EFFECT OF DIETARY CHROMIUM, SELENIUM AND VITAMIN C SUPPLEMENTATION TO THE DIET ON REPRODUCTIVE PERFORMANCE AND EGG QUALITY OF LAYING HENS DOKKI-4 UNDER EGYPTIAN SUMMER CONDITION

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SUMMARY

The study was performed to evaluate the effect of organic chromium (Cr), organic selenium (Se) or vitamin C (vit. C) alone or in combination on reproductive performance, and egg quality of Dokki-4 laying hens under hot summer conditions of Egypt. A total number of 240 hens plus 48 cocks from Dokki-4 strain at 30 weeks of age were randomly selected and distributed into eight groups with 3 replicates (10 hens + 1 cock) each. The remaining 24 cocks were also divided into 8 groups of 3 cocks each and housed separately for semen evaluation experiment. Treatments groups were fed a basal diet (control group) or the basal diet supplemented with either 400 µg Cr/kg diet (Cr group), 250 mg of L-ascorpic acid /kg diet (vit. C. group), 0.2 mg Se/kg diet (Se group), 400 µg Cr plus 250 mg of L-ascorpic acid/ kg diet (Cr + vit. C group), 400 µg Cr plus 0.2 mg Se/kg diet (Cr + Se group), 2 mg Se plus 250 mg L-ascorbic acid/kg diet (Se + vit. C. group) and 400 µ Cr plus 0.2 mg Se plus 250 mg ascorbic acid /Kg diet (Cr + Se + vit.C group). All groups were put under observation for 12 weeks. Results obtained can be summarized as follows: Supplementation of Cr, Se or vitamin C alone or in combinations significantly increased ($P < 0.05$) shell thickness, haugh units, albumin and shell to egg weight, sperm motility, ejaculate volume, sperm concentration, total sperm output, total motile sperm, live spermatozoa, semen quality factor and significantly decreased ($P < 0.05$) dead spermatozoa and seminal malondialdehyde (MDA) as compared with control group. The best result was obtained for layers fed diet containing the combinations of the three supplements (Cr, Se or vitamin C) as compared to other groups.

In conclusion, combinations of Cr, Se and vitamin C improved the most reproductive traits and egg quality of Dokki-4 strain under Egyptian hot summer.

Keywords: *Laying hens, chromium, selenium and vit. C supplementation, reproductive performance and egg quality.*

INTRODUCTION

Nowadays in Egypt there is necessity of increasing animal production to fulfill the insisting demand of animal protein. It is noticed that the price of animal protein is getting higher during the last few years. So, the increase in animal protein production may come from the poultry. Poultry feeding and management could be considered very reasonable in cost if compared with the other animals. One of the problems challenging in poultry industry is the high ambient temperature, which persists in Egypt for about 5 months per year (May to September), as it is can compromise the ability of birds to maintain homeostasis (Kadim *et al.*, 2008). It depresses body weight, egg production, weight, quality and hatchability and increase mortality (Pavlik *et al.*, 2009). Heat stress begins when the ambient temperature becomes higher than 27°C and is readily apparent above 30°C (Bollengier-lee *et al.*, 1998). Heat stress leads to generation of free radicals, such as O- and HO. These free radicals can damage cell membranes by inducing lipid peroxidation of polyunsaturated fatty acids in the cell membrane (NRC, 1994). Because radical reactions are exergonic, they contribute with failure of the thermoregulation process to the increase of body temperature observed during heat stress. As a result, dietary supplementation of these two antioxidant compounds would attenuate the deleterious heat-induced- oxidative stress. Heat stress increases the need for antioxidants, because birds can not synthesize enough ascorbate during hot conditions (Cheng *et al.*, 1990), and dietary supplementation with high dosages of antioxidants, such as vitamin C and E, have been conducted.

Various techniques are being practiced by the farmers to minimize the heat stress, such as adding antioxidant, vitamins or minerals such as selenium, vitamins C, E and folic acid, zinc and chromium (Sahin *et al.*, 2002).

On the other hand, vitamin C is an indispensable micronutrient required to maintain the physiological processes of poultry (McDowell, 1989). Kutlu and Forbes (1993) reported that ascorbic acid reduces the synthesis of corticosteroid hormones in birds. Similarly, Sahin *et al.* (2002) reported that lower concentrations of adrenocorticotropic hormone (ACTH) in quail reared at 32°C and fed a diet supplemented with vitamin C than in the heat-stressed controls. By decreasing synthesis and secretion of corticosteroids, vitamin C alleviates the negative effects of stress (McDowell, 1989). Desoky (2008) found that dietary supplementation of vitamin C showed high ability of alleviating the negative effect of heat stress and improved egg production and egg quality. Moreover, vitamin C itself plays important roles in cellular anti-oxidant defenses, not only by reacting with all oxygen species through formation of dehydroascorbyl, a particular inert radical, but also by transferring radical equivalents from lipid phases to the aqueous compartment. In complement, ascorbate participates in the regeneration of reduced glutatione from the oxidized form in the cytoplasm and allows tocopherol regeneration through a non-enzymatic reaction (Ciftci *et al.*, 2005).

Organic Cr compounds can be absorbed about 20-30 times more efficiently than inorganic forms (Piva *et al.*, 2003). Uyanik *et al.*, (2002) have shown that Cr absorption is higher when it is associated with a specific organic molecule. A number of organic Cr preparations are commercially available to protect animals from stress associated with environmental and management conditions of intensive livestock farming. Chromium-methionine (Cr-Met) chelate is a newly developed organic Cr which is able to directly cross the intestinal cell membrane and be metabolized without any prior digestion since it was chelated with amino acid. Thus, bioavailability of Cr-Met chelate is proposed to be higher than those of other organic Cr.

On the other hand, selenium (Se) has been recognized as an essential nutrient required for laying hens for normal growth, maintenance of health and physiological functions. The role of Se in biological systems has been associated with its antioxidant activity (Schwary and Foltz, 1957), while its physiological importance was recognized when it is found to be an essential structural of the glutathione peroxidase enzymes (Rotruck *et al.*, 1973) that destroy free radicals produced during normal metabolic activity (Wakebe, 1998). The use of seleno-yeast (SY) in laying hens is very effective for increasing the Se content of egg (Utterback *et al.*, 2005). However, Se can affect egg quality where, it can ameliorate some of the adverse effects of strong Haugh unit value of eggs (Pappas *et al.*, 2005). Also, it may affect metabolism and production because it is essential for the synthesis of active thyroid hormones, while, no differences in egg production, egg weight, feed intake or mortality by using organic SY which is very effective for increasing the Se content of eggs (Utterback *et al.*, 2005).Therefore, adding Se to laying diets improves their health , productivity and can also be a natural way to produce functional food respectivity the production of egg enriched with Se (Yaroshenko *et al.*, 2003 and Sara *et al.*, 2008), which represents a commercially valuable use for the future.

This study was carried out to establish the dietary supplementation of chromium, selenium and vitamin C either alone or in combinations on reproductive traits, egg quality, and economic efficiency of Dokki-4 strain, under hot summer conditions.

MATERIALS AND METHODS

The experimental work of this study was carried out at Sakha Poultry Research Station, Animal Production Research Institute, Egypt. The chemical analyses were carried out at Laboratories of the Animal Production Research Institute, Ministry of Agriculture, Egypt during summer season (from June to August, 2014). The average minimum and maximum ambient temperatures ranged between 21.75 and 40.65^{o}C, relative humidity from 31.8 to 80.7% and temperature-humidity index (THI) from 36.0 to 51.39% under under Kafr El-Sheikh Governorate, Egypt (Central Laboratory for Agricultural Climate) as show in Table 2 which was a burden on the chickens According to Marai *et al.* (2002) there is severe heat stress when THI is higher than 28.9.

The THI was calculated according to the formula by Marai *et al.* (2001) as follows:

THI = db^{o}c – [(0.31-0.31 RH) x (db^{o}c -14.4)]

Where: db^{o}c is dry bulb temperature in Celsius degrees, and RH is the relative humidity as a percentage.

A total number of 264 (240 laying hens + 48 cocks) chickens (Egyptian strain, Dokki-4), 30 weeks old were individually leg-banded, weighed and randomly distributed into 8 groups of 3 replicates (10 laying hens + 1 cock / replicate). The remaining 24 cocks were also divided into 8 groups of 3 cocks each and

housed separately for semen evaluation experiment. The birds were housed in floor pens in an open house and photoperiod of 17 hours daily. Birds were kept under the same managerial and hygienic conditions. Birds were healthy and examined against diseases and treated with antibiotics and vaccines.

A corn-soybean meal basal experimental layer diet (~16% and 2750 kcal ME/ kg diet) was formulated to cover all recommended nutrient requirements according to the Egyptian Feed Composition Table (2001) as shown in Table (1). Treatments groups were fed a basal diet (control group) or the basal diet supplemented with either 400 µg Cr/ kg diet (Cr group), 250 mg of L-ascorbic acid / kg diet (Vit. C group), 0.2 mg of Se / kg diet (Se group), 400 µg Cr plus, 250 mg of L-ascorbic acid / kg diet (Cr + Vit. C group), 400 µg Cr plus 0.2 Se/ kg diet (Cr + Se group), 0.2 mg Se plus 250 mg ascorbic acid / kg diet (Se + Vit C group) and 400 µg Cr plus 0.2 Se + 250 mg ascorbic acid / kg diet (Cr + Se + Vit. C group), respectively. Chromium-methionine {Cr $(C_5H_{10}NO_2S)_3$}, alight violet-red crystalline powder containing 1% Cr, was the source of the supplemental Cr. Vitamin C (ROVIMX® x STAY-C® 35) was provided by a commercial company (Roche, Levent-Istanbul, Turkey). Organic selenium was SelPlex™ in the form of selenium yeast (Alltech Inc.) contained 1000 ppm organic selenium and produced by the fermentation of yeast (*Saccharomyces cerevisiae*) in a high organic selenium medium.

Egg quality measurements:

Five representative eggs from each treatment were collected monthly throughout the experimental period in order to determine egg and shell quality.

Parameters measured for egg quality were egg shape index (%) was calculated according to Romanoff and Romanoff (1949) as an egg diameter divided by an egg length. Estimated as the ratio of the egg maximum width to its length, shell thickness was measured in mm using a micrometer. Yolk index was calculated according to Funk *et al.* (1958), as yolk height divided by yolk diameter. Haugh unit was calculated according to Eisen *et al.* (1962) using the calculation chart for rapid conversion of egg weight and albumen height. Arc-sine transformations were done for percentage of egg shape index and yolk, albumin and shell percentages before stimulation of the data. Haugh unit score was calculated for each individual egg according to Haugh (1937).

Egg shells were ground prior to ashing (600°C for 6 h). Calcium and phosphorus were determined according to the methods of the Association of Official Analytical Chemists (2005).

Effect of storage time on malondialdehyde concentration:

Malondialdehyde, yolk pH was studied at 0, 5, 10 and 15 days after storage of three eggs from each treatment at (20 to 23°C) and 60 % RH. Malondialdehyde was measured according to Marshall *et al.* (1994). The values of malondialdehyde were expressed in terms (mg/ kg egg yolk) as reported by Wang and Pan (2003). Yolk pH during storage was measured as reported by (Kirunda and McKee, 2000). The previous analyses were done at Lab. Of Food Science, Faculty of Agriculture, Cairo Univ., Giza. Five representative eggs from each treatment were collected monthly throughout the experimental period and yolk samples were separated from the broken eggs, calculated and extracted according to Folch *et al.*, (1957). Total lipids, cholesterol, LDL, HDL and triglycerides were colorimetrically determined in egg yolk.

Monthly semen sample was collected from cocks by the massage method from all cocks. Semen ejaculate volume was measured by tuberculin syringe graduated to nearest 0.01 ml according to Allen and Champion (1955). Sperm concentration was determined by using Thomes-Zeis haemocytometer (Kalamah *et al.*, 2000). Total sperm output was calculated by multiplying ejaculate volume and spermatozoa concentration. Percentage of live and abnormal sperms were determined after staining with eosine and nigrosine (Blom, 1950), then calculated as a percentage out of randomly chosen 100 sperm counted. Percentage of motile sperm was estimated by visual examination under low-power magnification (10x) using a phase-contrast microscope according to Melrose and Laing (1970). Total number of motile sperm (TMS) calculated by multiplying percentage of motile sperm and total sperm output. Semen quality factor (SQF), calculated according to the following pattern was used: SQF = (sperm concentration x ejaculate volume x live spermatozoa) / 100, Hydrogen ion concentration (pH) of semen was determined immediately after collection using pH paper. Seminal plasma was obtained by centrifugation of semen samples at 3500 rpm for 20 min at 4°C, and was stored at -20°C until later analysis. Malondialdehyde (MDA) in seminal plasma was measured in the form of thiobarbituric acid reactive substance (TBARS) as described by Richard *et al.* (1992).

Eggs of each group were daily collected at 5.00 pm during the last four days of each month, stored in egg room storage at 16-18 °C and incubated in an automatic electrical incubator at 99-100 °F and 60-70% relative humidity with turning at 2-hours intervals. After 14th day of incubation, eggs were transferred to the hatcher (turning was stopped, 96- 97 °F and 80-90% relative humidity). At hatching time, infertile eggs were examined to determine fertility % (fertile eggs/ total eggs x 100) and hatchability % was calculated as hatched chicks/ total eggs x 100.

Statistical analysis:

Data were analyzed using the GLM procedure of SAS® (SAS, 2003) using one-way ANOVA according to the following model:

$Xij=\mu+Ti+eij$

Where: Xij = any observation.

μ = Overall mean,

Ti = Treatments (i= 1, 2…and 8),

eij = Experimental error.

Before analysis, all percentages were subject to logarithmic transformation (log10 x+1) to normalize data distribution. Mean difference at $P\leq0.05$ was tested using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

***Egg quality**:*

Egg quality and egg components of Dokki-4 layers as affected by either dietary supplementation of chromium, selenium, vitamin C, or their mixtures during hot summer condition are presented in Table (3). Supplementation of Cr, vitamin C or Se, singly or in combinations were significantly increased ($P < 0.05$) the shell thickness, Haugh units, yolk index, shell to egg weight, albumen weight % calcium and phosphorus levels in shell compared to the control group. It is worth noting that, egg shape index, was not significantly affected by dietary supplementation of Cr, vitamin C and Se alone or with any combinations. These results agreed with those of Pavlik *et al.* (2009) who found that exposure of hens to high ambient temperatures resulted in a significant decrease in egg quality traits (shell weight, shell thickness, and specific gravity). These finding could be due to the reduction in feed consumption (data not published). The adverse effect of high environmental temperature on egg shell quality has been well documented (Mahmoud *et al.*, 1996). The decrease in shell quality in the current study may be partially due to a reduction in plasma calcium. It has been reported that plasma calcium level was significantly decreased in laying hens (Mahmoud *et al.*, 1996) when the birds were exposed to high temperatures. Also, the reduction in shell thickness may result from the insufficient intake of nutrients including minerals such as Ca due to reduced feed intake or insufficient HCO_3 level to form $CaCO_3$ due to excess expiration of CO_2 or both. The reduction in egg weight (Bollengier-Lee *et al.*, 1998) and shell thickness (Yardibi and Türkay, 2008) reflects the detrimental effects of heat stress on egg shell quality.

These findings agreed with those reported by Essa and Madian (2009) and El-Gendi *et al.* (1999) who found that eggs were characterized significantly higher ($P \leq0.05$) absolute and relative weights of shell and yolk by vitamin C supplementation. Also, Metwaly (2005) found that hens fed diet with vitamin C had a good quality of yolk, shell weight and shell thickness when compared to the control.

As well, Cheng *et al.* (1990) reported that shell weight per unit surface area showed a small increase with supplementary ascorbic acid (0, 100, and 200 ppm) and values (in Haugh units) were increased by ascorbic-acid at a level of 200 mg/ kg with low relative humidity. Desoky (2008) found that dietary supplementation of vitamin C (200 mg vitamin C/ kg diet) or vitamin E (150 mg vitamin E/ kg diet) alone or in combination showed high ability of alleviating the negative effect of heat stress and improved egg quality.

Similarly to the results of the present study, El-Boushy *et al.* (1998) reported that dietary vitamin C supplementation increased egg production and egg shell strength in stressed laying hens. It has been reported that ascorbic acid plays a role in bone maturation by improving hydroxyproline production which is required for collagen formation. Accordingly, in birds, it was postulated that ascorbic acid stimulates 1,25- dihydroxycholecalciferol and together these compounds increase calcium mobilization from bones, suggesting that vitamin C has an important role in egg shell formation (Sahin and Sahin, 2001). In the present study, vitamin C supplementation significantly increased egg shell thickness and egg shell weight. Chee *et al.* (2005) reported that vitamin C (200 mg/ kg) supplemented to the diet of broiler breeder hens could prevent drops in egg shell quality under highly stressful environmental temperatures. This may be due to an increased calcium mobilization from bones (Sahin and Sahin, 2001).

Concerning Selenium, egg quality traits were significantly effected ($P<0.05$) by using diets containing SY. These results are in agreement with Renema (2006) and Sara *et al.* (2008), they reported that the administration of organic Se in laying hen diets increased shell-thickness consequently improved egg shell quality. Similar reports by Spring (2006) and Hanafy *et al.* (2009). However, SY addition, reduced deterioration of the albumen quality which results in slower carbon dioxideloss and thus maintains

albumen quality after the egg is laid (Wakebe, 1998). Experimental results obtained are in harmony with those reported by Spring (2006) who indicated that organic Se supplementation in broiler and breeder layers improved egg quality and antioxidative properties.

Se supplementation was stated to influence the oestradiol dependent mechanisms by exerting a direct effect on oestradiol or an indirect effect through maintaining more normal function of cellular processes regulating oestradiol and restoration of estrogen secretion (Bolleengier- Lee *et al.*, 1998). Oestradiol has an effect on circulating calcium through its control synthesis of 1, 25 dihydroxy cholecalciferol and the active cholecalciferol metabolite that regulates calcium absorption (Taylor and Drake, 1984). Circulating calcium and estrogen concentration are highly correlated in laying hens (Tojo and Huston, 1980).

Concerning feeding Cr-supplemented diets significantly increased shell thickness, albumin weight, yolk index and yolk weight compared to control diet. Similar results were obtained in laying Japanese quail (Sahin *et al.* 2001, 2002, Yeşilbağ and Eren, 2009 and Abdel-Mageed and Hanan, 2012) and hens (Lien *et al.* 1999; Uyanık *et al*., 2002 and Yıldız *et al.*, 2004). 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 egg during the plumping process in the uterus., In Lohman White Laying hens chromium yeast supplementation increased albumen and yolk index (Eseceli *et al.*, 2010). Uyanik *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.

Also, the beneficial effect of organic Cr on external egg shell traits may be account for the indirect action of Cr in empowering the ascorbic acid transportation (Seaborn *et al.*, 1994), which has an important role in egg shell formation. Moreover increasing egg shell thickness may due to Cr stimulates and regulates the action of insulin (Anderson, 1994 and Mowat, 1994); thus increasing the effectiveness of insulin.

Egg yolk constituents:

Dietary supplementation of chromium, selenium, vitamin C, or their mixtures during hot summer condition decreased ($p \leq 0.05$) egg yolk content of total cholesterol, L.D.L., total lipids and triglycerides as compared with the control (Table 4). The results of egg yolk constituents were supported by Ali *et al.* (2007) who found that addition of thyme and anise as natural antioxidants, in layer diets (Inshas and Dokki-4) decreased LDL, total cholesterol, triglyceride and total lipids in yolk extract. Radwan *et al.* (2008) found that addition of natural antioxidants, in layer diets (El-Slam strain) significantly decreased yolk lipid in comparison to the control group.

Egg quality during storage:

Table (5) represent the effect of storage time on egg content pH and its and malonaldehyde content as response to different treatments.

Egg content pH in general increased with increasing storage time (Table 5). Addition of chromium, selenium, vitamin C, or their mixtures kept the pH of egg content lower than that of the control. Egg yolk malonaldehyde increased with increasing storage time (Table 5). Addition of chromium, selenium, vitamin C, or their mixtures resulted in less ($p \leq 0.05$) malonaldehyde than that of the control.

In this regard Radwan *et al.* (2008) found that addition of natural antioxidants in layer diets during laying period significantly decreased malonaldehyde formation in egg yolk and had a positive effect on oxidative stability of shell eggs storage at room temperature (16°C ±2). Samli *et al.* (2005) supported the effect of egg storage time on pH of albumen and yolk, where it increased in stored eggs compared with the fresh eggs. The authors observed rapidly increased pH in albumen with 2 d of storage time and extended from 7.47 to 9.2 at 29°C during 5 d of storage. They indicated that the increase in pH observed in yolk was not as large as in albumen and it differed from 5.75 to 6.08 during 10 d of storage at 29°C. They explained that most of these changes in egg quality were attributed to water loss by evaporation through the pores in the shell and the escape of carbon dioxide from albumen.

Semen physical characteristics*:*

Sperm motility, dead spermatozoa, sperm cell concentration and seminal MDA of Dokki-4 cockerels as affected by dietary supplementation of either Cr, vitamin C, Se or their mixtures during hot summer condition are presented in Table (6). Supplementation of Cr, vitamin C or Se singly or in combinations were significantly ($P < 0.05$) increased sperm motility and decreased dead spermatozoa and seminal MDA compared with the control group. While, supplementation of Cr, vitamin C and Se in combinations were significantly increased ($P < 0.05$) sperm cell concentration as compared with control group. These results are in agreement with Hood (1999) who reported that heat exposure caused an increase in the

percentage of dead sperm (29.1%) and a decrease in the sperm quality index (SQI) (10.2%). Whoever, Monsi and Onitchi (1991) supplemented the feed of heat-stressed broiler breeders with 0, 125, 250 or 500 ppm of ascorbic acid and who found that semen volume, total sperm, and motile sperm per ejaculate were significantly increased due to the addition of ascorbic acid. Noll (1997) reported that improved sperm cell concentrations in males and more eggs per hen when turkey breeder diets were supplemented with 200 mg per kg of vitamin C. This improved reproductive performance was noted in spite of environmental temperature fluctuations.

Furthermore, these results are in agreement with that reported by Simon (2004) who showed that when broiler breeders were fed diets supplemented with selenium improve sperm morphology and activity. Also, Surai *et al.* (1998) indicated that the need for defense against oxidative damage is clear in the male, where antioxidant enzymes play a key role in maintaining the sperm cells. Sperm cells contain large amounts of polyunsaturated fatty acids, which allow them to maintain flexibility relating to motility (Surai, 2002). However, this means they are also a target for lipid peroxidation. Cellular integrity is maintained by GSH-Px and other selenoenzymes which protect the cell membranes from oxidative damage (Flohe and Zimmermann, 1970). Similar results of a live sperm were obtained by Davtyan *et al.* (2006) who indicated that the number of spermatozoa was also increased significantly by the use of selenium. Sefton and Edens (2004), found that the sperm quality index was greater in semen samples collected from males fed Sel-Plex^{TM}. Also, normal sperm number was significantly increased while abnormal number was significantly decreased compared with group fed sodium selenite. Edens (2002) found that the inclusion of selenium in poultry diets enhances sperm number, and using an organic source (Sel-Plex ^{TM}) reduces production of defective sperm, thereby having a positive effect on the fertilizing potential of the male. Our results in sperm concentration are supported by Renema (2006) who found that feeding broiler breeder males (between 45- 65 weeks of age) 0.2 mg / kg Sel-Plex ^{TM} increased semen concentration. Also, Spring (2006) indicated significant improvements in spermatozoa concentration and activity, when fed diets were supplemented with Se yeast in comparison to selenite. Significant improvements in semen physical properties and seminal malondialdehyde concentration were observed as affected by supplementing Cr from Cr-Met compared with those for control group (Table 6). 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). 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 for the degree of sperm membranes integrity and their fertilizing ability (Long and Kramer, 2003).

Vitamin C has been demonstrated to be a powerful antioxidant that acts through a two-way mechanism, that is, through its conversion to L-dehydroascorbic acid, a particularly inert radical, this reaction is reversible and the interconversion of these molecules forms a redox system, and the basic physiology of their actions, as both show vitamin C activity. The other route, is the formation of an ascorbate radical that destroys free radicals generated by oxygen, which includes hydroxyl (OH·), mono-oxygen (O·) and the superoxides (O2·) and also in the transfer of radical equivalents from lipid phases to aqueous compartment. In realizing this function, the vitamin enters into a synergistic action with other protective antioxidant enzymes, such as: catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSHPx). Puthpongsiriporn *et al.* (2001) confirmed in vitro that the addition of vitamin C reduced the rate of proteolytic induction by hydrogen peroxide (H_2O_2) and the destruction of SOD.

Fertility and hatchability %:

Hatchability percentage and hatch chick weight of Dokki-4 layers as affected by dietary supplementation of either Cr, vitamin C, Se or their mixtures during hot summer condition are presented in Table (7). Supplementation of Cr, vitamin C or Se either individually or at any combination significantly ($P < 0.05$) increased hatchability percentage comparable to control birds.

Ciftci *et al.* (2005) reported that, exposure of Japanese quails and laying hens to high ambient temperatures caused reduction in reproductive activities and egg quality respectively. The reduction in reproductive performance associated with heat stress is a well-known phenomenon in domestic birds (Daghir, 2009). This is probably due to the direct debilitating effect of high ambient temperature on ovarian function in the birds (Rozenboim *et al.*, 2007). A possible mechanism for the reduction of ovarian function might be the reduction in blood flow to the ovary; a differential ovarian blood flow pattern was found in hens exposed to high ambient temperatures (Mashaly *et al.*, 2004).

The improvement in hatchability percentages could be attributed to the improvement in egg shell quality (Abdel-Mageed and Hassan 2012), since the poor hatchability in hot climate may be partially due to thin shell eggs (Daghir 1995). These results agree with those reported by Anderson (1994) who reported that Cr is thought to be essential for activating certain enzymes and for stabilizing proteins and nucleic acids which lead to increase fertility and hatchability percentages. Likewise Hanafy (2011) found that fertility and hatchability percentages significantly ($p \leq 0.05$) increased with the increase of dietary organic Cr levels from 250 to 1500 ppb Cr. Similarly, Contreras and Barajas (2001) reported that supplementing 400 µg Cr/ kg diet from Cr-Met improved hatchability in Japanese quail during winter season compared with the control (74 vs 64.8 % respectively). Also, Contreras *et al.* (2000) showed that 200 ppb Cr-Met supplementation improves hatchability in Japanese quail under weather controlled conditions in dry tropic areas (25 °C). However, Abd El-Samee *et al.* (2012) reported that supplementing diets with 600 or 1200 µg Cr-yeast/ kg diet had no effects on fertility and hatchability percentages in quail.

Vitamin C is known to decrease the use of corticosteroids released during stress (Sahin *et al.*, 2003), thus playing an important role in response to stress. Single or combined dietary supplementation with vitamin C, Cr and Se of laying hens exposed to heat stress in this study has significantly improved the fertility and hatchability parameters. Dietary additions of Se alone or with other feed additives appeared to be more beneficial for laying hens during heat stress, probably due to its concurrent function as a fertility factor (Kevin, 1982). In addition, Hassan (1990) reported that the effects of Se deficiency (at 0.03 mg / kg) in the diet of White Leghorn caused a significant reduction in hatchability. Oishi *et al.* (1988) reported that low- Se diet decreased LH hormone. Agate *et al.* (2000) showed that Se as Sel-Plex supplementation in laying hen diets improved the environment of the sperm storage tubules in the hen's oviduct, allowing sperm to live longer, increasing the length of time sperm can be stored and increasing the average number of sperm holes in the yolk layer. Also, Hanafy *et al.* (2009) reported that Se as Sel-Plex supplementation increased the hatchability of fertile eggs and number of hatched for laying hen. Osman *et al.* (2010) showed that Se supplementation increased hatchability, this may be due to improved anti-oxidant status. In this respect, Davtyan *et al.* (2006) and Petrosyan *et al.* (2006), reported that a primary effect is on the breeder performance through a higher number of viable chicks produced and lower mortality of embryos during incubation, while the second effect of Se is on the males by maintaining sperm quality long time when stored in sperm storage tubules after mating and quantity is of major interest.

The protective effects of Cr , Se or vitamin C are apparent especially during the highly oxidative state of late incubation and the first few days after hatch, concerning to Se, this result agrees with (Surai, 2000). Hatched chicks produced from hens fed dietary supplementation of either Cr, vitamin C, Se or their mixtures during hot summer condition were significantly heavier than those of the group fed the basal diet (Table 7). Similar results were reported by Sefton and Edens (2004) and Pappas *et al.* (2006) who found that the chick weights from parents fed diets high in Se were heavier at hatch than those hatched from parents fed diets low in Se.

In this experiment, the single or combined dietary supplementation with chromium, vitamins C and selenium in laying chickens exposed to summer tropical hot-humid climate, have significantly improved reproductive performance, egg quality profiles of: egg weight, egg yolk weight, egg albumen weight and eggshell weight. However, it is interesting to note that supplementation of these antioxidants appeared to be more beneficial for laying hens during heat stress. Probably, due to their synergistic actions in quenching free radicals generated during heat stress (Ciftci *et al.*, 2005). The synergic effects between these additives are particularly efficient for reducing production of reactive oxygen species in both aqueous and lipid phase of the cell membrane, and because radical reactions are exergonic, they contribute to the failure of thermoregulatory process in hyperthermia observed during heat stress (Mujahid *et al.*, 2005).

CONCLUSIONS

By increasing reproductive performance and egg quality profiles, the administration of both antioxidants demonstrated their positive effects at ameliorating the adverse conditions of the hot-humid summer period under which the birds were reared. It is therefore recommended that both antioxidants be incorporated into laying hen's daily routine feeds as a nutritional strategy towards sustaining reproductive performance and egg quality productions, particularly during the hot summer periods.

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Table (1). Composition of the basal experimental diet.

Ingredients	%
Yellow corn	66.00
Soybean meal (44%)	24.00
Limestone	7.59
Di-calcium phosphate	1.71
Sodium chloride	0.30
Vit.& Min. Mixture*	0.30
DL.Methionine	0.10
Total	100
Calculated analysis**	
Metabolizable energy (kcal/ kg)	2750
Crude Protein, %	16.43
Crude fiber, %	3.20
Ether extract, %	2.70
Calcium, %	3.33
Available phosphate, %	0.45
Total phosphorus, %	0.66
Lysine, %	0.86
Methionine, %	0.39
Determined analysis	
Crude Protein, %	16.45
Crude fiber, %	3.18
Ether extract, %	2.68
Calcium, %	3.50
Total phosphorus, %	0.70

**Supplied per kg of diet: vit.A, 10000 IU; D_3, 2000 IU; Vit.E, 10mg; Vit.K_3, 1mg; vit.B_1, 1mg; vit. B_2, 5mg; vit.B_6, 1.5mg; vit. B_{12}, 10mcg; Niacin, 30mg; Pantothenic acid, 10mg; Folic acid, 1mg; Biotin, 50µg; Choline, 260mg; Copper, 4mg; Iron; 30mg; Manganese, 60mg; Zinc, 50mg; Iodine, 1.3mg; Selenium, 0.1mg and Cobalt, 0.1mg.*
*** Calculated according to NRC. (1994).*

Table (2). Temperature (ºC) and relative humidity% during the experimental period from June to August, 2014*.

Items	AT (ºC)		RH (%)		THI	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
June	22.6	32.8	26.4	39.4	21.0	28.5
July	22.2	37.7	25.1	41.1	10.8	29.9
August	23.9	36.2	31.1	47.1	22.3	31.6
±SE	0.91	0.97	2.19	1.87	0.76	0.73

** Central Laboratory for Agricultural Climate.*

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Table (3). The effects of supplemental Cr, Se or vit. C alone or in combination on egg quality of laying hens reared during summer conditions.

Treatments	Egg quality									
	Egg Weight (g)	Yolk wt. %	Albumen wt. %	Shell wt.%	Egg Shape index	Yolk index	Shell thickness (mm)	Haugh unit	Shell calcium %	Shell phosphorus (ppm)
Control group	46.98	32.60^{a}	57.90^{c}	9.38^{e}	74.70	44.15^{c}	0.321^{d}	80.12^{c}	32.5^{c}	1200^{e}
Cr-group	47.44	29.86^{c}	60.61^{a}	9.5^{d}	74.85	44.98^{ab}	0.356^{b}	82.15^{b}	33.8^{b}	1345^{d}
Vit. C-group	46.98	29.03^{c}	61.32^{a}	9.64^{cd}	74.95	44.87^{b}	0.348^{c}	83.05^{a}	33.7^{b}	1378.5^{d}
Se-group	47.61	29.69^{c}	60.32^{a}	9.97^{cd}	75.5	44.95^{b}	0.355^{b}	82.5^{ab}	34.1^{ab}	1542.5^{c}
Cr+vit.C-group	47.49	31.03^{b}	59.57^{b}	9.89^{cd}	75.00	45.05^{ab}	0.354^{b}	83.75^{a}	34.7^{ab}	1560^{c}
Cr+Se-group	47.9	31.94^{b}	58.03^{bc}	10.02^{c}	74.85	45.62^{a}	0.360^{b}	82.95^{ab}	33.9^{b}	1780^{a}
Se+vit.C-group	47.88	31.28^{b}	56.82^{c}	11.88^{a}	75.11	45.7^{a}	0.358^{b}	82.68^{ab}	34.4^{b}	1698^{b}
Cr+Se+Vit.C- group	47.55	30.66^{ab}	58.88^{bc}	10.45^{b}	75.80	45.89^{a}	0.373^{a}	83.25^{a}	35.2^{a}	1788.5^{a}
SEM	0.231	0.069	0.082	0.059	0.193	0.257	0.003	0.193	0.069	0.525
p-value	0.10	0.004	0.006	0.0001	0.9	0.001	0.001	0.001	0.0001	0.0001

a.b…= Means on the same column differently superscripted are significantly different (p ≤0.05).
SEM = Standard error of means.

Table (4). The effects of supplemental Cr, Se or vit. C alone or combination on chemical composition of fresh egg yolks at the end of the experimental period.

Treatments	Egg yolk extract					
	Protein, %	Total lipid, %	Cholesterol, mg/ g	H.D.L. mg/ g	L.D.L. mg/ g	Triglycerides mg/ g
Control group	19.2	27.2	23.60^{a}	10.09^{c}	12.25^{a}	125^{a}
Cr-group	19.1	27.9	21.70^{b}	10.25^{b}	10.78^{b}	119^{b}
Vit. C-group	19.3	28.1	21.10^{b}	10.33^{b}	10.80^{b}	120^{b}
Se-group	19.4	28.2	21.60^{b}	10.42^{b}	10.24^{bc}	118^{bc}
Cr+vit.C-group	18.9	28.3	21.17^{b}	10.85^{a}	10.22^{bc}	117^{bc}
Cr+Se-group	19.3	27.3	20.50^{c}	10.79^{ab}	10.35^{bc}	115^{c}
Se+vit.C-group	19.2	27.6	21.44^{b}	10.87^{a}	10.05^{bc}	115^{c}
Cr+Se+Vit.C- group	19.7	27.2	20.50^{c}	10.93^{a}	9.55^{c}	112^{d}
SEM	0.213	0.144	0.477	0.07	0.32	0.94
p-value	0.47	0.39	0.002	0.0001	0.0001	0.0001

a.b…= Means on the same column differently superscripted are significantly different (p ≤0.05).
SEM = Standard error of means.

Table (5). The effects of supplemental Cr, Se or vit. C alone or combination on egg content pH and egg yolk maloaldehyde during storage time.

Treatments	Storage time				Storage time			
	Egg content pH				Egg yolk maloaldehyde (mg/ kg yolk)			
	Zero time	5 days	10 days	15 days	Zero time	5 days	10 days	15 days
Control group	7.60a	7.83a	7.89a	7.99a	0.532a	0.568a	0.642a	0.708a
Cr-group	7.45b	7.51b	7.55b	7.64b	0.425b	0.468c	0.531bc	0.601c
Vit. C-group	7.44b	7.49b	7.53b	7.62b	0.420b	0.452bc	0.523c	0.621b
Se-group	7.44b	7.50b	7.60b	7.62b	0.412c	0.500b	0.550b	0.600c
Cr+vit.C-group	7.43b	7.52b	7.58b	7.63b	0.408bc	0.489b	0.518c	0.588d
Cr+Se-group	7.40b	7.60b	7.61b	7.65b	0.412c	0.492b	0.538b	0.589d
Se+vit.C-group	7.43b	7.55b	7.62b	7.66b	0.408bc	0.485b	0.547b	0.585d
Cr+Se+Vit.C- group	7.42b	7.50b	7.53b	7.60b	0.401bc	0.478bc	0.521c	0.580d
SEM	0.011	0.029	0.031	0.030	0.001	0.005	0.010	0.009
p-value	0.001	0.008	0.01	0.009	0.001	0.0089	0.0008	0.0001

a.b…= Means on the same column differently superscripted are significantly different (p ≤0.05).
SEM = Standard error of means.

Table (6). The effects of supplemental Cr, Se or vit. C alone or combination on semen quality of laying hens reared during summer conditions.

Treatments	Ejaculate volume (ml)	Sperm concentration ($x10^6$ ml)	Total sperm output	Sperm motility (%)	Total motile sperm ($x10^6$ ml)	Live spermatozoa (%)	Dead spermatozoa (%)	Sperm abnormalities (%)	Semen quality factor	pH	MDA (nmol/ ml)
Control group	0.22c	630d	138.6d	60.00c	83.16e	62.54d	37.45a	17.5a	86.68d	7.90	1.78a
Cr-group	0.25b	686c	171.5bc	67.5bc	115.76cd	73.20bc	30.45b	13.6b	125.53c	7.78	1.22b
Vit. C-group	0.24bc	690c	165.8c	65.8bc	109.09d	73.00bc	28.50c	14.2b	120.88c	7.75	1.32b
Se-group	0.25b	685c	171.25bc	66.8bc	114.39cd	72.98c	29.00bc	14.00b	124.97c	7.78	1.18c
Cr+vit.C-group	0.25b	702b	175.5bc	72.5b	127.23c	75.00b	28.00c	12.6c	131.62bc	7.72	1.08c
Cr+Se-group	0.26ab	725ab	188.5b	72.0b	135.72b	75.80b	26.1d	12.0c	142.88b	7.68	1.10c
Se+vit.C-group	0.26ab	730ab	189.8b	73.0b	138.55b	75.67b	26.0d	12.0c	143.62b	7.70	1.00d
Cr+Se+Vit.C- group	0.28a	780a	218.4a	80.6a	210.6a	78.00a	23.60e	10.45d	170.35a	7.67	0.95d
SEM	0.08	106.22	42.28	7.29	42.88	3.65	1.93	2.35	26.25	0.11	0.075
p-vaue	0.001	0.001	0.0001	0.002	0.0001	0.001	0.0001	0.0001	0.001	0.9	0.0001

a.b…= Means on the same column differently superscripted are significantly different (p ≤0.05).
SEM = Standard error of means.

Table (7). The effects of supplemental Cr, Se or vit. C alone or combination on fertility, hatchability percentage and hatch body weight.

Treatments	Fertility %	Hatchability %		Chick hatch weight (g)
		Total egg set	Fertile eggs	
Control group	76.5	61.11^{c}	66.5^{c}	35.00
Cr-group	80.45	72.1^{ab}	80.25^{b}	35.15
Vit. C-group	80.9	72.8^{ab}	80.05^{b}	35.25
Se-group	81.0	71.8^{b}	80.5^{b}	35.12
Cr+vit.C-group	82.5	73.2^{a}	82.05^{ab}	35.85
Cr+Se-group	81.9	73.0^{a}	82.6^{ab}	35.67
Se+vit.C-group	82.6	72.9^{a}	82.9^{a}	35.48
Cr+Se+Vit.C- group	83.2	74.2^{a}	83.5^{a}	35.90
SEM	0.62	0.95	0.63	0.07
p-value	0.08	0.02	0.002	0.9

a.b…= Means on the same column differently superscripted are significantly different (p ≤0.05).
SEM = Standard error of means.

تأثير اضافة الكروميوم والسيلينيوم و فيتامين ج للعليقة على الكفاءة التناسلية و جودة البيض لسلالة دقى 4 المحلية تحت ظروف الصيف المصرية.

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تهدف الدراسة الى تقييم تأثير الكروميوم العضوي – السيلينيوم العضوي و فيتامين ج منفردة او متحدة معا على الكفاءة التناسلية و جودة البيضة لسلالة دقي4 البياض المحلية تحت درجات الحرارة العالية في ظروف الصيف في مصر.

تم استخدام عدد 240 دجاجة بياض مع 48 ديك من سلالة دقي 4 عمر 30 اسبوع و تم اختيارها عشوائيا و توزيعها الى 8 مجاميع كل مجموعة 3 مكررات كل مكررة (10 دجاجات + ديك) .

وتم تقسيم 24 ديك الباقية الى 8 مجاميع كل مجموعة 3 ديوك و تم تسكينها فرديا لجمع السائل المنوي و تقييمه تم تغذية المعاملات على العليقة الأساسية (معاملة كنترول) أو استخدام العليقة الأساسية مضاف اليها كلا من الاضافات الآتية 400 ميكروجرام كروميوم/ كجم عليقة (مجموعة الكروميوم) – 25ملجم حمض الاسكوربيك / كجم علف (مجموعة فيتامين ج) – 0.2 ملجم سيلينيوم عضوي / كجم عليقة (مجموعة السيلينيوم) – 400 ميكروجرام كروميوم + 250 ملجم حمض اسكوربيك / كجم علف (مجموعة الكروميوم و فيتامين ج) – 400 ميكروجرام كروميوم + 0.2 ملجم سيلينيوم / كجم عليقة (مجموعة الكروميوم و السيلينيوم) – 0.2 ملجم سيلينيوم + 250 ملجم حمض الاسكوربيك / كجم عليقة (مجموعة السيلينيوم و حمض الاسكوريك) وأخيرا 400 ميكروجرام كروميوم + 0.2 ملجم سيلينيوم 25 ملجم حمض الاسكوربيك / كجم عليقة (مجموعة الكروميوم و السيلينيوم و فيتامين ج) . تم وضع كل هذه المجاميع تحت الدراسة لمدة 12 اسبوع ويمكن تلخيص اهم النتائج المتحصل عليها كلاتى:

أدت أضافة الكروميوم والسيلينيوم وحمض الاسكوربيك منفردة او متحدة معا الى زيادة معنوية فى سمك القشرة ووحدات البياض والوزن النسبى لكل من البياض والقشرة والحركة التقدمية للسائل المنوى وحجم القذفة وتركيز الحيوانات المنوية مع انخفاض معنوى فى نسبة الحيوانات المنوية الميتة ونواتج الاكسدة فى بلازما الحيوانات المنوية (المالونالدهيد) بالمقارنة بمجموعة الكنترول ومن خلال النتائج يتضح ان افضل نتيجة كانت للدجاج البياض التى تغذت على عليقة تحتوى على الكروميوم والسيلينيوم وحمض الاسكوربيك بالمقارنة بباقى المعاملات.

الخلاصة

اضافة الكروميوم والسيلينيوم وحمض الاسكوربيك أحدثت تحسن فى معظم الصفات التناسلية وجودة البيض لسلالة دقى4 تحت ظروف الصيف فى مصر.