

EFFECT OF DIETARY CHROMIUM AND ASCORBIC ACID SUPPLEMENTATION ON PERFORMANCE OF LAYING HENS UNDER EGYPTIAN SUMMER CONDITIONS

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SUMMARY

A 8-wk study was conducted to evaluate the effects of supplementing laying hen diets with high levels of chromium (chromium chloride, $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$), vitamin C (L-ascorbic acid) and their combination on productive performance, egg quality and some blood constituents under Egyptian summer conditions (28.7-32.5°C). One hundred and twenty Hy-line White laying hens (32wk of age) were evenly distributed to four experimental groups of 30 hens with ten replicates of three hens each. The first group (control) was fed a basal diet without any supplementation. The second group was fed the basal diet containing 80mg Cr/kg and third group was fed 250mg vitamin C/ kg diet. Finally, the fourth group was fed a basal diet containing 80 mg Cr/kg and 250mg vitamin C/kg together.

It could be noticed that the chromium, vitamin C and their combination had no effect on body weight, egg weight and feed consumption. Inversely, the hens fed chromium, ascorbic acid and their combination significantly produced higher egg number and egg mass compared to control-fed group. The feed conversion ratio was significantly improved by chromium and ascorbic acid supplementations. With respect to egg quality, it could be noticed that the eggshell quality was significantly improved by added either chromium or vitamin C in the diet. Also, the internal egg quality was significantly improved. In accordance to hematological parameters, the plasma cholesterol, total lipids and glucose were significantly reduced when laying hens fed a diet containing chromium, vit.C or their combination compared to control-fed group. Opposite trend was noticed for plasma calcium, phosphorus and insulin. The T_3 hormone was significantly increased when hens fed a diet supplemented with chromium in a single manner or combined with ascorbic acid. On the other hand, there were no any effects of treatments on plasma protein, albumin and globulin.

From the obtained results, it could be concluded that the chromium and ascorbic acid supplementation at the studied levels is beneficial for laying hens during heat stress to improve the performance and hematological parameters.

Keywords: chromium, vitamin C, laying hens, productive performance, egg quality, blood

INTRODUCTION

Poultry are exposed to various factors of stress. Under Egyptian conditions, high ambient temperature is the major stress factor during summer season. High ambient temperatures have deleterious effects on growth and production of poultry. In laying hens, heat stress depresses egg production (Marsden *et al.*, 1987), egg weight (Peguri and Coon, 1991) and shell quality (Balnave and Muheereza, 1997). This negative effect of heat stress is primarily due to reduced feed intake (Savory, 1986). Heat stress also impairs antioxidant status, increases mineral excretion, and elevates lipid peroxidation products in serum and liver while decreasing serum and tissue levels of antioxidant vitamins and minerals in poultry (Sahin and Kucuk, 2003). It has been reported that some minerals and vitamin supplement such as chromium and vitamin C can be included into diet to prevent the negative effect of environmental stress (Fekete and Kellems, 2007 and McDowell, 2000). Chromium (Cr) is a trace element that appears to be an essential micronutrient for animals and humans. The primary role of Cr in metabolism is to potentiate the action of insulin through its presence in an organometallic molecule, called the glucose tolerance factor (GTF) (Anderson, 1987; Sahin *et al.*, 2001; Pechova *et al.*, 2002 and Sahin *et al.*, 2003). Moreover, chromium deficiency can disrupt the carbohydrate and protein metabolisms, reduce the insulin sensitivity in peripheral tissues, and also impair the

growth rate (Pagan *et al.*, 1995; Sahin and Sahin, 2002; Sahin *et al.*, 2003). Dietary chromium supplementation was found to accelerate body growth and to increase lean body mass in humans, experimental animals and domestic livestock (Mertz, 1993; Hasten *et al.*, 1997; Mowat *et al.*, 1993). Dietary chromium supplementation was reported to have a positive effect on the growth rate and feed efficiency in growing poultry (Cupo and Donaldson, 1987; Lien *et al.*, 1999), particularly in birds reared under heat or cold stress conditions (Sands and Smith, 1999; Sahin *et al.*, 2001; Sahin *et al.*, 2003). There is no specification for Cr requirements in poultry diets (NRC, 1994), and most poultry diets are basically composed of plant-origin ingredients, usually low in Cr (Giri *et al.*, 1990). In accordance to vitamin C, poultry have the ability to synthesize ascorbic acid, or vitamin C, in their body (Chang and Mowat, 1992); hence, no recommended requirement is established by the NRC (1994). However, environmental and pathological stressors are known to alter vitamin C use or synthesis or both in the fowl (Pardue and Thaxton, 1986). Although synthesis in the neonatal chick is apparently limited (Horning and Frigg, 1979), it is generally assumed that the endogenous synthesis is adequate to meet biological demands in poultry. During certain conditions, vitamin C supplementation provides benefit to poultry (Pardue and Thaxton, 1986). Several researchers observed significant improvement in growth of chicks by the addition of vitamin C under high temperature. Broilers fed diets containing vitamin C were less stressed

due to having reduced body temperature and respiratory rates (Pardue and Thaxton, 1986) and showed higher feed intake (Kutlu and Forbes, 1993; Mckee and Harrison, 1995) than control birds. Substantial available reports show under field conditions that feeding vitamin C enhanced productivity, immune response, disease resistance, and survivability under stressful conditions (Gross, 1988; Pardue *et al.* 1985; Zulkifli *et al.*, 1996). Therefore, the objective of the present study was to evaluate the effect of Cr and vitamin C supplementation on productive performance, egg production and some hematological parameters of laying hens reared under Egyptian summer conditions.

MATERIALS AND METHODS

The present study was carried out at Poultry Breeding Farm, Poultry Production Department, Faculty of Agriculture, Ain Shams University. This experiment was run during summer season from July to September 2006. The averages ambient temperatures (Min. &Max.) recorded during the experimental period were 28.7 to 32.5°C, respectively.

One hundred and twenty 32-wks old, Hy-line White laying hens were randomly assigned to four groups. The hens were maintained in layer batteries with three decks of five cages each until 40 wks of age. Birds were individually weighed and distributed randomly into four experimental groups of 30 hens with 10 replicates (cages) of three hens each.

The first group (control) was fed a basal diet without any supplementation. The second group was fed the basal diet containing 80mg Cr/kg ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$) and third group was fed 250mg vitamin C (Ascorbic acid)/ kg diet. Finally the fourth group was fed a basal diet containing 80mg Cr/kg and 250mg vitamin C/kg. Birds in all groups were subjected to 16L: 8D regime with free access to feed and water and reared under the same environmental, managerial and hygienic conditions. The hens received a typical layer diet containing 2800 kcal ME/kg and 18% CP to meet or slightly exceed the nutrient requirement by NRC (1994). The composition and calculated chemical analysis of the experimental diet are presented in Table (1).

Body weight and feed intake was recorded every 28 days and the average feed intake per hen was then calculated. The produced eggs per replicate cage were daily weighed and average egg mass per hen was then calculated at 36 and 40 wks of age. Feed conversion was calculated (g feed intake/ g egg mass).

At 40 wks of age, three eggs from each replicate cage (30 eggs from each treatment) were taken to determine the different measurements of egg quality. The egg quality measurements were albumin, yolk percentages, albumin height (by using micrometer), Haugh units, shell percentage, shell thickness (by using an electric caliper), breaking strength (by using an apparatus of Fathi and El-Sahar, 1996) and shape index.

Table (1): Percentage composition of the basal diet

Ingredient	%
Ground yellow corn	61.80
Soybean meal (44%)	19.30
Corn gluten meal	2.90
Decorticated cottonseed meal (41%)	2.00
Corn gluten feed	4.00
Bone meal	1.80
Limestone	7.42
NaCl	0.32
Vitamin and mineral premix*	0.40
DL-Methionine	0.04
L-Lysine HCl	0.02
Total	100
<u>Calculated chemical analysis</u>	
ME, Kcal /Kg	2800
Crude protein (%)	18.00
Crude fat (%)	2.90
Crude fiber (%)	2.80
Calcium (%)	3.75
Available phosphorus (%)	0.40

*Each 2.5 kg of vitamins and minerals premix contain: vit. A, 12 mIU; vit. D₃, 4 mIU; vit. E, 15g; vit. K, 2g; vit. B₁, 1g; vit. B₂, 8g; vit. B₆, 6g; vit. B₁₂, 10 mg; Niacin, 30g; Biotin, 150 mg; Folic acid, 1g; Pantothenic acid, 10g; Choline chloride, 40 mg; Zinc, 60g; Manganese, 70g; Iron, 15g; Copper, 5g; Iodine, 1g; Selenium, 0.15g.

Blood samples were withdrawn from the brachial vein of ten hens per group at 40 weeks of age using heparinized tubes. The blood samples were immediately centrifuged at 3000 rpm for 10 minutes to separate plasma. Plasma samples were frozen at -20°C until assayed for insulin, glucose, total protein, albumin, total lipids, cholesterol, calcium and phosphorus by using commercial kits and plasma concentrations of thyroid hormones (T₃ and T₄) were determined using immunoassay kits (Diagnostic products corporation DPC). Plasma globulin was calculated by the difference between total protein and albumin.

Data were subjected to a one-way analysis of variance with treatment group effect using the General Linear Model (GLM) procedure of SAS, (2001). Duncan's Multiple Range Tests (Duncan, 1995) was also used for the comparison among means of the experimental groups.

RESULTS AND DISCUSSION

Productive parameters:

The effects of supplemental chromium, vitamin C and their combination on the productive parameters of laying hens are summarized in Table (2). It could be noticed that supplemental chromium, vitamin C and their combination had no significant effect on body weight, feed consumption and egg weight of laying hens during the

experimental period. Opposite trend was noticed for egg mass, egg number and feed conversion ratio, whereas the of dietary chromium, vitamin C and their combination significantly increased egg mass and egg number and significantly improved the feed conversion ratio when compared with control-fed group. The effect of heat is well defined as evidenced by a 17% reduction in feed intake for every 10°C increase in ambient temperature above 20°C (Ensminger *et al.*,1990). Geraert *et al.*(1996) reported that feed efficiency is reduced when birds were exposed to 32 °C and suggested that it could be the result of changes in metabolic utilization of nutrients. Many stressors, including high or low ambient temperatures, influence the mineral and vitamin utilization (Sahin and Kucuk, 2003). Stress may increase chromium mobilization from tissues and its excretion and also depresses ascorbic acid synthesis (Pardue and Thaxton, 1986; McDowell, 2000; Anderson, 1987), thus may exacerbate a marginal chromium and vitamin C deficiency or increased their requirement, implying that both of them should be supplemented. Kultlu and Forbes (1993) reported that ascorbic acid reduces the synthesis of corticosteroid hormones in birds. By decreasing synthesis and secretion of corticosteroids, vitamin C alleviates the negative effects of stress such as hot stress-related depression in poultry performance (McDowell, 2000). In accordance with the present results, Sahin *et al.* (2004) reported that chromium supplementation restored the impairment in egg production and feed efficiency in laying Japanese

Table (2): Productive Parameters of laying hens as affected by chromium, vitamin C and their combination

Age (Wk)	Treatment				Pooled SE	Prob.
	Control	Cr	Vitamin C	Cr + Vit. C		
	Body weight, g					
32	1372	1351	1312	1339	25.62	NS
36	1382	1390	1351	1368	28.70	NS
40	1429	1439	1392	1397	30.54	NS
	Feed consumption, g					
32-36	2996	3098	3132	3102	27.14	NS
36-40	3101	3035	3142	3127	40.54	NS
32-40	6097	6133	6274	6229	61.13	NS
	Egg mass, g					
32-36	1280 ^b	1462 ^a	1471 ^a	1491 ^a	18.14	0.01
36-40	1292 ^b	1499 ^a	1504 ^a	1519 ^a	15.42	0.01
32-40	2572 ^b	2961 ^a	2975 ^a	3010 ^a	16.70	0.01
	Feed conversion ratio (g feed/ g egg mass)					
32-36	2.34 ^a	2.12 ^b	2.13 ^b	2.08 ^b	0.02	0.01
36-40	2.40 ^a	2.02 ^b	2.09 ^b	2.06 ^b	0.01	0.01
32-40	2.37 ^a	2.07 ^b	2.11 ^b	2.07 ^b	0.02	0.01
	Egg number, no.					
32-36	21.8 ^b	24.5 ^a	24.5 ^a	24.7 ^a	0.11	0.01
36-40	21.0 ^b	24.3 ^a	24.3 ^a	24.8 ^a	0.08	0.01
32-40	42.8 ^b	48.8 ^a	48.8 ^a	49.5 ^a	0.17	0.01
	Egg weight, g					
32-36	59.4	59.9	60.3	60.1	0.12	NS
36-40	61.7	61.6	62.1	61.7	0.15	NS
32-40	60.6	60.8	61.2	60.9	0.14	NS
	Egg production rate					
32-36	77.89 ^b	87.50 ^a	87.50 ^a	88.21 ^a	0.17	0.01
36-40	75.00 ^b	86.79 ^a	86.79 ^a	88.57 ^a	0.22	0.01
32-40	76.43 ^b	87.14 ^a	87.14 ^a	88.39 ^a	0.19	0.01

^{a,b} Means within the same row with different letters are significantly differed ($P < 0.05$). NS = non significant effect, Cr = Chromium

quails reared under heat stress. Lien *et al.* (1999) found that chromium supplementation markedly enhanced weight gain due to increased feed consumption in broilers. Steele and Rosebrough (1979) reported that 20 ppm Cr supplementation improved growth rate and feed efficiency. In the present experiment, 80 ppm Cr did not affect body weight as in the results of Cupo and Donaldson (1987) in chicks and Pahin *et al.* (2001) in rabbits, but improved feed efficiency. Egg weight were not affected by chromium supplementation (Lien *et al.*, 1996).

Internal egg and egg shell quality:

Effect of higher levels of chromium, vitamin C and their combination on internal and egg shell quality is presented in Table (3). There was no significant difference among treated groups for percentages of albumen, yolk and shell. Inversely, chromium, vitamin C and their combination significantly increased Haugh unit compared to control-fed group. Also, the shell thickness and shell breaking strength were significantly improved by added chromium, vitamin C and their. The results reported herein agree with the findings of Sahin *et al.* (2002) who reported that supplemental chromium increased eggshell thickness and Haugh unit in laying hens reared under exposed to ambient temperature stress. Similar to results of the present study, El-Boushy *et al.* (1968) reported that dietary vitamin C supplementation increased egg shell strength and interior egg quality in stressed-laying hens. Sahin *et al.* (2004) reported that chromium supplementation restored the impairment in egg quality in

laying Japanese quails reared under heat stress. In the contrary of our results, Lien *et al.* (1996) reported that shell thickness was not affected by chromium supplementation under thermo-neutral conditions. On the other hand, Uyanik *et al.* (2002) reported that chromium had no effect on specific gravity, shape index, shell thickness and Haugh unit, but increased shell breaking strength, albumen and egg yolk index values.

Blood constituents:

Hematological parameters of laying hens as affected by chromium, vitamin C and their combination are summarized in Table (4). It could be noticed that there was no significant difference among treated groups for plasma total protein, albumen and globulin. The last result could be attributed to the beneficial effects of chromium and ascorbic acid in debating the detrimental influence of glucocorticoid hormone on protein degradation as a result of stress-induced gluconeogenesis. This effect was also observed by Sahin *et al.*, (1999) and Uyanik *et al.*, (2005). The dietary chromium level in a single state or combined with vitamin C significantly increased both calcium and phosphorus in the plasma when compared to control-fed group. Similar trend was noticed for vitamin C alone. Moonsie-Shageer and Mowat (1993) found increases in serum Ca and Mg concentrations by supplemental chromium in calves. Chang and Mowat (1992) reported a relationship between Cr and Mg. Page *et al.* (1993) and Pahin *et al.*

Table (3): Interior and egg shell quality measurements of laying hens as affected by chromium, vitamin C and their combination.

Item	Treatment				Pooled SE	Prob.
	Control	Cr	Vitamin C	Cr + Vit. C		
Egg weight, g	60.74 ^b	63.30 ^a	62.40 ^a	63.03 ^a	2.15	0.01
Albumen, %	60.61	60.45	60.51	60.16	3.59	NS
Yolk, %	30.10	30.26	30.14	30.15	0.63	NS
Haugh unit	81.56 ^b	84.12 ^a	84.17 ^a	85.63 ^a	2.14	0.01
Shell, %	9.29	9.29	9.35	9.69	0.17	NS
Shape index	77.21	77.38	77.54	78.05	1.14	NS
Shell thickness, mm	0.331 ^b	0.360 ^a	0.354 ^a	0.355 ^a	0.01	0.01
Breaking strength, kg	2.96 ^b	3.16 ^a	3.25 ^a	3.67 ^a	0.12	0.01

^{a, b} Means within the same row with different letters are significantly differed ($P < 0.05$). NS = non significant effect, Cr = Chromium

Table (4): Hematological parameters of laying hens as affected by chromium, vitamin C and their combination.

Parameter	Treatment				Pooled SE	Prob.
	Control	Cr	Vitamin C	Cr + Vit. C		
Total protein (g/dl)	6.27	6.40	6.33	6.40	0.12	NS
Albumen (g/dl)	3.24	3.02	3.16	3.01	0.05	NS
Globulin (g/dl)	3.03	3.38	3.17	3.39	0.10	NS
Calcium (mg/dl)	20.45 ^b	23.43 ^a	23.51 ^a	24.16 ^a	1.16	0.01
Phosphorus (mg/dl)	8.59 ^b	8.57 ^b	9.21 ^a	9.14 ^a	0.98	0.01
Glucose (mg/dl)	222.7 ^a	184.0 ^c	201.5 ^b	173.7 ^d	7.15	0.01
Insulin (ng/ml)	3.20 ^c	4.32 ^b	3.64 ^c	5.10 ^a	0.14	0.01
T ₃ (ng/ml)	3.31 ^b	4.26 ^a	3.87 ^b	4.59 ^a	0.23	0.01
T ₄ (ng/ml)	11.38 ^a	10.41 ^b	10.03 ^b	9.45 ^c	0.76	0.01
Total lipids (mg/dl)	234.6 ^a	213.6 ^b	185.2 ^c	130.4 ^d	5.4	0.01
Cholesterol (mg/dl)	300.27 ^a	257.69 ^c	287.91 ^b	281.51 ^b	6.19	0.01

^{a-d} Means within the same row with different letters are significantly differed ($P < 0.05$). NS = non significant effect, Cr = Chromium

(2001) suggested that Cr had no effect on Ca and inorganic P levels.

Plasma glucose level was significantly depressed when hens were fed diets supplemented with vitamin C alone or combined with Cr. Similar trend, but not statistically significant, was noticed for Cr alone. Chromium is generally accepted as the active component in the glucose tolerance factor (GTF), which increases the sensitivity of tissue receptors to insulin, resulting in increased glucose uptake by cells. Research suggests Cr involvement in carbohydrate metabolism including glucose uptake, glucose utilization for lipogenesis, and glycogen formation (Anderson *et al.*, 1991). It was hypothesized that increased glucose uptake should increase oxidation of glucose which would be otherwise converted to fatty acids and stored as triglycerides in adipose tissues. Because the experimental birds were reared under summer season of Egypt (32°C), this stress may stimulate the hypothalamic-pituitary-adrenal axis to increase the secretion of glucocorticoids (corticosterone) from the adrenal cortex which in turn increase glucogenesis. Dietary vitamin C may reverse the previous changes by reducing the secretion of corticosterone (Kutlu and Forbes, 1993; Lein *et al.*, 1999). With respect to insulin concentration, it could be noticed that chromium significantly increased the insulin concentration. Similar trend was noticed in the laying hens fed diet containing chromium and vitamin C together. However, the effect of

vitamin C alone on insulin concentration was not significant. The primary role of Cr in metabolism is to potentiate the action of insulin through its presence in an organometallic molecule, called the glucose tolerance factor (GTF) (Anderson 1987; Sahin *et al.*, 2001; Pechova *et al.*, 2002; Sahin *et al.*, 2003). Insulin has been shown to increase the glucose and amino acid uptake into muscle cells, to regulate energy production, muscle tissue deposition, fat metabolism, and cholesterol utilization. If glucose cannot be utilized by body cells due to a low insulin level, it is converted into fat and stored in fat cells. Furthermore, if adequate amino acids cannot enter the cells, muscles cannot be built (Anderson, 1987). Moreover, chromium deficiency can disrupt the carbohydrate and protein metabolisms, reduce the insulin sensitivity in peripheral tissues, and also impair the growth rate (Pagan *et al.*, 1995; Sahin and Sahin, 2002; Sahin *et al.*, 2003).

The present result showed that the T_3 level was significantly increased by using chromium alone or with vitamin C compared to control-fed group. Opposite trend was noticed for T_4 concentration. This result may be explained by that the chromium or vitamin C supplementations increase the conversion rate of T_4 to T_3 , where the T_3 is the active form of thyroid hormone. Therefore, the performance of laying hens fed a diet containing either chromium or vitamin C in a single manner or combined together was higher compared to the control-group. The higher T_3 associated with dietary chromium and ascorbic acid may explain the higher

eggshell quality and egg performance traits.

Studies in human and various animal species regarding the influence of Cr supplementation on lipid parameters are conflicting. Amoikon *et al.* (1995) reported that fasting plasma cholesterol concentrations in pigs were increased by chromium picolinate. Different forms of Cr reduced total cholesterol in humans (Press *et al.*, 1990), pigs (Page *et al.*, 1993), lambs (Kitchalong *et al.*, 1995) and layers (Lien *et al.*, 1996). The present results showed that supplemental vitamin C or chromium in a single manner or combined significantly decreased total lipids compared to control-fed group. The decrease in plasma lipids could be resulted from depression of fatty acids synthesis due to mainly increasing demands of lipids for yolk formation. With respect to plasma cholesterol, the present result showed that all additives significantly reduced plasma cholesterol compared to control-fed group.

From the results obtained, it can be concluded that the chromium and ascorbic acid supplementation at higher levels is beneficial for laying hens during heat stress to improve the performance and hematological parameters.

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تأثير إضافة الكروم و حامض الاسكوربيك الى العلائق على أداء الدجاج البياض تحت ظروف حرارة جو الصيف في مصر

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اجريت هذه التجربة بغرض دراسة تأثير اضافة الكروميوم(كلوريد الكروميوم) وحمض الاسكوربيك(فيتامين ج) بمستويات مرتفعة الى علائق الدجاج البياض على الاداء الانتاجي وجودة البيض وبيض مقاييس الدم تحت ظروف الصيف المصري (28.7- 32.5 م). استخدم في هذه التجربة عدد 120 دجاجة بياضة من سلالة الهاي لاين الابيض عمر 32 اسبوع . تم توزيع الدجاجات عشوائيا على اربعة معاملات تجريبية تضم كل منها عدد 30 دجاجة موزعة على 10 مكررات بكل منها 3 دجاجات. غذيت المجموعة الاولى (مجموعة المقارنة) على الطليقة القاعدية بدون اى اضافات بينما غذيت المجموعة الثانية و الثالثة والرابعة على الطليقة القاعدية مضافا اليها 80 ملجم كروميوم او 250 ملجم حمض اسكوربيك / كجم عليقة او كليهما معا على الترتيب واستمرت التجربة حتى عمر 40 اسبوع . لوحظ عدم تأثر كل من وزن الجسم، وزن البيض والاستهلاك الغذائي بعكس النتائج المتحصل عليها من زيادة في عدد و كتلة البيض المنتج معنويا بأضافة كل من الكروميوم ، فيتامين ج او كليهما معا مقارنة بمجموعة المقارنة. تحسن معدل التحويل الغذائي وصفات الجودة الداخلية وجودة القشرة للبيض معنويا بأضافة الكروميوم وفيتامين ج. انخفض محتوى بلازما الدم لكل من الكوليسترول، الليبيدات الكلية والجلوكوز بينما زاد محتواه لكل من الكالسيوم ، الفوسفور والانسولين للدجاجات التي غذيت على جميع الاضافات مقارنة بمجموعة المقارنة. زاد مستوى هرمون التيروكسين الثلاثي اليود زيادة معنوية بأضافة الكروميوم بصورة منفردة او مع حمض الاسكوربيك. لم يتأثر محتوى بلازما الدم لكل من البروتين، الاليومين والجلوبيولين بالمعاملات المستخدمة.

نتخلص من النتائج السابقة أن اضافة الكروم وحمض الاسكوربيك بمستويات عالية له تأثير ايجابي معنوي على الاداء الانتاجي وصفات جودة البيض الداخلية والخارجية وكذلك مقاييس الدم تحت ظروف حرارة جو الصيف في مصر.