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EFFECT OF DIETARY CHROMIUM, SELENIUM AND VITAMIN C ON PRODUCTIVE PERFORMANCE AND SOME BLOOD PARAMETERS OF LOCAL STRAIN DOKKI-4 UNDER EGYPTIAN SUMMER CONDITIONS

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ABSTRACT: The study was performed to evaluate the effect of chromium (Cr), Selenium (Se) or vitamin C (Vit. C) alone or combination on productive performance, some blood constituents, oxidative stress marker (Malondialdehyde MDA), some physiological measurements (body temperature, respiration rate and blood PH) and immune response to sheep red blood cells (SRBC' s) of Dokki-4 laying hens under hot environmental summer condition in Egypt. A total number of 240 hens plus 24 cocks from Dokki-4 of 30 weeks old were randomly selected and distributed into eight groups with 3 replicates (10 hens+ 1 cock) each. Treatments groups were fed a basal diet (control group) or the basal diet supplemented with either 400 µ Cr/kg diet (Cr group), 25 mg of L-ascorpic acid /kg diet (vit. C. group), 0.2 mg Se/kg diet (Se group), 400 µ Cr plus 250 mg of L-ascorpic acid/ kg diet (Cr + Vit. C group), 400 µ 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 kept under observation for 16 weeks. Supplementing heat-stressed laying hens with Cr, Se or Vit. C alone or combination improved productive performance compared to the control group. Digestability of dry matter, organic matter, crude protein and ether extract were highest in the treated treatments and lowest in the control group (p 0.05). Retention of Ca, P, Zn and Cu were highest in the treated groups compared to the control group. Furthermore, serum malonialdehyde (MDA) , cholesterol and glucose concentration decreased , whereas, serum total protein, albumin, globulin, Ca and P concentrations increased with dietary Cr, Se, Vit. C alone or combination compared with the control group. However, body.

Key Words: Chromium , Selenium , Vitamin C and Blood Parameters

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temperature, respiration rate and blood pH were lower in treated groups and higher in the control group. The results showed that high temperature reduced antibody titer against sheep red blood cells. However, Cr, Se or Vit.C supplementation enhanced humeral antibody response against SRBC. The results of this study indicate that, separately or in combination Cr, Se and Vit. C supplementation attenuates the decline in performance and antioxidants status caused by hot climate . Such supplementation may offer protection against hot climate-related decline in performance of local laying hens

INTRODUCTION

Stress is defined as the interaction between stress factors and protective reactions. Factors causing stress include physiological factors, such as climate, environment, nutrition and diseases and physical conditions, such as cage density and transport (Freeman, 1987). Under stress, rapid and temporary changes occur in the body initially, with continuous stress, these are followed by permanent and irreversible changes. Finally, a decline in yield and resistance to diseases may occur. Animals under stress become ill more easily and excess medicine may be necessary to maintain health. As a result of that, drug residues increase in animal products and threaten public health directly. Stock health and welfare management are key factors in animal health and food safety. For this reason, stress conditions in animal need to be examined carefully (Onbasilar and Aksoy, 2005). The suitable temperature for poultry is between 16-25ºC (Cerci et al., 2003). Hot climate begins when the ambient temperature climbs above 25ºC and is readily apparent above 30ºC. Hot climate in laying hens is prompted by combination of environmental temperature and humidity that prevent the bird's thermoregulatory process from effectively dissipating the heat produced during metabolism (Webster, 1983). High environmental temperature is the major problem faced by laying hens as well as by poultry farmers, usually in summer months.

Hot climate in laying hens reduces live weight gain, feed intake, feed efficiency, production and quality of eggs and increases mortality (Giftci et al., 2005). Researchers have tried to minimize the effect of hot climate by changing the environment and diets of laying hens. Environmental approaches include increasing the airflow over birds to increase heat loss, by increasing ventilation rates or by using evaporative cooling systems in enclosed houses and lowering stocking densities. Nutritional modifications usually made are the optimization of diets to meet the altered needs of stressed birds for protein and energy and for providing some additional nutrients. Because it is expensive to cool poultry houses methods are focused mainly on nutritional modifications. For this aim, antioxidants such as chromium, selenium and vitamin C are used in the poultry diet because of their anti-oxidant effects and also because their synthesis is reduced during heat (Tatli Seven et al., 2008).

Chromium (Cr) has been considered as an anti-stress factor to ameliorate the effects of environmental stress (Sahin et al., 2002). Chromium is not currently considered an essential trace element for poultry and an appropriate recommendation on Cr requirement of poultry has not been made (NRC, 1994). In addition, most poultry diets are basically composed of plant origin ingredients, which have low Cr content (Giri et al., 1990). Furthermore, stress condition increase Cr metabolisation from the tissues that is irreversibly excreted through the urine in humans and farm animals (Anderson, 1994). And thus may exacerbate a marginal Cr deficiency or an increased Cr requirement.

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, but very limited information is currently available compared to other organic Cr complexes.

Selenium (Se) is an essential trace element which is involved in thyroid metabolism. Thyroid function is known to be altered by many environmental factors, such as energy intake and dietary composition in addition to ambient temperature (Chang et al., 2005). Se is essential for appropriate thyroid hormone synthesis, activation and metabolism as the three isoenzymes of iodothyronine 5-deiodinase are selenoenzymes (Behne et al., 1990).

Vitamin C has been supplemented to diets of poultry reared under stress. In addition, several studies revealed a beneficial effect of vitamin C supplementation on growth rate, egg production, egg shell strength and thickness in stressed laying hens and broilers (Bains, 1996 and Tatli-Seven, 2006).

The objective of this study to determine the possible beneficial effects of dietary supplementation with chromium, selenium, vitamin C and their combinations on productive performance and some blood parameters of Dokki-4 laying hens under Egyptian summer condition.

MATERIALS AND METHODS

Experimental birds, diets and design: The present study was conducted at Sakha, Animal Production Research Station and Laboratories, APRI, ARC, Egypt during summer months (from June to August, 2014).

Total number of 264 (240 laying hens + 24 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 birds were housed in floor pens in an open house. 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.

All birds received feed and water ad libitum during the experimental period. Lighting Schedule was 17: 7 light dark cycle.

Temperatures and humidity were recorded at a particular time daily (6.00, 12.00, 18.00 and 24.00 h within the experimental house).Table (2) shows indoor climatic conditions, changes in ambient temperature (AT, ^{o}C) and relative humidity (RH, %) were recorded using electronic digital thermo-hygrometer. Temperature-humidity index (THI) was calculated according to Marai et al. (2002).

THI = db^{o}c – [(0.31-0.31x 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. The THI values were classified as absence of heat stress (< 27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9 – 29.9) and very severe heat stress (> 30.0).

Performance variables: Body weights were recorded at the beginning and at the end of the study to determine body weight changes. During the experimental period, egg number and egg weight were recorded daily per hen. The average daily egg production and the daily feed consumption per replicate were calculated for fortnight intervals. The value of feed conversion ratio (feed consumption / egg mass) were calculated.

Digestibility coefficients: At the end the feeding trail, three birds per treatment were randomly assigned to determine the retention and excretion of dietary nutrients. Nutrient was the amount of nutrient retained per hens which was calculated based on the availability of nutrient and feed intake. Excreta of layers were totally collected for three days. Diets and excreta were analyzed according to Chemical Procedures of AOAC (1995) for proximate analysis. Urinary nitrogen was determined according to Jakobson et al. (1960). Urinary organic matter was evaluated according to Abou-Raya and Galal (1997).

Mineral retention ratio was calculated using the following equation:

$$\text{Mineral retention ratio} = \frac{(WFI \times EF) - (WEV \times EE)}{(WFI \times EF)} \times 100$$

where:

WFI = weight of feed intake

EF = concentration of element in feed

WEV = weight of total excreta voided

EE = concentration of element in total excreta

Physiological measurement:The rectal temperature (RT) of three birds randomly selected out of each replicate was measured with a digital thermometer (0.1^{o}C accuracy) inserted into the rectum (colon) of the birds for one minute as previously described by Yahav and McMurtry (2001). Respiratory rate (RR) of the birds was taken as the number of breaths per minute. Data on RT and RR were collected two consecutive days in every week.

Some blood constituents:At the end of the study, blood samples were collected from nine birds (three per replicate) randomly chosen from each treatment group. Blood samples were centrifuged at 3000 rpm for 20 min and sera were collected. Serum total protein, albumin, cholesterol, glucose, alkaline phosphatase, creatinine, calcium and phosphorus were recommendations of manufactures. Lipid peroxidation was assessed as malonidialdehyde (MDA) concentration in serum by the method of Placer et al. (1966). Serum was separated to measure triodothyronine (T3) hormone level, Radioimmunoassay (RIA) kits (diagnostic products corporation, Los Angeles, USA) were used for the assays.

Immunization and titration: Sheep red blood cells (SRBC) were used as test antigens to quantitatively analyze specific antibody response as a measure of humeral immunocompetence. Three birds from each treatment groups were injected intravenously with 0.5 ml of 10% SRBC suspension prepared in 0.9% sterile saline. At 3, 6 and 9 days, post immunization, blood samples were collected to determine the primary antibody response. Antibody levels were quantitated using a micro titration hemeagglutination technique (Van der Zijpp and Leenstra, 1980).

Statistical analysis:Data were statistically analyzed using one-way ANOVA (SAS, 1996). Before analysis, all percentages were subjected to logarithmic or arcsine values transformation (log 10 x^{+1}) to approximate normal distribution. Significant differences among means were ($p \leq 0.05$) detected by Duncan's new multiple range test (Duncan, 1955).

RESULTS

Laying performance:During the experiment, laying house temperature ranged between 23 and 38ºC, while the maximum and minimum values of its relative humidity were 45% and 78%. Table (3) shows the effects of dietary supplementation with Cr, Se or Vit. C supplementation singly or in combination, on productive performance of Dokki-4 laying hens during summer. Average of initial body weight was similar between groups (p<0.05). However, Cr, Se or Vit. C alone or their combination compared with the control group resulted in higher body weight change, egg number, egg weight, egg mass, egg production, feed intake and improved feed efficiency (p<0.05). The highest values of performance were obtained when combination between Cr, Se and Vit. C.

Digestibility coefficients of nutrients: Digestibility of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE) and NFE were higher with Cr, Se and Vit. C singly or in combination (Table 3), but no effect on crude fiber was detected between groups (p<0.05). Supplementing the diet with Cr, Se or Vit. C increased the retention of minerals Ca, P, Zn and Cu which was highest in the groups fed combination of Cr, Se or Vit. C and lowest in control group (p<0.05). On the other hand, the excretion of minerals was lower in the treated groups than in the control group (p<0.05, Table 4).

Body temperature and respiration rate: Supplemental Cr, Se, Vit. C singly or in combination decreased body temperature and respiration rate (p<0.05) in heated-stressed birds compared with control group (Table 5).

Blood pH:In general, blood pH of treated groups (Cr, Se or Vit. C) was decreased as compared with control group (Table 5).

Antibody titer against SRBC's: The influence of hot climate and Cr, Se or Vit. C supplementation on antibody against SRBC's is shown in Table (5). Throughout the days post immunization control group produced the lowest antibody levels, whereas Cr, Se or Vit. C alone or their combination had higher SRBC antibody levels as compared to control group.

Serum parameters: Separately or in combination, supplemental Cr, Se or Vit. C increased serum concentrations of total protein, albumin, globulin, calcium , phosphorus and T3 (p<0.05) but decreased cholesterol, glucose and MAD concentrations (p<0.05) (Table 6) compared with the control group, while no effect on creatinene kinases were detected between groups (p<0.05).

DISCUSSION

Significant negative effects on egg weight, egg production, feed intake and feed conversion and some blood constituents as well as on nutrient digestibility and immune response to SRBC occur in local laying hens exposed to the high ambient temperature. In the present study, Cr, Se or Vit. C singly or in combination increased feed intake and improved the productive performance indicating that the three supplements alleviate the negative effects of the hot climate. Performance and feed intake decreased when ambient temperature rises above the thermoneutral zone (Siegel, 1995 and Ensminger et al., 1990). The reduced feed intake in the present study (Table 2) during summer season may be caused by a direct effect on various regions of the brain acting on feed intake control mechanism. Also, the blood flow and the motility of the intestine decreased, which may result in an increase of food passage time and delay in the thermogenic effect of food intake (Van Handel-Hruska et al., 1997). El-Tantawy et al. (1998) found that the feed consumption was lower in high environmental temperature by about 36-43%. At such temperature, corticosteroid secretion increases (Brown and Nestor, 1973). Kultu 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 (McDowell, 1989). These results were supported by Attia et al. (1997) who reported that dietary supplementation with ascorbic acid improved the productive performance. The benefit effect of vitamin C supplementation may be attributed to vitamin C in activating thyroid gland which influenced the fed intake (El-Fiky, 1998). Also, vitamin C scavenges free oxygen radicals which are important, however, to prevent stress caused by oxidation of cell membrane in the digestive system and restore efficient fed utilization (Jaffy, 1984). As well as effects of vitamin C on feed conversion may be due to that vitamin C helps to control the increase in body temperature and plasma corticosteron concentration. It also protects immune system (Roma et al., 2002). Similarly, Kim et al. (1997) and Liu et al. (1999) in laying hens and Sahin et al. (2002) and Abdel-Maged et al. (2012) in Japanese quail observed that feeding diets supplemented with Cr-picolinate enhanced egg production, egg weight and feed intake as well as improved feed conversion ratio under hot climate. Also, Sahin et al. (2002) reported that Cr supplementation caused increasing insulin concentration that increased glucose utilization, consequently feed efficiency improved.

It is known that Cr is involved in protein synthesis and there is an interaction of Cr with DNA templates that resulted in a significant stimulation of RNA synthesis. The oligopeptide low-molecular-weight Cr-binding protein (chromoduline) tightly binds four chromic ions before the oligopeptide obtains a conformation required for binding to the tyrosine kinase active site of the insulin receptor. Thus, chromodulin appears to play a role in an auto amplification mechanism in insulin signaling (Sahin et al., 2002).

Rosebrough and Steele (1981) have stated Cr as a cofactor for insulin activity and that it is necessary for normal glucose utilization and healthy animal growth. Insulin regulates metabolism of carbohydrate, fat and protein, stimulating amino acid uptake and protein synthesis as well as glucose utilization in tissues (Sahin et al., 2001a).

The main reason for the decreased egg production of the control group may be due to the decrease in feed intake. At high temperatures, birds increase their respiration rate to regulate heat loss through water evaporation from their lungs (Okela et al., 2003). This panting behavior increases CO_2 loss from lungs and partial pressure of CO_2 in blood was reduced causing a decrease in HCO_3 concentrations due to the increase in HCO_3 excretion with a reduction of H^+ excretion by the kidneys to maintain the acid-base balance in the bird. Lowered H^+ concentration raises the level of blood plasma pH, a leading to respiratory alkalosis (Borges et al., 2007). This acid-base imbalance alters Na: Cl ratio thus reduced feed consumption (Naseem et al., 2005) Environmental stress increases mineral excretion (Smith and Teeter, 1987). El-Husseiny and Creger (1981) reported significantly lower rates of retention of minerals such as Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn in broilers subjected to environmental stress. High environmental temperature significantly decreases the true digestibility of protein and amino acids in broilers (Zuprizal et al., 1993).

Sahin and Kucuk (2001) reported that utilization of dry matter, crude protein and an ether extract in broiler quails kept at high ambient temperature is significantly decreased and that such negative effects were restored by vitamin C and Cr supplementation. Stress increase Cr and Se mobilization from tissues and its excretion and also depresses ascorbic acid synthesis (McDowell, 1989), this stress may exacerbate a marginal chromium, Se and ascorbic acid deficiency or an increased Cr, Se and ascorbic acid requirment, implying that Cr, Se and ascorbic acid should be supplemented as shown in the present study.

Since, Cr, Se and Vit. C (postulated to be antioxidants) have a protective effect on pancreatic tissue against oxidative damage (McDowell, 1989 and Preuss et al., 1997), they may help pancreas to function properly including secretions of digestive enzymes, thus improving retention of nitrogen and minerals. Metwally (2004) found that increase in retention and a decrease in excretion of nitrogen ash, calcium, phosphorus and iron were proved for the birds fed vitamin C as compared to those fed the control diet under high temperature. The results of the current metabolic trial showed that retention of Ca, P, Zn and Fe is improved and excretion decreased by supplemental Cr, Se or Vit. C singly or in combination. Kornegay et al. (1997) found that the digestibility of dry matter and N-retention were increased by supplemental Cr in pigs, which was speculated to be due to increased secretion of digestive enzymes. The results of nutrient digestibility in the present study also support the beneficial effect of dietary Cr, Se and Vit. C in laying hens reared under a high temperature.

It is well known that hot climatee increases MDA (malondialdehde) concentration as a lipid peroxidation indicator (Halliwell et al., 1989 and Sahin et al., 2002). Antioxidant system (glutathione peroxidase, superoxide dismutase and vitamin E, C and A) are important in scavenging free radicals and their metabolic products, as well as in maintaining normal cellular physiology restoring depletion of various antioxidants in stressed poultry (Halliwell et al., 1989). Tatli-Seven et al. (2009) found that plasma MDA level was significantly decreased in vit. C group compared to the control group. Separately or as a combination supplemental vit. C and Cr resulted in a decrease in MDA concentration (Tawfeek et al., 2014). It has also been reported that serum glucose and cholesterol decreased, whereas total protein, albumin, globulin, calcium and phosphorus concentrations increased when dietary Cr, Se and Vit. C singly or in combination were supplemented. Similar to our results, Sahin et al. (2002) reported that serum glucose, triglycerides and cholesterol concentrations decreased, whereas total protein and albumin concentrations increased vitamin C. Similarly, Kutlu and Forbes (1993) reported that vitamin C supplementation increased plasma protein whereas blood glucose and cholesterol markedly decreased in heat-stressed (36^{o}C) broilers. A likely mechanism by which vitamin C causes a reduction in corticosterone concentration is through inhibitory effect of vitamin C on glucocorticoid synthesis, and it has been postulated that the improved performance of poultry results from a decrease in protein – derived glucogenesis (McDowell, 1989). Increase in concentrations of glucose may be attributed to increased glucocorticoid secretion, which increases glucogensis (McDowell, 1989). Dietary vitamin C may reverse these changes, presumably by reducing the secretion and/or synthesis of glcocorticoids. Sahin et al. (2001b) found that Cr supplementation markedly decreased blood glucose, corticosterone and cholesterol concentrations. Also, Shain and Onderci (2002) found that adding Cr to broiler diet increased serum Ca and P. A possible explanation for the effect of Cr supplementation on Ca metabolism may be due to that this mineral (Cr) compete for the same binding sites.

Kucuk et al. (2003) reported that Se resulted in an increased total protein and globulin but decreased glucose and cholesterol concentration. Similar results were obtained with the supplementation of Sahin et al. (2003) and Gursu et al. (2004) and with Cr by Sahin et al. (2002) in broilers and quails. Tawfeek et al. (2014) showed that supplementation of Zn, Se and Cr significantly increased total protein while serum cholesterol and glucose decreased in broiler reared under hot climate. The lower circulatory glucose concentration in the Cr supplemented birds was perhaps indicative of an increased turnover rate and utilization of glucose at the tissue level (Tawfek et al., 2014)

Thyroid gland is involved in control of growth and development and exerts primary control of metabolic rate. Any treatments like hot climate that changes metabolic rate affect thyroid activity (May and McNaughton, 1980). The present study showed that T3 level was reduced significantly during hot climate. This means that thyroid hormone is an important factor in response to hot climate. Exogenous thyroid hormone has a shorter survival time during hot climate (Fox, 1980 and Bowen et al., 1984). Also, thyroid size and thyroid activity was reduced by high temperature and increased by low temperature in chickens (Huston et al., 1982). The present study Cr, Se or Vit. C singly or in combination has been shown to attenuate these negative responses in poultry to hot climate by increasing thyroid activity.

Selenium is an essential trace element which is involved in thyroid metabolism. Thyroid function is known to be altered by many environmental factors, such as energy intake and dietary composition in addition to ambient temperature (Chang et al., 2005). Se is essential for appropriate thyroid hormone synthesis, activation and metabolism as the three isoenzymes of iodothronine 5-deiodinase are selenoenzymes (Behne et al., 1990).

Chicken like all birds, is a homothermia, it keep its body temperature at a relatively constant level by thermoregulation. The body temperature and respiration rate of chicken depending on bird size, environmental temperature, age and sex (Strackie, 1986). The rise in body temperature in response to high environmental temperature in the present study was also reported previously (El-Gendy and Washbum, 1995 and Osman, 1996). They considered that rectal temperature is a good indicator of both hot climate and acclimation. Elevation of blood pH was concomitant with increase in respiration rate in the present study was interpreted by Balnavave and Gorman (1993). The current study indicated that Cr, Se or Vit. C singly or in combination improved body temperature, respiratory and blood pH.

Previous studies showed that in vitro hot climate suppress the activity of T and B-lymphocytes and macrophages (Atta, 1996). Higher antibody titer against SRBC at 6 days post immunization in Cr, Se or Vit. C alone or in combination may explain the benefits of Cr, Se or Vit. C supplementation on humoral immune response, especially during hot climate. Dietary supplementation of vitamin C increased antibody response to SRBC that were suppressed by hot climate (Pardue et al., 1985).

Selenium has been shown to play a major role in the development and maintenance of the defense system (Bird and Boren, 1999). Se is important for building antibodies and defense mechanisms against diseases heat and other stress (Yu, 1994 and Spears, 1999).

El-Hommosany (2008) demonstrated that total antibody and IgG titers against SRBCs were significantly higher in quail chicks received Cr compared with those of control at secondary immune responses. Also, it has been reported that chromium modulates the immune response through its effect on cytokine release (Wang et al., 1996). Cytokines are small proteins or glycoproteins messenger molecules transporting information among cells. Cytokinase, together with their receptors are playing a role as control regulators of immune system by affecting the activity of other cells (Davison, 2003).

The enhancement of immune response via Cr, Se or Vit. C supplementation may be due to their antioxidant property. It reasons to protect immature lymphocytes from damage by free radical due to oxidation (Amaky-Anim et al., 2000).

These results revealed additive effects of Cr, Se and Vit. C indicating that Cr, Se and Vit. C act synergistically. In addition, antioxidant activity was reported to be more efficient when antioxidants are used in combination (Gall-Torres, 1980). Sahin et al. (2002b) reported that the combination of ascorbic acid and chromium caused more significant changes than either Vit. C or chromium alone and speculated about the synergistic action of Vit. C and chromium. Similarly, Carol et al. (1994) found an interaction between Cr and Vit. C in bone and brain Mn retention and distribution in guinea-pigs, and postulated that dietary Cr may influence ascorbic acid metabolism by protecting ascorbic from oxidative destruction. In addition, insulin is known to play a role in ascorbic acid transpiration in red blood cells, and glucose competitively inhibits ascorbic acid transport (Mann and Newten, 1995). Through increasing the effectiveness of insulin, Cr indirectly promotes the ascorbic acid transportation (Seaborn et al., 1994).

Interaction of vitamins and minerals has been described in several metabolic situations and continues to be investigated by (Vannucchi, 1991).This interaction occurs in different ways i. e. starting from the ction of vitamins on mineral metabolism, from the action of both types of nutrients in the protection of the organism and from the action of minerals on vitamin metabolism.

These results revealed additive effects of ascorbic acid and chromium work together or act synergistically. Similarly, Carol et al. (1994) found an interaction between Cr and vitamin C on bone and brin Mn retention and distribution in guinea pigs, and stated that dietary Cr may influenced ascorbic acid metabolism via protecting ascorbae from oxidative destruction. In addition, insulin is known to play a role in ascorbic acid transportation in red blood cells, and glucose competitively inhibits ascorbic acid transport (Mann and Newton, 1995). Through increasing the effectiveness of insulin, chromium indirectly promotes the ascorbic acid transportation (Seaborn et al., 1994). In addition antioxidant activity was reported to be more efficient when antioxidants are used in combination (Gallo-Torres, 1980).

In conclusion, the present results suggested that dietary supplementation of either chromium, selenium, vitamin C or their blends have the potential to alleviate the detrimental effects of hot summer season on laying performance and some blood parameters . Therefore, the magnitude of effects was greater with their combinations .

Table (1): Composition and calculated chemical analysis 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)

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

Items	AT (ºC)		RH (%)		THI	
	Maximum	**Minimum**	**Maximum**	**Minimum**	**Maximum**	**Minimum**
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.

Table (3): The effects of supplemental Cr, Se or vit. C alone or combination on productive performance of laying hens reared during summer season**.**

Treatments	Initial body weight (g)	Final body weight (g)	Body weight changes (g)	Egg number (egg/h/16 wks)	Average egg weight (g)	Egg mass (g/h/d)	Egg production (%)	Feed intake (g/h/d)	Feed conversion (g feed : g egg)
Control group	1363	1480	117^{d}	62.5^{d}	43.5^{b}	24.24^{c}	55.80^{d}	85.5^{c}	3.52^{a}
Cr-group	1367	1490	123^{c}	65.6^{c}	44.6^{ab}	26.12^{b}	58.57^{c}	87.6^{b}	3.35^{b}
Vit. C group	1354	1486	132^{b}	65.0^{c}	43.8^{ab}	28.47^{b}	58.03^{c}	86.5^{bc}	3.04^{d}
Se group	1362	1503	141^{a}	64.5^{c}	43.5^{b}	25.05^{bc}	57.58^{c}	87.8^{b}	3.50^{a}
Cr + Vit. C group	1363	1492	129^{c}	67.0^{b}	44.3^{b}	26.50^{b}	59.82^{b}	90.2^{a}	3.40^{ab}
Cr + Se group	1358	1500	142^{a}	66.5^{b}	44.9^{a}	26.66^{b}	59.37^{b}	88.5^{b}	3.32^{c}
Se + Vit C group	1360	1505	145^{a}	67.5^{b}	45.2^{a}	27.24^{a}	59.37^{b}	89.6^{a}	3.29^{c}
Cr + Se + Vit. C group	1362	1510	148^{a}	69.6^{a}	44.9^{a}	27.90^{a}	62.14^{a}	90.0^{a}	3.23^{c}
SEM	15.67	77.52	24.33	0.64	0.84	2.65	6.36	0.61	0.25
Significant	NS	NS	*	*	*	*	*	*	*

a-d Means within each column with different superscript are significantly different ($p \leq 0.05$)

Table (4): The effects of supplemental Cr, Se or vit. C alone or combination on nutrient digestibility, mineral retention and excretion of laying hens reared during summer season.

Treatments	Nutrient digestibility %				Retention				Excretion			
	CP	CF	EE	NFE	Ca g/h/d, M	P g/h/d,DM	Zn g/h/d,DM	Cu g/h/d,DM	Ca g/h/d,DM	P g/h/d,DM	Zn g/h/d,M	Cu g/h/d,DM
Control group	74.33^{b}	21.17	73.33^{c}	73.03^{b}	2.1^{b}	0.20^{b}	1.00^{b}	0.23^{b}	3.17^{a}	0.69^{a}	10.67^{a}	2.87^{a}
Cr-group	76.57^{a}	21.95	74.60^{b}	73.25^{ab}	2.5^{ab}	0.26^{ab}	1.22^{ab}	0.30^{a}	2.85^{b}	0.56^{b}	9.55^{b}	2.56^{b}
Vit. C group	76.00^{a}	22.00	74.63^{b}	73.75^{ab}	2.8^{a}	0.25^{ab}	1.36^{a}	0.28^{a}	2.90^{b}	0.52^{b}	9.60^{b}	2.60^{b}
Se group	75.86^{ab}	21.80	73.85^{bc}	74.00^{a}	2.7^{a}	0.27^{a}	1.30^{ab}	0.25^{ab}	2.80^{b}	0.50^{b}	9.45^{b}	2.50^{b}
Cr + Vit. C group	76.12^{a}	22.15	74.05^{b}	73.90^{a}	2.8^{a}	0.28^{a}	1.38^{a}	0.30^{a}	2.65^{bc}	0.46^{c}	9.36^{b}	2.36^{c}
Cr + Se group	76.00^{a}	21.96	73.96^{b}	74.08^{a}	2.6^{a}	0.30^{a}	1.40^{a}	0.28^{a}	2.50^{bc}	0.40^{c}	9.25^{b}	2.20^{c}
Se + Vit C group	76.25^{a}	22.00	74.00^{b}	75.00^{a}	3.0^{a}	0.27^{a}	1.38^{a}	0.30^{a}	2.55^{c}	0.40^{c}	9.30^{b}	2.30^{c}
Cr + Se + Vit. C group	76.30^{a}	21.90	75.95^{a}	74.88^{a}	2.8^{a}	0.30^{a}	1.42^{a}	0.35^{a}	2.16^{d}	0.45^{c}	9.25^{b}	2.08^{d}
SEM	2.25	5.25	0.58	1.25	0.46	0.32	0.46	0.25	0.36	0.26	0.38	0.42
Significant	*	NS	*	*	*	*	*	*	*	*	*	*

a-d Means within each column with different superscript are significantly different ($p \leq 0.05$)

Table (5): The effects of supplemental Cr, Se or vit. C alone or combination on body temperature, respiration rate, blood pH and antibody titer against SRBC of laying hens reared during summer season.

Treatments	Body temperature (°C)	Respiration rate	Blood pH	Days post immunization		
				3 days	6 days	9 days
Control group	42.0^{a}	82.00^{a}	7.77^{a}	3.65^{c}	4.33^{c}	3.35^{c}
Cr-group	41.30^{b}	87.50^{b}	7.70^{a}	4.25^{b}	4.60^{b}	4.56^{a}
Vit. C group	41.16^{b}	76.10^{c}	7.67^{a}	4.15^{ab}	4.55^{b}	4.16^{b}
Se group	41.26^{b}	75.10^{c}	7.60^{ab}	4.08^{ab}	4.62^{b}	4.06^{b}
Cr + Vit. C group	41.06^{b}	77.18^{bc}	7.63^{ab}	4.50^{a}	5.05^{a}	4.37^{a}
Cr + Se group	41.08^{b}	76.50^{c}	7.50^{b}	4.36^{a}	5.00^{a}	4.42^{a}
Se + Vit C group	41.10^{b}	78.00^{b}	7.60^{ab}	4.63^{a}	5.15^{a}	4.52^{a}
Cr + Se + Vit. C group	41.07^{b}	76.10^{c}	7.55^{b}	4.56^{a}	5.20^{a}	4.58^{a}
SEM	0.175	0.890	0.036	0.336	0.405	0.261
Significant	*	*	*	*	*	*

a-d Means within each column with different superscript are significantly different ($p \leq 0.05$)

Table (6): The effects of supplemental Cr, Se or vit. C alone or combination on some blood constituents of laying hens reared during summer season.

Treatments	Total protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	Glucose (gm/dl)	Cholesterol (mg/dl)	Ca (mg/dl)	P (mg/dl)	Creatinine (mg/dl)	T3 (ng/dl)	MDA (µmol/ml)
Control group	3.55^{c}	1.57^{c}	1.98^{c}	152.29^{a}	169.53^{a}	12.70^{c}	6.30^{c}	1.14	159.14d	3.10^{a}
Cr-group	4.18^{abc}	1.71^{b}	2.47^{abc}	120.63^{b}	160.55^{ab}	13.76^{bc}	6.60^{ab}	0.09	162.53^{c}	2.50^{b}
Vit. C group	4.15^{bc}	1.76^{ab}	2.39^{abc}	122.46^{b}	166.02^{a}	13.85^{bc}	6.52^{ab}	1.05	162.37^{c}	2.60^{b}
Se group	4.13^{bc}	1.79^{ab}	2.34^{bc}	120.66^{b}	162.89^{ab}	13.80^{bc}	6.65^{b}	1.02	164.51^{b}	2.46^{b}
Cr + Vit. C group	4.38^{ab}	1.74^{ab}	2.64^{ab}	101.66^{c}	137.89^{bc}	14.00^{b}	6.86^{a}	1.10	165.11^{b}	2.30^{bc}
Cr + Se group	4.66^{ab}	1.84^{a}	2.82^{ab}	100.49^{c}	138.28^{bc}	15.25^{a}	6.78^{ab}	1.02	166.10^{a}	2.36^{bc}
Se + Vit C group	4.37^{ab}	1.71^{b}	2.66^{ab}	100.09^{c}	137.11^{bc}	15.10^{a}	6.80^{a}	1.05	167.00^{a}	2.20^{bc}
Cr + Se + Vit. C group	4.89^{a}	1.91^{a}	2.98^{a}	100.91^{c}	132.03^{c}	15.16^{a}	6.85^{a}	1.11	166.90^{a}	2.12^{c}
SEM	0.28	0.21	0.18	5.25	7.62	0.58	1.10	0.28	8.09	0.07
Significant	*	*	*	*	*	*	*	NS	*	*

a-d Means within each column with different superscript are significantly different ($p \leq 0.05$)

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الملخص العربى

تأثير أضافة الكروميوم و السيلنيوم وفيتامين ج على الكفاءة الأنتاجية وبعض قياسات الدم فى لسلالة دجاج دقى٤ تحت ظروف الصيف الحار فى مصر

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اجريت هذه الدراسة لمعرفة تاثير الكروميوم و السيلينيوم و فيتامين ج منفردة او معا على الاداء الانتاجي و بعض مكونات الدم و مضادات الأكسدة (المالونالدهيد) و بعض القياسات الفسيولوجية (درجة حرارة جسم الطائر –معدل التنفس – حموضة الدم) و الاستجابة المناعية وكرات الدم الحمراء للاغنام لسلالة دقي٤ تحت ظروف المناخ الحار خلال شهور الصيف في مصر.
وقد استخدم ٢٤٠ دجاجة بياضة + ٢٤ ديك من سلالة دقى ٤ عمر ٣٠ اسبوع تم توزيعها عوائيا الى ثمنية مجموعات متساوية وكل مجموعة ٣ مكررات وكل مكررة ١٠ دجاجات + ديك وتم ترتيبها تحت ظروف متماثلة وكانت المجموعة تغذت على عليقة اساسية (مجموعة الكنترول) او تغذت على على العليقة الأساسية مضاف اليها كلا من ٤٠٠ ميكروجرام كروميوم/كجم علف (مجموعة كروميوم) و ٢٥٠ ملليجرام حمض الاسكوربيك /كجم علف (عليقة فيتامين ج) و ٠,٢ ملليجرام سيلينيوم / كجم علف (مجموعة السيلينيوم) و ٤٠٠ ميكروجرام كروميوم مع ٢٥٠ ملليجرام حمض الاسكوربيك/ كجم علف (عليقة كروميوم وفيتامين ج) و ٤٠٠ ميكروجرام كروميوم مع ٠,٢ ملليجرام سيلينيوم / كجم علف (مجموعة كروميوم و سيلينيوم) و ٠,٢ ملليجرام سيلينيوم مع ٢٥٠ ملليجرام حمض الاسكوربيك / كجم علف (مجموعة السيلينيوم و فيتامين ج) وتغذت على ٤٠٠ ميكروجرام كروميوم مع ٠,٢ ملليجرام سيلينيوم مع ٢٥٠ ملليجرام حمض الاسكوربيك / كجم علف (مجموعة كروميوم و سيلينيوم و فيتامين ج). وذلك لمدة ١٦ اسبوع من يوليو الى اغسطس اثناء فصل الصيف الحار وكانت النتائج كالآتي :
اضافة الكروميوم و السيلينيوم و فيتامين ج منفردة او مجتمعة الى الدجاج البياض المجهد حراريا ادى الى تحسين الاداء الانتاجي بالمقارنة بمجموعة المقارنة.
معاملات هضم للمادة الجافة – المادة العضوية – البروتين الخام و المستخلص الايثيري اعلى من المجاميع التي تغذت على الاضافات الغذائية.وكات أقل في مجموعة المقارنة في حين ان الاملاح المحتجزة (كالسيوم – فوسفور – زنك – نحاس) كانت اعلى في المجاميع المعاملة بالاضافات الغذائية وكانت اقل في مجموعة المقارنة .
انخفضت تركيزات السيرم من المالونالدهيد - الكوليسترول – الجلوكوز في حين ان البروتين الكلي – الالبيومين-الجلوبيولين – الكالسيوم و الفوسفور زادت مع اضافة الاضافات الغذائية بالمقارنة مع مجموعة المقارنة .
انخفضت درجة حرارة جسم الظائر و معدل التنفس وحموضة الدم في المجاميع الجزيئية وكانت أعلى في مجموعة المقارنة .أظهرت النتائج ان التعرض للاجهاد يقلل الاجسام المضادة و كرات الدم الحمراء للاغنام بينما اضافة الكروميوم و السيلينيوم و فيتامين ج منفردا او متحدة معا زادت الاستجابة المناعية لكرات الدم الحمراء للاغنام .
دلت النتائج في هذا البحث ان اضافة الكروميوم و السيلينيوم و فيتامين ج منفردا او متحدة معا تعدل من الانخفاض في الاداء الانتاجي ومضات الاكسدة الناتج من الاجهاد الحراري و مثل هذه الاضافات يمكن ان تعطي حماية ضد الاجهاد الحراري المرتبط بالانخفاض في الاداء الانتاجي للدجاج البياض المحلي.