicity in neuronal culture. Exp Neurol. 141:201-206 .*

Mc Namara, J. P. and Valdez, F. (2005). *Adipose tissue metabolism and production responses to calcium propionate and chromium propionate. J. Dairy Sci. 88: 498-507.*

Mowat, D. N. (1994). *Organic chromium. A new nutrient for stressed animals. In: Biotechnology in the Feed Industry (Lyons, T. P. & Jacques, K. A., eds.), pp. 275–282. University Press, Nottingham, UK.*

Nichols, E. L.; Marion, W. W. and Balloun, S. L. (1963). *Effect of egg yolk size on yolk cholesterol concentration. Proceedings Society for Experimental Biology and Medicine 112: 378-380.*

National Research Council (1997). *The role of chromium in animal nutrition. committee on animal nutrition board on Agriculture National Research Council, National Acad. Press, Washington, DC.*

Piva, A.; Meola, E.; Gatta, P. P.; Blagi, G. C.; Mordenti, A. L.; Luchansky, J. B.; Silva, S. and Mordenti, A. (2003). *The effect of dietary supplementation with trivalent chromium on production performance of laying hens and the chromium content in the yolk. Anim. Feed Sci. Tech. 106: 149-163.*

Prasad, C. S. and Gowda, N. K. S. (2005). *Imortance of trace minerals and relevance of their supplementation in tropical animal feeding system. A review. Ind. J. Anim. Sci. 75: 92-100.*

Preuss, H. G.; Grojec, P. L.; Lieberman, S. and Anderson, R. A. (1997). *Effects of different chromium compounds on blood pressure and lipid peroxidation in spontaneously hypertensive rats. Clin. Nephrol. 47: 325–330.*

Ramnath, V. and Rekha, P. S. (2009). *Brahma Rasayana enhances in vivo antioxidant status in cold-stressed chickens (gallus gallus domesticus). Indian J Pharmacol. 41: 115–119.*

Richard, M. J.; Portal, B.; Meo, J.; Coudray, C.; Hadjian, A. and Favier, A. (1992). *Malondialdehyde kit evaluated for determining plasma and lipoprotein fractions that react with thiobarbituric acid. Clin. Chem. 38: 704–709.*

Sagher, B. M. (1975). *The Effect of cold stress on muscle growth in young chicks. Growth 39: 281-288.*

Sahin, K. and Onderci, M. (2002). *Optimal dietary concentration of vitamin C and chromium for alleviating the effect of low ambient temperature on serum insulin, corticosterone and some blood metabolism in laying hens. J. Trace Elem. Exper. Med., 15: 153-161.*

Sahin, K.; Kucuk, O. and Sahin, N. (2001a). *Effects of dietary chromium picolinate supplementation on performance, insulin and corticostrerone in laying hens under low ambient temperature. J. Anim. Physiol. Anim. Nutr. 85: 142–147.*

Sahin, K.; Kucuk, O.; Sahin, N. and Ozbey, O. (2001b). *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 Sahin, K. (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. 85: 47–58.*

Sands, J. S. and Smith, M. O. (1999). *Broilers in heat stress conditions: effects of dietary manganese proteinate or chromium picolinate supplementation. J. Appl. Poult. Res. 8: 280– 287.*

Sari, M. (1993). *Çevrenin evcil hayvanlarda besin maddesi ihtiyaçlari üzerine etkileri. Yüksek Lisans Ders Notlari, Elazig.*

SAS Institute. (1996). *SAS User's Guide. Release 6.12 Edition: SAS institute Inc., Cary, NC.*

Seaborn, C. D.; Cheng, N.; Adeleye, B.; Owens, F. and Stoecker, B. J. (1994). *Chromium and chronic ascorbic acid depletion effects on tissue ascorbate, manganese, and 14C retention from 14C-ascorbate in guinea pigs. Biol. Trace Elem. Res. 41: 279-294.*

Spinu, M. and Degen, A. A. (1993). *Effect of cold stress on performance and immune responses of Bedouin and White Leghorn hens. Br. Poult. Sci. 34: 177-185.*

Surai, P. F.; Blesbois, E.; Grasseau, I.; Chalah, T.; Brillard, J. P.; Wishart, G. J.; Cerolini, S. and Sparks, N. H. C. (1998). *Fatty acid composition, glutathione peroxidase and superoxide dismutase activity and total antioxidant activity of avian semen. Comp. Biochem. Physiol. B, Biochem. Mol. Biol. 120: 527-533.*

Trout, J. M.; Mashaly, M. M. and Siegel, H. S. (1996). *Changes in blood and spleen lymphocyte populations following antigen challenge in*

immature male chickens. Br. Poult. Sci., 37: 819-827.

Underwood, E. J. and Suttle, N. F. (1999). *The mineral nutrition of livestock, 3rd Ed. CAB International, Wallingford, UK., pp: 517-518.*

Uyanik, F.; Kaya, S.; Kolsuz, A. H.; Eren, M. and Sahin, N. (2002). *The effect of chromium supplementation on egg production, egg quality and some serum parameters in laying hens. Turk. J. Vet. Anim. Sci. 26: 379-387.*

Wang, J. Y; Tsukayama, D. T.; Wicklund, B. H. and Gustilo, R. B. (1996). *Inhibition of T and B proliferation by titanium, cobalt, and chromium: role for IL-2 and IL-6. J. Biomed. Mater. Res. 32: 655–661.*

Yıldız, A. O.; Parlat, S. S. and Yazgan, O. (2004). *The effects of organic chromium supplementation on production traits and some serum parameters of laying quails. Revue. Med. Vet. 155: 642-646.*

الملخص العربى

تأثير أضافة الكروم العضوى فى العلف على الصفات الانتاجيه ومكونات السيرم والحالة المناعية لدجاج البندرة البياض و الصفات الطبيعية للسائل المنوى للديوك فى فصل الشتاء

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أستخدم فى هذه الدراسة ١٥٠ دجاجة بياضة و ٤٠ ديك من سلالة البندرة المحليه عمر ٣٢ أسبوع خلال فصل الشتاء وزعت الطيور عشوائياً فى أقفاص فردية إلى خمسة مجموعات تجريبية (٣٠ دجاجة و ٨ ديوك فى كل مجموعة) وتكون الغذاء التجريبى من أضافة الغذاء الأساسى مع صفر ، ٢٥٠ ، ٥٠٠ ، ١٠٠٠ ، ١٥٠٠ جزء فى البليون كروم المضاف من خميرة الكروم و أستمرت التجربة حتى عمر ٤٤ أسبوع .

أوضحت النتائج أن الكروم ليس له تأثير على وزن الجسم و الغذاء المستهلك للدجاجات والديوك و لكن أستخدام الكروم حسن معنويا انتاج البيض ووزن البيضة و النسبة المئوية للبياض و القشرة و سمك قشرة البيضة ووحدة هيو و معامل الصفار ووجد أن أستخدام الكروم لم يؤثر معنويا على معامل شكل البيضة بينما أنخفض النسبة المئوية للصفار مقارنة بمجموعة الكنترول. بزيادة أضافة الكروم يزداد معنويا الأستجابة المناعية الأولية و الثانوية و مكونات السيرم من البروتين الكلى و الجلوبيولين و الكالسيوم و الأنسولين بينما أنخفض معنويا تركيز الدهون الكلية و الكولستيرول والجلوكوز و هرمون الكورتيكوستيرول فى السيرم. كما وجد ان تركيز الألبيومين فى السيرم لم يتأثر بأضافة الكروم مقارنة بمجموعة الكنترول وبالأضافة إلى ذلك أدى أضافة الكروم إلى تحسن فى الصفات الطبيعية و تركيز المالونالدهيد فى السائل المنوى للمعاملات باستثناء المستوى المنخفض من الكروم (٢٥٠ جزئ فى البليون) لم يغير معنويا من حجم القذفة مقارنة بمجموعة الكنترول ،كما تحسنت معنويا النسبة المئوية للخصوبة و الفقس للدجاج البياض مع أضافة الكروم من خميرة الكروم.

يمكن التوصية بأن أضافة الكروم بمستوى ١٥٠٠ جزئ فى البليون يوصى به خاصة فى فصل الشتاء لتحسين الصفات الانتاجية سابقة الذكر و الصفات المناعية و جودة السائل المنوى و الخصوبة والفقس.