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EFFECT OF VITAMIN E, THYROXINE HORMONE AND THEIR COMBINATION ON HUMORAL IMMUNITY, PERFORMANCE AND SOME SERUM METABOLITES OF LAYING HENS DURING SUMMER SEASON.

By

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Abstract: *An experiment was conducted to investigate the effect of supplemental vitamin E, thyroxine hormone or their combination on performance, immunity and some serum constituents of laying hens during summer months. One hundred twenty, ISA Brown layers in their 28th weeks of age were randomly assigned to six dietary groups. The first group was served as control group. Whereas, the other experimental five groups were given the basal diet with additional provision of either vitamin E (VE), thyroxine or their combination as follows; the 2nd and 3rd groups were supplemented with 250 and 500ppm VE respectively; the 4th group was supplemented with 0.25ppm thyroxine; whereas the 5th and 6th groups were supplemented with 250ppm VE plus 0.25ppm thyroxine and 500ppm VE plus 0.25ppm thyroxine, respectively. Treatments were lasted for eight weeks.*

Results showed that all supplemented groups had better (P<0.001) total secondary and IgG anti-SRBC's as compared to the control one. Supplemental VE either singly or in combination with thyroxine had insignificant effect on alanine aminotransferase (ALT), and phosphorus (P) levels. On other hand, a slight reduction was obtained for serum concentration of aspartate aminotransferase (AST) in particular, hens given either 500ppm VE alone or in combination with thyroxine. Comparable to all supplemented groups, the control group recorded significantly the lowest serum concentration of both alkaline phosphatase (AP) and calcium (Ca). Thyroxine (T_4) concentration was significantly (P≤0.001) increased in both individual VE 250ppm group and its combination with thyroxine. But, T_3 level did not statistically differ among all groups. Serum concentration of uric acid was reduced in VE and/or thyroxine; moreover the reduction was dose dependent. Average values of egg mass and egg production percentage were significantly (P ≤0.01) increased in whole supplemented groups. The improvements were more pronounced for layers fed 250 or 500ppm VE plus

0.25ppm thyroxine. Haugh units, yolk height, shell thickness and shell surface area (ESA, cm^2) were significantly ($P \leq 0.01$) improved with adding VE, thyroxine hormone or their combinations. Higher egg yolk percentages were achieved using 0.25ppm thyroxine followed by 250ppm VE and 500ppm VE plus thyroxine. These results suggested that better dealing to heat stress during summer in laying hens could be evoked using these supplements in particular, with the blend of 0.25ppm thyroxine plus 250ppm VE.

INTRODUCTION

Stress is a general subjective term used to describe the sum of nonspecific responses or defense mechanisms of the body when faced with abnormal or extreme demands. Many factors, including environmental, nutritional and pathological disturbances, can generate a state of stress and evoke a combination of behavioural, biochemical and physiological adaptations which generally result in a reduction in the performance of poultry (Bollengier-Lee *et al.*, 1998). In summer time, high environmental temperature is the major problem faced by laying hens as well as poultry farmers not only because of mortality, but also because of the reduction in the number and quality of the eggs produced during heat stress (Franco-Jimenez and Beck, 2007). Heat stress begins when the ambient temperature climbs above 25°C and is readily apparent above 30°C. Mashaly *et al*. (2004) reported that heat stress impaired immunocompetence and reduce egg laying performance of hens. Thermal stress can lead to over production of oxygen free radicals OH· and O2 (Slater, 1984). Free radicals can cause metabolic disturbances, cell injury and changes in enzyme activity (Sahin *et al.,* 2002a, b). Also, heat stress has been shown to depress blood total and ionized calcium, reduces thyroid activity and the egg yolk precursor (vitellogenin) (Odom *et al.*, 1985 and Sahin, 2002c). Blood T_3 and T_4, important metabolic regulators in animals, are associated with ambient temperature (McNabb and King, 1993). Because it is expensive to cool poultry houses, Yang *et al*. (2000) reported that, enhancement of immune competence of poultry could be achieved through development of vaccination, breeding, nutrition, and management programs.

Vitamin E (VE) is a metabolic and immunomodulator nutrient that has received a lot of attention with respect to its importance to the immune response in poultry. This vitamin appears to be an immune system "booster." It seems to exert a complementary effect on the immune system by inhibiting the synthesis of prostaglandins as a response to inactivation of free radicals (Sahin *et al*., 2002a). Several studies showed positive effects of VE on immunity of poultry (Haq *et al*., 1996; Gore and Qureshi, 1997; and Erf *et al*., 1998). Leshchinsky and Klasing (2001) reported that

supplemental VE (25, 50 IU/kg diet) showed better humoral response against red blood sheep cells, infectious bronchitis virus and Brucella abortus. In addition, dietary VE could elevate T_3 and T_4 concentrations and reduce serum level of corticosterone (Sahin *et al.,* 2002a).

Another one is the thyroxine hormone that has been well known as an important metabolic factor. Thyroid hormones are necessary for reproductive system development and reproductive function, but high concentrations of thyroid hormones have antigonadal effects (Decuyere and Verheyen, 1986 and Elnagar *et al*., 2005). El-Husseiny *et al*. (2000) cited that, oxygen consumption was significantly increased by T_3 or T_4 thereby, increased growth and egg production and improved egg shell quality. The neuroendocrine-immune interactions may alter the immune response during thermal stress since, heat stress has been found to be linked with the increased plasma corticosterone level resulting in excess catabolism and decreased T_3 concentration (Durgun and Keskin, 1998 and Abdel-Fattah, 2006). Marsh and Scanes, (1994) revealed that numerous studies confirmed the enhancement role of thyroid hormones on cell mediated immune response. However their effects on humoral immunity showed contradict results.

The present study was conducted to asses the effect of dietary VE, thyroxine or their combination on humoral immune response, laying performance and some serum metabolites of laying hens during summer season.

MATERIALS AND METHODS

The present study was carried out at the Poultry Nutrition Research Section, Department of Poultry Production, Faculty of Agriculture, Ain Shams University, during the summer season.

One hundred twenty, ISA Brown layers 28 wks of age, were randomly assigned to six dietary groups. There were two replicates of ten individually caged hens for each group. The first group was served as control and received a basal corn-soybean diet (Table 1), balanced to meet NRC requirements of laying hens (NRC, 1994). The other experimental five groups were given the basal diet with additional provision of either VE (VE), thyroxine or their combination as follows; the 2^{nd} and 3^{rd} groups were supplemented with 250 and 500ppm VE respectively; the 4^{th} group was supplemented with 0.25ppm thyroxine; whereas the 5^{th} and 6^{th} groups were supplemented with 250ppm VE plus 0.25ppm thyroxine and 500ppm VE plus 0.25ppm thyroxine, respectively. Treatments were lasted for eight wks, till the age of 36 wks. Hens were kept in a windowed house with a light

cycle regimen of 16h light: 8h darkness. Throughout the experimental period, feed (mash form) and water were provided for *ad libitum* consumption.

Measurements and Observations

Productive Traits:-

Eggs were collected daily throughout the experimental period. Average egg number, egg weight and egg mass were calculated and summarized at the base of four wk interval. Weekly individual live body weight of hens, feed consumption and feed conversion ratio (g feed /g egg mass) per hen were recorded and also summarized as previously mentioned.

Egg Quality:-

Egg quality was evaluated twice through the experimental period, at the end of fourth (32 wk old) and again at eighth (36 wk old) wk of treatments. Five eggs per replicate were colleted at random for the evaluation of egg traits, giving a total of 120 eggs (6 treatments x 2 replicates/treatment x 5 eggs/replicate x 2 times). Egg width and length were measured using a digital caliper to calculate egg shape index. The thickness (mm) was measured using a dial gauge micrometer. Shell surface area (SA) and shell weight per unit surface area (SWUSA) were evaluated as suggested by Peebles *et al*. (1994). Shell density (SD) and Haugh units were determined according to Curtis *et al*. (1985).

Immunization and Titration against Sheep Red Blood Cells (SRBC's):-

At 31 wk of age, hens of all groups were injected intramuscularly (im) with 0.5 ml of 10% saline suspension of Sheep Red Blood Cells (SRBC's). Blood sample of individual hens were collected from brachial vein seven d after SRBC's challenge. Four wk post the first challenge, hens were given a second challenge of the same antigen and blood samples were collected seven d later to quantify anti-SRBC antibody titers. The total mercaptoethanol-sensitive (MES, presumably IgM) and mercaptoethanol-resistant (MER, presumably IgG) anti-SRBC's antibody titers were determined using a micro-heamagglutination technique as described by Yamamoto and Glick (1982) and Dix and Taylor (1996). The antibody data were expressed as the log2 of the reciprocal of the highest dilution giving visible agglutination.

Serum Metabolites:-

At the end of the experimental period (36 wk), six hens were randomly taken from each treatment and slaughtered. Blood samples were

collected, during their exsanguination, and centrifuged at 4000 rpm for 15 min. Serum samples were obtained and stored at -20°C until biochemical analyses were done. Commercial kits were used for colorimetrically determination of the following blood serum constituents according to the procedure outlined by the manufacturer. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Diamond Diagnostic, Cairo, Egypt), alkaline phosphatase (AP) (Bio-Merieux , Morcyl Etiols, France), calcium (Ca), inorganic phosphorus (P) and uric acid (Spinreact, S.A. Spain). Serum concentration of triiodothyronine (T_3) and thyroxine (T_4) were determined using commercial enzyme immunoassay test kit produced by Cal-Tech Diagnostics, Inc. Chino, California, USA.

Statistical Analysis:-

Data were subjected to one way analysis of variance with treatments effect using general linear model (GLM) procedure of SAS User's Guide, (SAS, 1998). Differences among means were tested using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSIONS

1- Humoral Responses

The SRBC is a T cell-dependent antigen which helps B cells to produce antibodies (Nelson *et al.,* 1995). It is known that helper T cells (Th2) help B cells in producing specific antibody through the production of cytokines which promote activation and growth of B-cells, and, consequently, enhance humoral immunity. The assessment of humoral immune response through the production of hemagglutinin antibodies to SRBC is commonly used because it is rapid and does not involve the use of pathogenic agents.

Results in Table (2) showed the effect of dietary supplementation of VE, thyroxine and their combination on total primary and secondary antibodies production. Although, the control group had the lowest total antibody titers estimated seven d post the first challenge with SRBC's, insignificant differences were found among whole experimental groups. Seven d post second challenge with SRBC's, data revealed that single provision of VE, at both levels, thyroxine and their combinations improved significantly the humoral immune responses. Hence, the control group was significantly ($p<0.005$) lower than the other experimental groups, except those on the blend of thyroxine plus 500ppm VE. With respect to MER and MES antibodies, dietary supplementation of VE, thyroxine or their combinations did not significantly affect on both MER and MES anti-

SRBC's seven d post primary challenge. Similar trend was obtained for MES post secondary challenge. Secondary MER antibodies, showed nearly similar aforementioned trend for total secondary anti-SRBC'.

Our findings are in agreement with (Gore and Qureshi, 1997 and Singh *et al*., 2006) they reported that VE supplementation in poultry increased antibody titers. On the other hand, some other studies (Boa-Amponsem *et al*., 2000; Sijben *et al*., 2002 and Amiri Andi *et al*., 2006) postulated that VE supplementation did not affect antibody titer after antigen challenge.

With regard to the effect of thyroxine on antibody production, the existed results are in accordance with those of Abaza *et al*. (2003) who found better total antibody, IgM and IgG titers against SRBC's in mature Alexandria cockerels fed diet supplemented with 0.5ppm thyroxine. Conversely, Marsh *et al*. (1984) reported that supplementing euthyroid chickens with additional thyroid hormone has been found to be generally ineffective in enhancing the antibody response.

The present results pointed out that, individual supplementation of VE at either high (500ppm) or low (250ppm) level was more potent in enhancement of humoral immune response (Table. 2) as compared to its combination with thyroxine. Moreover, the observed downshift in the secondary immune responses might imply that there was a predictable reduction in the humoral responses that could be accomplished by using higher levels of VE (over 500ppm) either alone or in combination with thyroxine.

These results are in full agreement with those of Leshchinsky and Klasing (2001) who showed that supplementing broiler diets with VE at 25 to 50 IU/Kg of diet was not immunomodulatory and higher levels of VE supplementation to the diet were less effective. Recently, Abdukalykova and Ruiz-Feria1 (2006) found that VE supplementation levels, eight times higher than those recommended by the NRC (1994) greatly improved the humoral immune response of birds challenged with SRBC. However, very high levels of VE had a tendency to reduce the antibody response as compared with normal levels of VE.

Several interpretations were suggested to explain the stimulus effect of dietary VE on immune responses in birds. First, as previously discussed by Gershwin *et al*., (1985) that VE like other nutritional factors, affects the development and maintenance of immunocompetence through multiple factors, either by acting directly on the immune cells or by indirectly altering metabolic and endocrine parameters, which in turn influence

immune function. Second, it seems to exert a complementary effect on the immune system by inhibiting the synthesis of prostaglandins, through modulating of arachidonic metabolism via cyclooxygenase and lipoxygenase pathways (Blumberg, 1994). These prostaglandins are produced in the cells following the oxidation of cellular membranes and are responsible for inhibiting the inflammation and immune response. VE prevents oxidation and thus, the production of prostaglandins (Williams, 2005). Third, the main mechanism of the bioactivity of VE could refer to its antioxidant potential in reducing free radical-induced pathology during normal metabolic stress and immune challenge. VE affects free radical-mediated signal transduction events and ultimately modulates gene expression caused by free radical signaling (Packer and Suzuki, 1993).

In this connection, Marsh and Scanes (1994) cited that a primary indication of the mechanism (s) through which the thyroid hormones may affect immune development and function is provided by the observation that thyroid hormone may control the production of or responsiveness to a variety of immune mediators. Therefore, thyroid hormones have stimulating effects on interleukin-2 (IL-2) production. IL-2 is known to be produced by all helper T cells and some cytotoxic T cells to stimulate proliferation and differentiation of all activated T cells and B cells (Chandratilleke and Marsh, 1993).

2-Serum Metabolites:-

2. a. Enzyme activities. Aspartate aminotransferase (AST) is not specific for hepatocellular damage but is highly sensitive in detecting liver damage. Alanine aminotransferase (ALT) is found in hepatocyte cytosol as well as in muscle and other tissues of birds. ALT has poor specificity for liver disease, and the clinical relevance of an increased ALT value is decreased (Harr, 2002).

From Table (3) it is clearly observed that, insignificant differences were found among all experimental groups including the control one for ALT activity. Although AST activity showed nearly similar trend observed for ALT, the control group attained numerically the highest level as well, supplemental 500ppm VE either singly or in combination with thyroxine slightly lowered ($P \leq 0.08$) AST level as compared to the other experimental groups. Thereby, the obtained liver enzyme activities may put forward trend toward improving liver functions. On the other hand, the obtained results of alkaline phosphatase (AP) activity indicated that control group was significantly the lowest comparable to the remaining groups. Thus, dietary provision of VE and/or thyroxine increased ($P \leq 0.0001$) AP, moreover the

effect was more pronounced in birds given thyroxine alone or in combination with 250ppm VE (Table. 3).

The present results are in partial agreement with those found in heat stressed broilers (Sahin *et al*., 2002b) and in Japanese quail (Gursu *et al*., 2003), they found that serum activities of both ALT and AST were not influenced by dietary VE supplementation but, serum activity of alkaline phosphatase increased (P = 0.001). Therefore, the present results confirmed those of Elnagar *et al* (2001) who demonstrated that clinical induction of hyperthyroidism in broilers as a result of dietary DL-Thyroxine (0.5ppm) was accompanied by a significant reduction in serum concentration of AST. On the contrary, Abdel-Maksoud (2006) postulated that supplemental VE (375-500ppm) increased plasma activities of AST and ALT of hens kept under heat stress of desert conditions. El-Kaiaty and Hassan (2004) cited that increasing activity of AP enzyme is associated with high egg production for some laying hen's strains. This may interpret the higher egg production in groups with higher AP level.

Through its known properties as an intra-membrane antioxidant, vitamin E may protect tissue membranes from lipid peroxidation caused by free radical attack. It could, therefore, reduce the associated loss of integrity of function of cell membranes and associated increased cellular permeability and play a role in alleviating the effect of heat stress in laying hens. It is also accepted that stress can lead to over production of oxygen free radicals H_2O_2, $OH\cdot$ and O_2 (Slater, 1984). Free radicals can cause metabolic disturbances and cell damage. This damage can be particularly serious in organs such as muscle and liver because of their high metabolic activity (Fowler, 1990). A reactive free radical formed close to DNA may produce a change in the molecular structure resulting in a mutation or cytotoxicity (Collins *et al.*, 1994) and cause profound changes in enzyme activity. Vitamin E prevented the associated rise in specific antioxidant enzymes and markers of cellular damage (McIntosh *et al.*, 1993).

As well, established thyroid hormones are major regulators of development, metabolism and homeostasis in birds, hence they may help reducing the effect of high ambient temperature (Sturkie, 1986). So that the interaction between VE and thyroxine could be more efficient in avoiding muscle and liver injury.

2. b.Thyroid Activity

Dietary supplementation of VE, thyroxine or their combination had no significant effect on serum concentrations of triiodothyronine (T_3) (Table. 3). But, serum thyroxine (T_4) concentration was significantly (P

≤0.001) elevated in those fed diet with 250ppm VE either alone or in blend with thyroxine. Additionally, control group had numerically the lowest T_4 concentration. These results are in partial agreement with Sahin *et al*. (2002a) who found that serum concentrations of T_3 and T_4 increased whereas, adrenocorticotropic hormone (ACTH) concentration decreased as dietary VE increased up to 250ppm, but further increases in dietary vitamin E supplementation up to 500ppm, did not change the concentrations of T_3, T_4, and ACTH.

These results may be due to the positive effects of vitamin E in alleviating the harmful effects of heat stress. Several researchers reported reduced concentrations of T_3 and T_4 in heat-stressed chickens (McNabb and King, 1993; Yahav *et al*., 1997, and Sahin *et al*., 2002a). In accordance with the results of the performance data of the present study, greater T_4 concentrations with dietary vitamin E supplement supported a greater performance. Jonier and Huston (1957), and Huston and Carmon (1962) reported at high temperatures that, thyroid size and thyroid secretion rate decreased, and subsequently metabolic rate might be reduced.

In addition, the significant elevation in serum T_4 concentration noted herein could be attributed to lower conversion of T_4 into T_3 to regulate the metabolic rate and then heat production under heat stress conditions (Abou El-Soud and Younis, 1999). Lien and Siopes (1989) suggested that T_4 may play a role in synchronizing the biological clocks necessary to initiate physiological functions required to begin an egg laying cycle.

2. c. Mineral Concentrations

Regarding serum concentration of both calcium (Ca) and phosphorus (P), data illustrated in Table (3) showed that, birds whose diets were supplemented with 250ppm VE achieved significantly ($P<0.01$) higher serum Ca concentration compared with the other groups except those on 500ppm VE. Therefore, neither single administration of 0.25ppm thyroxine nor its combination with either levels of VE (250 or 500ppm) had significant effects on Ca level when compared to control group. Despite that, insignificant differences were noted among various experimental groups in P level. The lowest serum P concentration was recorded for the control one. In agreement with our results, Bollengier-Lee *et al.* (1998) reported that plasma calcium concentration was raised in hens subjected to heat stress and given 500ppm VE. Also, in heat-stressed broilers and Japanese quails, Sahin *et al*. (2002a and 2002c) attained similar trend. Elnagar *et al.* (2001) failed to achieve significant effects of dietary 0.5ppm thyroxine on serum calcium concentration of broiler chicks; however P was

elevated by about 9% of euthyroid concentration. The present results may indicate that the fluctuation in blood phosphorus level was less pronounced at thermal stress of summer season.

Vitamin E supplementation was claimed 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 of synthesis of 1, 25 dihydroxy cholecalciferol (Taylor and Drake, 1984), and the active cholecalciferol metabolite that regulates calcium absorption. Circulating calcium and estrogen concentration are highly correlated in laying hens (Tojo and Huston, 1980) and oestradiol concentration had been shown to be depressed in hens subjected to heat stress (Tojo and Huston 1980; and Mahmoud *et al.*, 1995).

Data of uric acid, which is the major end product of protein metabolism in poultry (Sturkie, 1986), indicated that dietary addition of 500pp VE, 0.25ppm thyroxine and their combination resulted in a significant ($P \leq 0.05$) reduction in serum level of uric acid. It was noteworthy that, the reduction of uric acid concentration was dose dependent (Table. 3). These results are in harmony with those obtained in broiler chicks by Sahin *et al*. (2001 and 2002a), they found that dietary VE reduced ($P \leq 0.001$) serum concentration of uric acid.

In this respect, Daghir (1995) reported a marked elevation in circulating level of corticosterone following heat stress exposure as a result of increasing secretion of adrenocorticotropic hormone (ACTH). Consequently, through its greater catabolic effect, corticosterone induced gluconeogenic effects, yielding higher serum uric acid (Sahin *et al*., 2002a). Furthermore, elevated temperatures stimulate the endogenous synthesis of heat shock proteins (Hsp) or stress proteins (Franco-Jimenez and Beck, 2007). These proteins exert a cytoprotective effect, protecting the cells against harmful insults, thus making the cells resistant to apoptosis (Coronato *et al*., 1999).

Sahin *et al*. (2002a, b) stated that Serum concentration of ACTH and subsequently corticosterone concentration were lower with dietary VE, probably indicating lowered effects of heat stress with supplemental VE. The beneficiary effects of dietary thyroxine might be mostly accounted for by the actions of thyroid hormone on muscle protein synthesis and body fat synthesis (El-Husseiny *et al.*, 2000). These facts might point out the presence of a synergistic interaction between both VE and thyroxine.

3- Performance Data

Data of performance of laying hens are listed in Table (4). Supplementation of VE, thyroxine or combination of 0.25ppm thyroxine plus 250ppm VE insignificantly increased live body weight and body weight change as compared to the control group and those on combination of 500ppm VE plus thyroxine. However, layers fed diet containing 0.25ppm thyroxine and 250ppm VE observed the heaviest value for body weight and body weight change. Abdel-Maksoud (2006) showed that, VE supplementation at different levels in laying diets recorded insignificantly heavier values of the final body weight and body weight changes when compared with the control group. These results were confirmed by (Sahin *et al*., 2005 and 2002c) who reported that VE supplementation up to 250ppm increased body weight significantly and reduced the negative effects of heat stress in Japanese quail. Combination of 125ppm VE and 200ppm vitamin C in laying hen diets that were exposed to a chronic heat stress increased significantly body weight gain (Ciftci *et al*., 2005).

The improvement of growth rate by VE supplementation might be a result of stimulating thyroid hormones secretion in laying hens exposed to high ambient environmental temperature. Thyroid hormones are major regulators of development, metabolism and homeostasis in birds. They influence body weight and the growth of muscles, the chondrous and the bones (King and May, 1984). In addition, they are essential to stimulate growth rate in animals in association with ambient temperature (McNabb and King, 1993). The enhancement effects of VE supplementation in laying diets reared under heat stress on body weight gain and plasma concentration of T_3 and T_4 were confirmed by Whitehead *et al*. (1998); in broiler, Sahin *et al*. (2001) and (2002a); in Japanese quail, Ciftci *et al*. (2005)) and Sahin *et al*. (2005). In this respect, Wentworth and Ringer (1986) demonstrated in laying hens that, thyroid hormone administration in small doses improved growth of chickens, and when administered beyond physiologic doses, however, it depressed growth rate.

Layers received diets supplemented with VE, thyroxine or their combinations recorded significantly ($P \leq 0.01$) higher egg production percentage throughout the experimental period. There was insignificant increase in egg production percentage with supplements into layer diets during the second experimental period. However, the layers group fed 0.25ppm hormone plus 500ppm VE had the highest egg production percentage (more eggs number) as compared to the control group and other treatments during the first and the whole experimental periods. In this connection, Metwally (2003) showed that, egg laying rate (%) was

significantly higher by 18.56% during the period of stress in birds fed the highest level of VE than control birds. Egg production and quality were enhanced by VE supplementation (310ppm VE/kg). Also, Kirunda *et al*. (2001) reported that supplementation of diets with 60 IU VE/kg feed improved egg production under heat stress conditions. El-Husseiny *et al*. (2000) cited that oxygen consumption was significantly increased by T_3 or T_4, hence increased growth and egg production and improved egg shell.

As shown in Table (4), the results indicated that, layers fed diets supplemented by 250 or 500ppm VE had significantly ($P \leq 0.01$) better egg weight than those received the other treatments except those fed the combination of 0.25ppm thyroxine plus 250ppm VE. It is worthy that, layers given 0.25ppm thyroxine alone had higher egg weight as compared to the control group.

Results showed that, the average values of egg mass were significantly ($P \leq 0.01$) increased when laying hens fed diets containing different levels of VE, thyroxine or their mixtures as compared to the control group during the different experimental periods. Abdel-Maksoud (2006) indicated that, the inclusion of VE 375ppm during summer, insignificantly improved egg laying performance. Supplemental VE, thyroxine, or their combination increased egg production (%) by alleviating the adverse effects of high ambient temperature in laying hens during summer months or in hens reared under heat stress, thus maintaining the releasing concentrations of egg yolk precursors. Therefore, VE supplementation may have enhanced synthesis of egg yolk precursors in liver through protecting the liver from lipid peroxidation and damage to cell membranes. This was previously confirmed in hens by Bollengier–Lee *et al*. (1998 and 1999), they reported that hens fed VE supplementation from 125 to 500ppm/kg laid more eggs and had higher plasma concentration of egg yolk precursors under heat stress. Similar trend were obtained in laying Japanese quail reared under heat stress (34°C) by (Sahin *et al*., 2002c; Abdel Galil and Abdel Samad, 2004 and Ciftci *et al*., 2005). Elnagar *et al*. (2005) found that egg number and egg weight of Silver Montazah and Gimmiza (two local strains) were increased significantly with 50 and 100 µg T_4/kg body weight. The increase in egg weight may be due to the improvement in the metabolic process by direct stimulatory effect of thyroxine, or the improvement in egg shell thickness (Khalifa *et al*., 1983).

Laying hens fed diets supplemented with either 250ppm VE or its combination with 0.25ppm thyroxine, achieved the higher values of feed consumption (FC) compared to those fed 0.25ppm thyroxine diet or the control diet, during the different periods. The remaining groups had

intermediate values for FC. The obtained results are in agreement with those reported by Abdel-Maksoud (2006) who found that, the inclusion of VE increased feed consumption of laying hens under heat stress.

During the first period of this experiment, laying hen groups received diets containing 0.25ppm thyroxine either individually or in combination with 500ppm VE had the best ($P \leq 0.01$) values of FCR as compared to the control group and the other treated groups. Layer groups fed diets supplemented by 500ppm VE, 0.25ppm thyroxine alone or their mixture insignificantly improved FCR as compound to the other groups during the second experimental period and overall the experimental period (28-36 weeks of age). Similar results were obtained by Sahin *et al*. (2002c), in Japanese quail with VE (250 to 500ppm). Ciftci *et al*. (2005) reported that dietary supplementation with 125ppm VE plus 200ppm vitamin C in laying hens exposed to chronic stress (35°C) improved feed intake and feed efficiency. An explanation of these finding had been done by Bollengier-Lee *et al*. (1998) who stated that high environmental temperature could reduce nutrient metabolism and feed utilization due to increase of membrane lipid peroxidation, cell liver damage and other organs dysfunction. They added that, VE might play an important role through the protection of liver or other organs against oxidative damage (Abdel-Maksoud, 2006). Through its role as a metabolic regulator, thyroxine hormone might increase the utilization of consumed food.

No dead laying hens due to any of the experimental treatment groups were observed throughout the experimental period of the present study, whereas, the viability rate for layers was 100.0% from 28 to 36 weeks of age.

4-**Egg quality measurements:-**

As shown in Table (5), the results indicated that, the highest values for egg weights have been recorded for groups fed either 500ppm VE singly or those fed 250ppm VE in combination with 0.25ppm thyroxine hormone as compared to those received the control diet. However, the other treatment groups recorded intermediate values during the interval periods. Neither dietary VE, thyroxine supplementation nor their combinations had significant effects on egg shape index, albumen weight percentage and shell density throughout the 32 th or 36 th week of hens' age.

Values of Haugh units, yolk height, shell thickness and shell surface area (ESA, cm^2) were significantly ($P \leq 0.01$) improved with adding VE, thyroxine hormone or their combinations to layer diets as compared to the control group during both 32 th and 36 th week of hens age. Hens whose diets were supplemented with VE, thyroxine or their mixtures had significantly

($P \leq 0.01$) higher albumen height values than the control group. Metwally (2003) showed that, hens fed VE at level of 310 ppm had a significant increase in shell thickness and Haugh units during heat stress. Results obtained agreed with the findings of Sahin *et al*. (2002c) who found a significant improvement in shell thickness in Japanese quails when the diet contained VE (250 to 500ppm) under heat stress. Engelmann *et al*. (2001) showed that, eggshell and shell thickness were significantly influenced by VE 1000ppm in laying diet under heat stress. Abdel-Galil and Abdel Samad (2004) in the local breeds under hot climate conditions reported that shell quality was improved by supplemental VE.

The achieved improvements in shell thickness and shell surface area could be due to enhancement of calcium bioavailability by the action of supplemental VE and/or thyroxine. These facts confirmed the results of increased serum Ca concentration that has been established in the present study (Table. 3).

Generally, there were not significant effects ($P>0.05$) in values of albumen height, shell percentage and shell weight per unit surface area (SWUSA, mg/cm^2). Additionally, no significant effect in yolk weight percentage at 36 weeks old was observed due to treatments. Supplementation of 0.25ppm thyroxine alone in the layers diet significantly ($P \leq 0.01$) decreased SWUSA (mg/cm^2) compared to the other experimental groups.

The higher value of yolk weight percentage achieved for the laying hens fed 0.25ppm thyroxine alone followed by 250ppm VE and combination of 0.25ppm hormone and 500ppm VE while, the lower value recorded for both the high dose of VE and the control group. These results were in agreement with Ciftci *et al*. (2005) who found that a combination of 125ppm VE and 200ppm vitamin C under heat stress increased yolk weight percentage significantly. Similar results were found by Puthpongsiriporn *et al*. (2001). In this respect, Abdel-Galil and Abdel Samad (2004) reported that, VE supplementation improved yolk index of local breeds during summer season. However, the increase of VE up to 500ppm showed insignificant increase in yolk weight when compared with the other experimental groups (Abdel-Maksoud, 2006).

In conclusion, the present results suggested that dietary supplementation of either VE, thyroxine or their blends have the potential to alleviate the detrimental effects of hot summer season on laying performance. Therefore, the magnitude of effects was greater with their combinations in particular, 250ppmVE plus 0.25ppm thyroxine.

Table 1: Composition and calculated analysis of the laying hens basal diet.

Ingredients (%)	**(%)**
Yellow corn	63.00
Soybean meal (44% CP)	18.00
Corn gluten meal (60% CP)	8.00
Limestone ($CaCO_3$)	7.50
Bone meal	2.50
Vit & Mineral Premix*	0.30
Salt (NaCl)	0.40
L-Lysine HCl	0.15
DL-Methionine	0.15
Total	100.00
Calculated analysis:	
Crude protein %	18.08
ME (KCal/Kg diet)	2810
Calcium %	3.67
Availabile phosphorous %	0.43
Lysine %	0.73
DL-Methionine %	0..35

***** Each one kg of the vitamin-mineral mixture contains: Vit A 7500000 IU, Vit D3 1650000 IU, Vit E 33000 mg, Vit k3 2500 mg, Vit B1 1250 mg, Vit B2 4950 mg, Vit B6 3300 mg, Nicotinic acid 20000 mg, Pantothenic acid 9000 mg, Vit B12 10 mg, Biotin 80 mg, Folic acid 400 mg, Choline chloride 600g, Iron 40g,Manganese 50g, Copper 4 g, Iodine 0.4 g, Zinc 30g, Selenium 0.24 g and $CaCO_3$ was used as a carrier.

Table 2: Effect of dietary vitamin E (VE), thyroxine (T_4) and their combination on humoral immune response of ISA Brown laying hens during summer months.

Trait	Treatments						Sig.
	Control	250ppm VE	500ppmVE	0.25ppmT_4	250ppm VE +0.25ppmT_4	500ppm VE +0.25ppmT_4	
Total Primary Antibodies	3.75±0.29	4.00±0.40	4.00±0.41	3.75±0.47	4.25±0.25	3.75±0.25	NS
Primary IgM	3.50±0.25	3.75±0.25	3.50±0.20	3.25±0.25	4.00±0.40	3.25±0.47	NS
Primary IgG	0.25±0.05	0.25±0.02	0.50±0.08	0.50±0.04	0.25±0.03	0.50±0.07	NS
Total Secondary Antibodies	5.50^{c}±0.29	8.25^{a}±0.62	8.00^{ab}±0.41	7.25^{ab}±0.25	7.25^{ab}±0.62	6.75^{bc}±0.25	***
Secondary IgG	1.00±0.41	1.00±0.40	0.50±0.06	1.25±0.25	1.00±0.41	1.25±0.25	NS
Secondary IgM	4.50^{d}±0.29	7.25^{ab}±0.55	7.50^{a}±0.29	6.00^{bc}±0.41	6.25^{abc}±0.25	5.50^{cd}±0.28	***

[a-d] Means within a row with different superscripts are significantly different. NS = Not significant ***= P≤0.001

Table 3: Effect of dietary vitamin E (VE), thyroxine (T_4) and their combination on some serum metabolites of ISA Brown laying hens during summer months.

Parameter	Treatments						Sig.
	Control	250ppm VE	500ppmVE	0.25ppmT_4	250ppm VE +0.25ppmT_4	500ppm VE +0.25ppmT_4	
AST (U/I)	117.24^{a}±6.95	115.55^{ab}±6.34	95.78^{c}±v4.89	105.64^{abc}±7.24	99.98^{abc}±5.34	97.38^{bc}±5.73	*
ALT (U/I)	33.13±1.45	35.39±8.90	30.69±1.26	35.50±1.93	39.48±5.05	32.95±1.61	NS
AP (U/L)	178.40^{c}±9.98	209.83^{b}±6.25	212.14^{b}±3.91	256.01^{a}±6.42	270.12^{a}±7.51	200.60^{b}±4.63	***
T_3 (ng/ml)	0.73±0.07	0.91±0.05	0.73±0.01	0.79±0.08	0.75±0.07	0.71±0.06	NS
T_4 (µg/dl)	4.17^{c}±0.63	9.45^{a}±0.97	5.30^{c}±0.37	6.19^{bc}±0.51	8.08^{ab}±0.91	5.38^{c}±0.24	***
Ca (mg/dl)	15.20^{c}±0.84	19.59^{a}±0.25	18.45^{ab}±0.50	16.18^{c}±0.45	16.98^{bc}±1.18	16.23^{c}±0.36	**
P (mg/dl)	5.19±0.61	7.69±0.60	5.63±0.53	6.91±0.68	5.63±0.80	7.31±0.42	NS
Uric acid (mg/dl)	5.76^{a}±0.29	5.38^{ab}±0.24	4.53^{bc}±0.36	4.76^{bc}±0.31	4.89^{abc}±0.33	4.30^{c}±0.17	*

$^{a-d}$ Means within a row with different superscripts are significantly different.
NS = Not significant *= $p \leq 0.05$; **= $p \leq 0.01$; ***= $p \leq 0.001$.

Table 4: Effect of dietary vitamin E (VE), thyroxine (T_4) and their combination on body weights, egg production% and egg weight, egg mass, feed consumption (g/ hen/day) and feed conversion ratio of ISA Brown laying hens during summer months.

tems	Control	250mg VE	500mg VE	0.25ppmT_4	250ppm VE +0.25ppmT_4	500ppm VE +0.25ppmT_4	Sig.
ive body weight (g)							
t 28th week	1535.8±37.9	1536.9±36.6	1530.2±40.5	1535.8±37.5	1527.6±41.9	1528.3±27.8	NS
t 36th week	1763.7±32.6	1793.6±21.2	1787.1±40.6	1789.4±40.1	1807.7±44.8	1755.9±33.1	NS
ody change							
8-36 weeks	227.8±16.2	256.8±27.7	256.9±16.5	253.6±35.8	280.1±18.4	227.6.1±15.4	NS
gg production %							
8-32 weeks	87.6^{c}±2.1	91.1^{bc}±1.1	92.1^{ab}±1.1	90.9^{bc}±0.9	92.9^{ab}±1.1	95.7^{a}±1.1	**
2-36 weeks	93.5 ±1.7	96.1 ±1.3	95.2 ±1.2	95.0 ±0.8	96.7 ±0.8	95.5 ±1.1	NS
8-36 weeks	90.6^{b}±1.7	93.5^{ab}±0.9	93.6^{ab}±0.9	93.0^{ab}±0.8	94.9^{a}±0.8	95.7^{a}±0.8	*
gg weight (g)							
8-32 weeks	57.1^{d}±0.8	60.4^{ab}±1.2	61.2^{a}±0.9	58.3^{cd}±0.7	61.0^{ab}±1.0	58.0^{cd}±0.9	**
2-36 weeks	58.5^{b}±1.1	63.1^{a}±1.2	63.4^{a}±1.0	60.9^{ab}±0.6	61.2^{ab}±1.1	59.8^{b}±1.0	**
8-36 weeks	57.8^{c}±0.8	61.7^{ab}±1.1	62.3^{a}±0.9	60.1^{abc}±0.5	61.6^{ab}±0.9	59.1^{bc}±0.9	**
gg mass (g/hen/day)							
8-32 weeks	51.4^{b}±1.2	55.0^{a}±1.1	56.8^{a}±0.8	53.6^{ab}±0.7	55.5^{a}±1.2	56.8^{a}±1.0	**
2-36 weeks	54.6^{b}±1.4	59.6^{a}±1.5	60.3^{a}±1.1	57.9^{ab}±0.8	59.1^{a}±0.9	57.7^{ab}±1.2	**
8-36 weeks	53.3^{b}±1.2	57.3^{a}±1.1	58.6^{a}±0.8	56.6^{a}±0.6	57.5^{a}±0.9	57.7^{a}±0.9	**
eed consumption (g/day)							
8-32 weeks	99.1^{bc}±2.0	109.3^{a}±2.6	103.3^{ab}±2.5	92.8^{c}±3.3	105.6^{ab}±1.9	99.4^{bc}±1.4	**
2-36 weeks	102.5±3.9	106.6±5.3	103.0±4.2	99.7±4.0	110.0±2.3	101.1±4.4	NS
8-36 weeks	100.9^{ab}±2.4	107.9^{a}±2.7	103.2^{ab}±2.7	96.2^{b}±3.0	107.8^{a}±1.7	100.3^{ab}±2.6	*
eed conversion ratio							
8-32 weeks	1.95^{ab}±0.06	1.99^{a}±0.05	1.81^{bc}±0.04	1.75^{c}±0.07	1.91^{ab}±0.06	1.76^{c}±0.04	**
2-36 weeks	1.89±0.08	1.79±0.08	1.71±0.08	1.73±0.07	1.87±0.05	1.75±0.09	NS
8-36 weeks	1.88±0.06	1.89^{bc}±0.05	1.75±0.05	1.75±0.07	1.88^{c}±0.04	1.78±0.07	NS
iability 28-36 weeks	100.0	100.0	100.0	100.0	100.0	100.0	

Means within a row with different superscripts are significantly different. NS = Not significant *= $p \leq 0.05$; **= $p \leq 0.01$.

Table 5: Effect of dietary vitamin E (VE), thyroxine (T_4) and their combination on egg quality measurements of ISA Brown laying hens during summer months.

Traits	Hens age	Control	250mg VE	500mg VE	0.25ppmT_4	250ppm VE +0.25ppmT_4	500ppm VE +0.25ppmT_4	Sig.
Egg weight (g)	32	55.90^{c}	58.72^{abc}	60.96^{ab}	59.78^{ab}	61.14^{a}	57.26^{bc}	*
	36	57.37^{b}	60.66^{ab}	61.37^{a}	60.37^{ab}	62.04^{a}	59.60^{ab}	*
Egg shape index	32	0.81	0.78	0.79	0.79	0.79	0.79	NS
	36	0.80	0.78	0.78	0.79	0.79	0.79	NS
Albumen %	32	65.24	64.21	66.04	64.69	65.80	64.10	NS
	36	66.20	65.01	65.24	65.07	65.71	65.46	NS
Yolk %	32	24.24^{ab}	25.80^{a}	23.80^{b}	26.16^{a}	24.70^{ab}	25.72^{a}	*
	36	23.60	24.88	24.80	25.91	24.79	24.97	NS
Shell %	32	10.34	9.99	10.17	9.15	9.49	10.19	NS
	36	10.20^{a}	10.11^{a}	9.96^{ab}	9.02^{c}	9.50^{bc}	9.57^{bc}	**
Albumen height	32	6.60	7.80	7.47	6.78	7.54	7.28	NS
	36	8.15^{b}	10.23^{a}	9.57^{a}	9.25^{ab}	9.60^{a}	10.44^{a}	**
Yolk height	32	17.87^{b}	18.79^{ab}	18.44^{ab}	18.07^{b}	19.87^{a}	18.50^{ab}	**
	36	18.06^{b}	19.16^{a}	19.08^{ab}	18.95^{ab}	19.91^{a}	18.93^{ab}	**
Haugh units	32	69.17^{b}	77.98^{a}	74.75^{ab}	69.13^{b}	75.16^{ab}	74.31^{ab}	*
	36	80.55^{b}	92.16^{a}	88.46^{a}	87.10^{ab}	88.71^{a}	93.54^{a}	**
Shell thickness	32	0.34^{b}	0.40^{a}	0.40^{a}	0.38^{a}	0.39^{a}	0.39^{a}	**
	36	0.35^{c}	0.41^{a}	0.39^{a}	0.38^{ab}	0.38^{ab}	0.37^{bc}	*
Shell density	32	0.32	0.33	0.33	0.31	0.33	0.31	NS
	36	0.31	0.34	0.32	0.32	0.32	0.31	NS
Shell surface area (ESA, cm^2)	32	69.37^{c}	71.66^{b}	73.44^{ab}	72.51^{ab}	73.60^{a}	70.47^{bc}	*
	36	70.55^{b}	73.20^{ab}	73.76^{a}	72.97^{ab}	74.31^{a}	72.34^{ab}	*
SWUSA (mg/cm^2)	32	0.08	0.08	0.08	0.08	0.08	0.08	NS
	36	0.08^{a}	0.08^{a}	0.08^{a}	0.07^{b}	0.08^{a}	0.08^{a}	**

$^{a-c}$ Means within a row with different superscripts are significantly different. NS = Not significant

*= $p \leq 0.05$; **= $p \leq 0.01$;.

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الملخص العربي

تأثير فيتامين هـ (E)، هرمون الثيروكسين (T4) وكلاهما معا على المناعة الذاتية والأداء الإنتاجي وبعض مكونات سيرم الدم في الدجاج البياض أثناء موسم الصيف.

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أجريت التجربة لدراسة تأثير فيتامين هـ(E) وهرمون الثيروكسين (T4) وكلاهما معا على المناعة الذاتيةو الأداء الإنتاجي وبعض مكونات سيرم الدم في الدجاج البياض أثناء موسم الصيف.

أستخدم في هذه التجربة عدد 120 دجاجة بياضة عمر 28 أسبوع من سلالة ايزا براون (ISA Brown layers) . قسمت عشوائياً إلي 6 مجموعات تجريبية . استخدمت المجموعة الأولي كمجموعة مقارنة (كنترول) غذيت علي العليقة الأساسية بدون إضافات، بينما عوملت المجموعات الخمس الأخرى الأتي : أعطيت المجموعة الثانية والثالثة 250، 500 ملجم/ كحم فيتامين هـ (E) علي التوالي.في حين غذيت المجموعة الرابعة على 0.25 ملجم ثيروكسين وأعطيت المجموعة الخامسة خليط من الهرمون والفيتامين بمعدل 250 ملجم/كجم مع 0.25 ملجم ثيروكسين كما أعطيت المجموعة السادسة خليط من الهرمون والفيتامين بمعدل 500 ملجم/ كجم مع 0.25 ملجم ثيروكسين.

وكانت أهم النتائج كالأتي:

سجلت المجاميع المضاف إليها سواء الفيتامين أو الهرمون أوكلاهما أفضل استجابة مناعية للحقن بكرات دم الغنم. لم يتأثر مستوى إنزيم ALT وكذلك الفوسفور. بينما لوحظ

انخفاض غير معنوي في مستوى إنزيم AST وخاصة في المجموعات المغذاة على 500 ملجم فيتامين هـ بمفرده أو مع هرمون الثيروكسين. سجلت المجموعة المقارنة أقل قيم لمستوى الكالسيوم ونشاط إنزيم الألكالين فوسفاتيز بالمقارنة ببقية المجاميع التجريبية . زاد مستوى هرمون الثيروكسين زيادة معنوية في المجاميع المغذاة على عليقة مضاف إليها 250 ملجم فيتامين/ كجم وحده أو بخلطه مع هرمون الثيروكسين بينما لم يتأثر مستوى هرمون التراى أيودو ثريونين. انخفض تركيز حمض اليوريك بإضافة الفيتامين بمفرده أو/ ومع هرمون الثيروكسين. زاد مستوى قيم كتل البيض ومعدل الإنتاج معنويا في كل المجاميع التجريبية مقارنة بالمجموعة المقارنة، وخاصة المجموعة المغذاة على 250 أو 500 ملجم فيتامين مع ثيروكسين. تحسنت معنويا قيم Haugh units وارتفاع صفار البيض وسمك القشرة ومساحة سطح قشرة البيضة (ESA) بإضافة الفيتامين أو الهرمون أوكلاهما معا .كما سجلت المجموعات المغذاة على علائق احتوت علي 0.25 ملجم ثيروكسين أو فيتامين 250وتلك المغذاة علي خليط من الهرمون مع 500 ملجم من الفيتامين أعلى متوسط لوزن الصفار.

وقد خلصت الدراسة إلى أن الدجاجات البياضة أظهرت أفضل استجابة إنتاجية ومناعية أثناء فصل الصيف الحار باستخدام هذه الإضافات وننصح باستخدامها.