CHROMIUM CHLORIDE AND ASCORBIC ACID RELIEVE THE CONSEQUENCES OF HIGH TEMPERATURE STRESS OF SUMMER IN BROILER

Journal

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ABSTRACT

Seventy–two Hy-line white hens with 32 weeks old were maintained in layer batteries with three decks to the fortieth week of age from July to September where the maximum ambient temperatures was 34 ^{o}C ±2. Birds were individually weighed and distributed randomly into four experimental groups of 18 hens with six replicates (cages) of three hens each. The laying hens of each group were fed the basal diet alone as control (group 1) or the basal diet supplemented with 80 mg $CrCl_3.6H_2O$ (group 2), or with 250 mg vitamin C (group 3) and with the combination of Cr and Vit C (group 4). The basal diet was formulated to contain 18% crude protein and 2800 Kcal ME ⁄ kg of diet. The obtained results showed significant increasing in plasma concentration of T3 hormone by the supplementation with Cr especially when it added together with vitamin C. All supplementations; Cr, vitamin C and their combination increased significantly the plasma concentration of T4 hormone. The same effect could be observed by the supplementation with Cr and became higher when added together with vitamin C on increasing significantly the plasma concentration of insulin. The MDA (malondialdhyde) levels in plasma were decreased significantly especially by the application of Cr together with vitamin C, followed by Cr application individually and then by vitamin C alone. Supplementations with Cr, vitamin C separately and in combination have pronounced effects on the plasma concentrations of glucose, total lipids and cholesterol, but their effect on total protein and albumin was not significant. Plasma calcium (Ca) and inorganic phosphorous (Pi) were significantly changed by dietary supplements of Cr and vitamin C, since, Ca significantly increased in all treatments, however, Pi had inconsistent trend. The activities of ALP, AST and ALT enzymes showed pronounced changes under the effect of the addition of Cr and vitamin C either separately or in combination together. ALP activity decreased significantly by the adding of Cr alone and also in combination with vitamin C. AST activity decreased significantly by vitamin C supplementation, while the ALT enzyme activity increased significantly by the supplementation with vitamin C and became higher when supplemented together with Cr. As results of the pronounced changes in plasma hormones, metabolites, minerals and enzymes activities under the effect of the supplementation with Cr and vitamin C, the productive performance of the laying hens were affected diversely. No significant effect on body and egg weights of laying hens along the experimental period could be detected under all treatments. Feed intakes significantly increased by adding Cr and/or vitamin C. Significant improvement in feed conversion ratio could be obtained by Cr, vitamin C and Cr + vitamin C supplementation along the whole experimental period The supplementations with Cr or vitamin C separately or in combination together were significantly increased egg number and egg mass of laying hens along the experimental period in addition to an enhancement in egg shell thickness (mm) of treated hens. The result indicates that the combination between Cr and vitamin C as dietary supplementation could achieve good results better than their individual supplementation.

Keywords: Laying hens - Cr – vitamin C – T3 – T4 – insulin – MDA levels – glucose – total protein – cholesterol – Ca – Pi – ALP – AST – ALT – body weight – feed intake – feed conversion ratio – egg mass – egg number.

INTRODUCTION

The main consequence of high ambient temperature on laying hens is the reduction in feed intake, feed conversion ratio and egg production (Sahin and Kucuk, 2003). The rate of feed intake reduction is approximately 17% per every 10 ^{o}C increases in ambient temperature above the thermo neutral zone (18-24 ^{o}C) which recommended for optimum egg production (Kutlu and Forbs 1993). As the ambient temperature increase, birds respond by reducing plasma thyroid hormones (Sahin, *et. al*., 2003).

Several methods are available to reduce the negative effects of high summer temperature on egg production. Since, it is expensive to cool bird buildings or using low-pressure misting equipments with mixing fans. Recently, nutritional manipulation of the diet offers great advantages, especially for overcoming problems associated with reduction of productivity. These include using of vitamin C (Kutlu and Forbes, 1993 and Sahin, *et. al*., 2003). More recent studies were focused on chromium supplementation to poultry diets for alleviating the negative impact of heat stress (Uyanik, *et. al*., 2002 in laying hen diets, Sahin, *et. al*., 2003 in broiler and poultry diets and Sahin, *et. al*., 2004 in laying quails). Dietary chromium (Cr) was reported to improve insulin action by stimulating its receptors (McMurty *et. al*., 1983) and enhances the metabolism of carbohydrates, lipids and proteins (Lien, *et. al*., 1999 and Uyanik, *et. al*., 2005). Although chromium is an essential trace element for human and laboratory animals as a glucose tolerance factor (Mertz, 1993), the dietary intake of Cr often suboptimal because its low content in all plant products which considered the main nutrients for poultry (Anderson, 1993). The recommended level of Cr in poultry diets is not well known. It has been reported that dietary vitamin C and Cr requirements for poultry was significantly affected by stress conditions, and the negative effects could be prevented by both vitamin C and Cr supplementation (Sahin and Kucuk, 2003, Sahin, *et. al*., 2003 and Uyanik, *et. al* ., 2005). In addition, several investigations supported the findings that both vitamin C and Cr are similar in their role as growth enhancers and as antioxidants (Carol, *et. al.,* 1994, Sahin and Sahin 2002 and Sahin, *et. al*., 2003). An additive effect of both Cr and vitamin C supplementation was found in bone mineralization, in per-oxidation of lipids and in regulation of biochemical reactions in blood which in turn caused an increase in productivity of laying hens (Sahin, *et. al*., 2004, and Uyanik, *et. al*., 2005).

Based on the above mentioned literatures, this study was conducted to evaluate the biochemical roles of Cr and vitamin C as dietary supplements on plasma levels of insulin, T3 and T4, lipid per-oxidation status and some blood metabolites in laying hens and their response on the productive performance and egg production of laying hens reared under high temperature stress of summer season in Egypt.

MATERIALS AND METHODS

1- Birds and diets

A total of seventy–two Hy-line white hens, 32 weeks old were maintained in layer batteries with three decks for eight weeks (until 40 weeks of age) during the period from July to September where the maximum ambient temperatures recorded 34°C ± 2°C. Birds were individually weighed and distributed randomly into four experimental groups of 18 hens with six replicates (cages) of three hens each.

The laying hens of each group were fed as follow:

Group 1: fed on basal diet (control group)

Group 2: fed on basal diet supplemented with 80 mg of $CrCl_3.6H_2O$ / Kg of diet

Group 3: fed on basal diet supplemented with 250 mg of vitamin C / Kg of diet

Group 4: fed on basal diet supplemented with 80 mg of $CrCl_3.6H_2O$ and 250 mg of vitamin C / Kg of diet.

Table 1: Percentage Composition and Calculated Analysis of the Basal Diet

Ingredients	%	Ingredients	%
Ground yellow corn	61.801	L-Lysine	0.02
Soybean meal	19.30	Total	100
Corn gluten meal	2.90	Calculated Composition (% except energy)	
Decorticated cottonseed meal	2.00	ME, Kcal /Kg	2775
Corn gluten feed	4.00	Crude protein	18.00
Bone meal	1.80	Crude fat	2.90
Limestone	7.42	Crude fiber	2.80
Salt	0.32	Calcium	3.75
Vitamin and premix*	0.40	Available phosphorus	0.40
DL-Methionine	0.04		

***Each 2.5 kg of vitamins and minerals premix contain:** vit. A: 12 mIU; vit. D_3: 4 mIU; vit. E:15g,; vit. K: 2g; vit. B_1: 1g; vit. B_2: 8g; vit. B_6: 6g; vit: B_{12}: 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.

The basal diet was formulated to contain 18% crude protein and 2800 Kcal ME / kg of diet as the recommended requirements of laying hens according to the National Research Council (NRC, 1994). The composition of the basal diet is shown in table.

2- Methods of analysis

2-1- Performance and Productivity of Hens

Birds were subjected to16 hours light/ 8 hours dark regime with free access to feed and water.

- **Body weight** was recorded every four weeks of egg production period.
- **Feed intake** was determined every four weeks of age.
- **The average feed intake** per hen was calculated.
- **The produced eggs** per replicate cage were daily weighed and average egg mass per hen was then calculated at 36 and 40 weeks of age.
- **Feed conversion** was calculated (g of feed intake / g of egg mass).
- **Shell parameters** as shell weight, percentage and thickness by using electric caliper were determined at 36 and 40 wks of hen age, by using three eggs from each replicate cage (18 eggs from each treatment).

2-2- Biochemical analysis

Blood samples were withdrawn from the brachial vein of nine hens per group at 40 weeks of age by using heparinized syringe. The blood samples were immediately centrifuged at 4000 rpm for 10 minutes to separate plasma.

Plasma samples were frozen at -20 ^{o}C until assayed for:

- **Total Protein** (g/dl) determined according to Henry (1974).
- **Albumin** (g/dl) estimated based on the method described by Doumas *et. al.* (1971).
- **Globulin** (g/dl) calculated as the different between plasma albumin and plasma total protein.
- **Total lipids** (g/L) were determined according to Knight *et. al.* (1972).
- **Cholesterol** (mg/dl) was determined according to Richmond (1973).
- **Total Bilirubin** (mg/dl) determined by method described by Jendrassik (1938).
- **ALP** (E.C.3.1.3.1) (Alkaline Phosphatase activity) by method of Brooks and Purdy (1972).

- **AST** (E.C.2.6.1.1) (Aspartate Transaminase activity) by Reitman and Frankel (1957).
- **ALT** (E.C.2.6.1.2) (Alanine Transaminase activity) by the same method for AST.
- **Plasma insulin** (ng ⁄ ml) was measured by RIA, commercial kits, Lumunotec Technicon, Raxt, Los Angeles, CA as described by McMurtry *et. al.* (1983).
- **The radioimmunoassay** (RIA) method was used for the determination of triiodothyronine (T3) and thyroxin **(T4).**
- **Plasma T3 and T4** were determined by RIA kits (gamma coat ^{125}I RIA kits, Clinical Assay Cambridge, Medical Diagnostics, Boston, MA) as reported by Akiba *et. al.* (1982).
- **Phosphorus** was determined spectrophotometrically by using available commercial kits (Spineract, S.A. Sant Esteve De Bas, Spain) according to Varshney and Kale (1990)
- **The degree of lipid Per-oxidation** was estimated through the measurement of the concentration of the major product of lipid per-oxidation process, malondialdhyde (MDA), by using spectrophotometric method described by Varshney and Kale (1990).
-**The degree of lipid Per-oxidation** was expressed as mmol / malondialdhyde (MDA) / mL blood.

2-3- Statistical Analysis

- **Data were statistically analyzed** by using the general liner Model (GLM) procedure of SAS (SAS institute, 2001). Significant difference among treatment means were determined by Duncan's multiple range test (Duncan, 1955) with a 5% level of probability.

RESULTS AND DISCUSSION

Since the objective of this study is to enhance the productive performance and egg production of laying hens reared under high temperature stress of summer season in Egypt (34 ^{o}C ± 2) by using Cr and vitamin C as dietary supplements, the following discussion is focusing on the biochemical roles of Cr and vitamin C on plasma levels of insulin, T3 and T4, lipid per-oxidation status and some blood metabolites and then their responses on the productive performance and egg production of laying hens.

1- Effect of Supplemental Chromium, Vitamin C and Their Combination on Blood Constituents of Laying Hens Under Heat Stress

1-1- Effect of Supplemental Cr, Vit. C and Their Combination on Plasma Hormones and Malondialdehyde (MDA)

The effects of dietary chromium (Cr), vitamin C and their combination (Cr + Vit. C) on plasma concentration of thyroid gland hormones (T3 and T 4) and insulin are presented in Table (2). Plasma concentration of T3 was significantly ($P \leq 0.05$) higher in hens fed Cr + Vit. C and Cr diets in comparison with those fed the control diet. However, no significant effects were found in hens fed Vit. C diet. Concerning the T4 concentration, the control hens had the lowest level of T4 in comparison with all treated groups. Moreover, the differences between treatments were not in significant matter in general while all of them were in significant increasing than the control. Only slight increase in T4 level in hens fed Cr + Vit. C (14.23 ng /ml) followed by those fed Cr diet (13.74 ng /ml) and the Vit, C group (13.15 ng /ml) were detected. It is suggested that under heat stress condition, Cr supplementation could stimulate the release of thyroidal hormones especially by increasing the rate of T4 to T3 conversion, which the later is the more active hormone in birds. In this respect several researchers elucidated that the thyroid activity is depressed under high environmental temperature as an adaptation mechanism of the bird to combat heat stress (Sahin, *et. al*., 2003 and Uyanik, *et. al*., 2005).

Concerning the plasma insulin concentration, results clearly showed that the higher level ($P\leq 0.05$) was in hens fed on chromium supplemented diets. In this respect, Cr + Vit. C supplementation also improved insulin level since the obtained value in hens fed Cr + Vit. C was 4.41 vs 4.32 ng/ml for those fed on Cr individually supplemented diet. Vitamin C individually was shown to have a slight effect on insulin level. Chromium supplemented diet alone or combined with vitamin C which indicates the positive role of Cr in potentiating the action of insulin in glucose uptake by muscles and liver cells are in agreement with those obtained by Carol, *et. al*., 1994 and Sahin, *et. al*., (2004). Several studies explained the proposed mechanism for the activation of insulin receptor activity by Cr. Cr play a crucial role in metabolism by potentiating the action of insulin through its presence in an organometallic molecule called glucose tolerance factor (GFT). The oligopeptide low-molecular-weight chromium-binding protein (chromodulin) binds Cr ions in response to an insulin-mediated chromic ion flux, and the metal-saturated oligopeptide can bind to an insulin-stimulated insulin receptor, activating the receptor`s tyrosine kinase activity. Thus, chromodulin appears to play a role in an autoamplification mechanism in insulin signaling (Sahin, *et. al.,* 2003 and Vincent, 2006).

Table 2: Effects of Supplemental Chromium (Cr) and Vitamin C on Plasma Hormones and MDA Levels in Laying Hens Reared Under Heat Stress (34 ^{o}C ± 2)

Items	Treatments			
	Control	Cr	Vit. C	Cr + Vit. C
T3 (ng/mL)	3.31^{c} ± 0.16	4.16^{ab} ± 0.34	3.65^{bc} ± 0.20	4.53^{a} ± 0.15
T4 (ng/mL)	11.38^{b} ± 0.33	13.74^{a} ± 0.38	13.15^{a} ± 0.44	14.23^{a} ± 0.46
Insulin (ng/mL)	3.20^{b} ± 0.09	4.32^{a} ± 0.17	3.64^{b} ± 0.18	4.41^{a} ± 0.12
MDA (mmol/mL)	2.92^{a} ± 0.10	2.11^{b} ± 0.19	2.15^{b} ± 0.09	1.28^{c} ± 0.12

- a-c Means within same row with different letters are significantly differed ($P \leq 0.05$)
- Control: Basal diet, Cr: control diet + 80 mg of Cr/kg of diet.
- Vit. C: control diet + 250 mg of vitamin C/kg of diet,
- Cr + Vit C: control diet + 80 mg of Cr/kg of diet + 250 mg of vitamin C/kg of diet.

The obtained results showed significant decreases in MDA plasma levels especially in hens that fed composite of Vit.C + Cr diet, which consider as indicator for improving lipid peroxidation. These findings could be supported by the antioxidant activity theory of Vit. C in addition to the role of Cr in preventing damage of cell membranes (Sahin, *et. al.*, 2004).

Based on the above mentioned results we could summarize that the combination between Cr and Vit. C as dietary supplements is the best for hens to combat the heat stress and still productive in begetting eggs. In general we could find that the combination between Vit. C and Cr is the best since dietary Cr may influence ascorbic acid metabolism via protecting ascorbic acid from oxidative destruction. Moreover, insulin is known to play a role in ascorbic acid transport. Through increasing the effectiveness of insulin, Cr indirectly promotes the ascorbic acid transportation (Carol, *et. al.,* 1994).

1-2- Effect of Supplemental Cr, Vit. C and Their Combination on Some Plasma Metabolites

Results show that dietary supplementations of Cr, Vit. C and their combination have pronounced effects on the plasma concentrations of glucose, total lipids and cholesterol, but their effect on total protein and albumin was not significant (Table 3).

It is clear from the results that plasma glucose level was significantly decreased when hens were fed diets supplemented with Vit. C + Cr or vitamin C alone. It appears also that supplemental Cr alone has insignificant effect in reducing plasma glucose level, inspite of the higher insulin concentration recorded from this treatment (4.32 ng /ml) as shown in Table (3).

These findings could be attributed to the heat stress since the experimental birds were reared under summer condition of about 35 ^{o}C ± 2. This heat 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 Vit. C may reverse the previous changes, by reducing the secretion of corticosterone. Our results are in concomitant with those reported by Kutlu and Forbes, (1993) and Lien, *et. al.*, (1999).

Plasma total protein, albumin and globulin were not significantly affected by all treatments. This may reflect the beneficial effects of both Cr and vitamin C in debating the detrimental influence of glucocorticoid hormone (s) on protein degradation as a result of stress-induced gluconeogenesis. This effect was also observed by Sa*hin, et. al.*, (2003) and Uyanik *et. al*., (2005).

Supplemental Vit. C and Cr + Vit.C decreased significantly plasma total lipids in comparison with individual Cr supplement and the control fed hens (Table 3). This trend was not observed for the plasma cholesterol concentration where the lowest value was recorded for the Cr supplemented diet compared with the other treatments. In general, the obtained results showed significant decreases in plasma lipid parameters, which could resulted from the depression of fatty acid synthesis due mainly to increasing demands of lipids for egg yolk formation. These results are consistent with those of Lien, *et. al.,* 1996 and Lien, *et. al.,* (1999), Uyanik, *et. al*., (2002) and Uyanik, *el. al*., (2005), who suggested that Vit, C or Cr supplementation to laying hens or quail diets could reduce all the blood plasma lipid parameters but not egg yolk total lipids and cholesterol.

Table 3: Effects of Supplemental Chromium (Cr) and Vitamin C on Plasma Metabolites of Laying Hens Reared Under Heat Stress (34 °C ± 2)

Items	Treatments			
	Control	Cr	Vit. C	Cr +Vit. C
Glucose (mg/dl)	222.72^{a} ±13.11	212.33^{ab}±16.55	183.98^{bc} ±6.61	173.69^{c}±4.64
Total protein (g/dl)	6.27 ± 0.25	6.40 ± 0.12	6.33 ± 0.12	6.40 ±0.12
Albumin (g/dl)	3.24 ±0.20	3.02 ± 0.12	3.16 ± 0.11	3.01 ±0.13
Globulin (g/dl)	3.03 ± 0.29	3.38 ± 0.26	3.17 ± 0.15	3.39 ±0.18
A/G	1.12 ± 0.14	0.92 ± 0.10	1.0 ± 0.08	0.91 ±0.08
Total lipids (g/L)	23.46^{a} ±1.25	21.36^{a} ± 0.90	18.52^{b} ± 0.25	13.04^{c} ± 0.66
Cholesterol (mg/dl)	300.27^{a} ±7.00	257.69^{b} ±10.30	287.91^{a} ± 6.60	281.51^{a} ±6.50

- a-c Means within same row with different letters are significantly differed ($P \leq 0.05$)
- Control: Basal diet, Cr: control diet + 80 mg of Cr/kg of diet.
- Vit. C: control diet + 250 mg of vitamin C/kg of diet,
- Cr + Vit C: control diet + 80 mg of Cr/kg of diet + 250 mg of vitamin C/kg of diet.

1-3- Effect of Supplemental Cr, Vit. C and Their Combination on Some Plasma Minerals and Enzymes

Plasma calcium (Ca) and inorganic phosphorous (Pi) are the two main important minerals for eggshell formation in laying birds. They significantly changed by dietary supplementation with Cr and vitamin C (Table 4). Plasma Ca significantly increased in all treated hens. Pi had significant increase under the influence of Cr while the other treatment had no significant effect on Pi plasma content (9.56 vs 5.28 mg /dl).

Alkaline Phosphatase activity (ALP) and Aspartate Transaminase activity (AST) were decreased significantly by all treatments except for AST which showed the same activity as control under the influence of Cr + Vit. C supplementation. On the contrary, Alanine Transaminase activity (ALT) showed significant increasing by the supplementation of Vit. C and Cr + Vit. C combination while no change in its activity was showed by Cr supplementation individually.

Since the typical plasma levels of ALP are 20-70 IU/L, therefore, the supplemental Cr and Vit. C either separately or in combination has good influences on optimizing the plasma levels of ALP in hens under heat stress as they decreased significantly the ALP levels in plasma of hens under stress from 90 IU/L (control; basal diet) to 36 IU/L (Cr diet), 78 IU/L (Vit. C. diet) and to 47 IU/L (Cr + Vit. C diet). Concerning the activity of AST and ALT, no pronounced changes could be observed by all treatments which refer to normal liver function of hens especially with the referenced ranges of activities obtained by all treatments (Gaze, 2007).

Table 4: Effects of Supplemental Chromium (Cr) and Vitamin C on Plasma Ca and Pi Levels and ALP, AST and ALT Activities of Laying Hens Reared Under Heat Stress (34 ^{o}C ± 2)

Items	Treatments			
	Control	Cr	Vit. C	Cr +Vit. C
Plasma Ca (mg/dl)	13.00 ±3.08^{c}	25.43 ±1.92^{a}	23.52 ±1.43^{b}	24.17 ±1.77^{ab}
Plasma Pi (mg/dl)	6.0 ±1.47^{ab}	9.56 ±1,22^{a}	5.28 ± 0.91^{b}	6.09 ± 0.90^{ab}
ALP (IU/L)	90.21±10.62^{a}	36.0 ± 1.52^{c}	78.91 ± 14.44 ^{ab}	47.6 ± 9.64^{bc}
AST (IU/L)	109.43 ± 3.13	99.96 ± 4.95	95.28 ±6.28	110.75 ± 6.51
ALT (IU/L)	8.87 ± 1.57	8.39 ± 1.02	14.36 ±1.25	15.37 ± 0.39

- a-c Means within same row with different letters are significantly differed ($P \leq 0.05$)
- Control: Basal diet, Cr: control diet + 80 mg of Cr/kg of diet.
- Vit. C: control diet + 250 mg of vitamin C/kg of diet,
- Cr + Vit C: control diet + 80 mg of Cr/kg of diet + 250 mg of vitamin C/kg of diet.

2- Effect of Supplemental Chromium, Vitamin C and Their Combination on Productive Performance and Egg Production of Laying Hens under Heat Stress

Data presented in Table (5) showed the effects of Cr and Vit.C supplementations either separately or in combination on productive performance of laying hens. The results showed that all treatments had no significant effects on body and egg weights of laying hens along the experimental period. Feed intakes significantly increased during the first 4 wk of the experimental period by Cr or Vit.C supplementation and the differences were disappeared during the second 4 wk of the experimental period. However, the groups fed Cr or Vit C separately gave higher feed intake value compared to the control hens by the end of the experimental period but the difference was not statistically significant.

On the other hand, it could be observed that Cr, Vit.C or a combination of both of them significantly increased egg number/hen by about 14.8, 14.1 and 15.0 respectively compared to control-fed group (Table 6). With respect to egg mass it could be noticed that supplementation with Cr, Vit. C or Cr + Vit. C significantly increased egg mass of laying hens by about 395, 399 and 436 g/hen, respectively compared to control–fed group along the whole period of experiment (32 - 40 wk). The result indicates that the combination between Cr and Vit. C supplementation to the diet gave better results than they supplemented separately. Concerning feed conversion ratio, the dietary Cr, Vit. C and Cr + Vit C supplementation showed a significant improvement along the whole experimental period. It has been reported that the supplementation of 20 ppm chromium chloride ($CrCl_3$) to the diet of broilers resulted in higher body weight (Steele and Rosebrough, 1979). Also, Lien *et. al.* (1999) found that chromium picolinate (Cr pic.) markedly enhanced weight gain due to the increasing of feed consumption in broilers. In this study, 80 ppm Cr did not affect the body weight of hen under this investigation which it is in concomitant with the results of Cupo and Donaldson (1987) in chicks and Sahin, *et. al.* (2001) in rabbits, but it reduced feed consumption and improved feed efficiency. Egg production and egg weight were not affected by chromium supplementation (Lien *et. al.,* 1996).

Table 5: Effects of Supplemental Chromium (Cr) and Vitamin C on Body Weight, Feed Intake and Feed Conversion Ratio of Laying Hens Reared Under Heat Stress (34 °C ± 2)

Age (Wk)	Treatments			
	Control	Cr	Vit. C	Cr +Vit. C
Body weight (g)				
32	1367±22	1344±33	1301±23	1335±44
36	1384±27	1385±35	1344±28	1366±39
40	1433±27	1443±33	13823±18	1390±41
Feed intake (g/hen)				
32-36	2990^{b} ±51	3100^{a} ±22	3128^{a} ±35	3087^{a} ±27
36-40	3091^{ab}±48	3028^{b} ±33	3146^{a} ±42	3125^{ab} ±37
32-40	6081 ±91	6128 ±50	6265 ±22	6212 ±61
Feed conversion ratio (g feed/g egg)				
32-36	2.36^{b} ±0.11	2.14^{a} ±0.09	2.13^{a} ±0.06	2.07^{a} ±0.04
36-40	2.41^{b} ±0.06	2.02^{a} ±0.05	2.10^{a} ±0.04	2.07^{a} ±0.03
32-40	2.38^{b} ±0.08	2.07^{a} ±0.05	2.12^{a} ±0.04	2.07^{a} ±0.03

- a-c Means within same row with different letters are significantly differed ($P \leq 0.05$)
- Control: Basal diet, Cr: control diet + 80 mg of Cr/kg of diet.
- Vit. C: control diet + 250 mg of vitamin C/kg of diet,
- Cr + Vit C: control diet + 80 mg of Cr/kg of diet + 250 mg of vitamin C/kg of diet.

Table 6: Effects of Supplemental Chromium (Cr) and Vitamin C on Egg Number, Weight and Mass of Laying Hens Reared Under Heat Stress (35 ^{o}C ± 2)

Age (Wk)	Treatments			
	Control	Cr	Vit. C	Cr +Vit. C
Egg number/hen				
32-36	21.7^{b} ±1.14	24.4^{a} ±0.96	24.4^{a} ±0.42	24.8^{a} ±0.34
36-40	20.9^{b} ±0.47	24.2^{a} ±0.65	24.2^{a} ±0.53	24.7^{a} ±0.41
32-40	42.6^{b} ±1.52	48.9^{a} ±1.38	48.6^{a} ±0.87	49.4^{a} ±0.59
Egg weight (g)				
32-36	59.2 ±0.06	59.9±1.05	60.0±1.09	60.2 ±0.60
36-40	61.6 ±0.56	61.4±0.88	62.2±0.86	61.4±0.48
32-40	60.4 ±0.53	60.6 ±0.85	61.1±0.92	60.8±0.51
Egg mass (g/hen)				
32-36	1283^{b} ±73.6	1463^{a} ±59.7	1467^{a} ±40.1	1493^{a} ±34.5
36-40	1287^{b} ±34.9	1503^{a} ±40.3	1503^{a} ±35.0	1514^{a} ±31.8
32-40	2571^{b} ±84.3	2966^{a} ±81.7	2970^{a} ±64.8	3007^{a} ±56.5

- a-c Means within same row with different letters are significantly differed ($P \leq 0.05$)
- Control: Basal diet, Cr: control diet + 80 mg of Cr / kg of diet.
- Vit. C: control diet + 250 mg of vitamin C / kg of diet,
- Cr + Vit C: control diet + 80 mg of Cr / kg of diet + 250 mg of vitamin C / kg of diet.

The egg shell thicknesses (mm) were enhanced in all treated hens in comparison with the controls (Table 7). This improvement was insignificant, however, it suggest that chromium supplementation could affect this parameter, probably via its interference with calcium and phosphorus metabolism since they (Cr and Vit. C.) increased markedly plasma Ca content and moderately plasma Pi content as shown in Table 4.

These obtained results are in agreement with those found by Sahin and Sahin (2002) who reported that supplemental chromium increased eggshell thickness. Also, similar to our results, Sahin *et. al.* (2004) reported that chromium supplementation restored the impairment in egg quality in laying Japanese quails reared under heat stress. On the contrary, Lien *et. al.* (1996) reported that shell thickness was not affected by chromium supplementation under thermo-neutral conditions.

Since the main aim of this investigation is to reduce well known negative effects of high summer temperature (heat stress) on laying hens such as reduction in egg production in general which is consequent of reduction in feed intake and feed conversion ratio, the obtained results of this study showed that Cr and/or vitamin C supplementation could reduce these negative effects through improving their metabolism via increasing T3 and T4 plasma level together with the improving in insulin level. ALP, AST and ALT, also affected in addition to Ca and Pi plasma level which worked together to increasing feed intakes and feed consumption ratio of hens under heat stress. The sum of all obtained results is significantly improving in egg mass production with moderate improvement in egg shell especially from healthy laying hens fed basal diet supplemented with Cr + Vit. C.

Table 7: Effects of Supplemental Chromium (Cr) and Vitamin C on Shell Weight, Thickness and Percentage of Laying Hens Reared Under Heat Stress (34 °C ± 2)

Age (Wk)	Treatments			
	Control	Cr	Vit. C	Cr + Vit. C
Shell weight (g)				
36	5.56±0.15	5.62±0.10	5.58±0.19	5.47±0.13
40	5.56±0.09	5.61±0.24	5.74±0.11	5.74±0.07
Shell percentage				
36	9.53±0.29	9.47±0.21	8.91±0.26	9.22±0.17
40	9.18±0.15	8.85±0.12	9.20±0.18	9.12±0.14
Shell thickness (mm)				
36	0.352±0.011	0.365±0.005	0.364±0.010	0.363±0.004
40	0.361±0.004	0.362±0.008	0.374±0.009	0.364±0.006

2- a-c Means within same row with different letters are significantly differed ($P \leq 0.05$)
3- Control: Basal diet, Cr: control diet + 80 mg of Cr/kg of diet.
4- Vit. C: control diet + 250 mg of vitamin C/kg of diet,
5- Cr + Vit C: control diet + 80 mg of Cr/kg of diet + 250 mg of vitamin C/kg of diet.

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كلوريد الكروم وحمض الأسكوربيك يخففان أثار إجهاد درجات حرارة الصيف العالية فى دجاج البيض

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تم تقسيم 72 دجاجة بياضة ذو عمر 32 أسبوع إلى 4 محموعات حسب طريقة التغذية المقدمة إليهم كالأتى:
1- مجموعة التغذية القياسية والتى تتغذى على الوجبة الأساسية (كنترول)
2- محموعة تتغذى على الوجبة الأساسية + 80 مجم كلوريد كروم
3- مجموعة تتغذى على الوجبة الأساسية + 250 مجم حامض أسكوربيك (فيتامين سى)
4- مجموعة تتغذى على الوجبة الأساسية + 80 مجم كلوريد كروم + 250 مجم حامض أسكوربيك

أدت المعاملات السابقة إلى أهم النتائج الأتية:
1- حدوث زيادة معنوية فى تركيز هرمون T3 فى بلازما الدم
2- حدوث زيادة معنوية فى تركيز هرمون T4 فى بلازما الدم
3- حدوث زيادة معنوية فى تركيز هرمون الأنسيولين فى بلازما الدم
4- حدوث إنخفاض معنوى فى أكسدة الليبيدات وذلك بإنخفاض مستويات MDA فى بلازما الدم
5- عدم حدوث أى تأثير معنوى فى نسبة كلا من الجلوكوز أو الليبيدات الكلية أو الكوليسترول فى بلازما الدم
6- حدوث تغيير معنوى فى محتوى كلا من الكالسيوم (Ca) والفوسفور الغير عضوى (Pi) فى بلازما الدم
7- حدوث تغيير معنوى فى نشاط إنزيمات الكبد مثل : ALP, AST and ALT

أدت التغيرات المعنوية التى حدثت فى بلازما الدم للدجاج البياض تحت تأثير الكروم وفيتامين سى إلى الأتى:
1- عدم حدوث تغيير معنوى فى وزن الجسم للدجاج أو وزن البيضة الناتجة من الدجاج
2- حدوث زيادة معنوية فى معدل إستهلاك الغذاء وفى معامل التحويل طوال فترة التجربة
3- حدوث زيادة معنوية فى عدد البيض الناتج من كل دجاجة وبالتالى زيادة معنوية فى وزن البيض الإجمالى
4- حدوث تحسن معنوي فى سمك قشرة البيض الناتج

أستنتجت الدراسة أن:
إستخدام الكروم وفيتامين سى فى شكل مخلوط (ليس بشكل فردى كلا على حده) يدعم به الوجبة الأساسية للدجاج البياض بالتركيزات المستخدمة فى هذة الدراسة وخصوصا فى فصل الصيف من شأنه أن يحافظ على وزن جسم الدجاج ويزيد من شهيته للأكل ويزيد من مقاومته لحرارة الجو بل ويزيد من معدل إنتاجه للبيض كناتج أجمالى وذلك عن طريق زيادة عدد البيض الناتج ككل مع حدوث تحسن فى قشرة البيضة.