EFFECT OF SUPPLEMENTED DIFFERENT LEVELS OF VITAMINS E AND C TO LAYERS HEN DIETS ON: 2- IMMUNE RESPONSE AGAINST AVIAN INFLUENZA VACCINE AND SOME PHYSIOLOGICAL PARAMETERS.

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SUMMARY

This study was carried out at the Poultry Research Station, El-Azab, Fayoum, to study the effects of two dietary levels of vitamin E (Vit. E) (10 or 20 mg/Kg diet), vitamin C (Vit. C) (200 or 400 mg/Kg diet) and their mixtures on teeter influenza virus, some blood plasma constituents and semen traits of El-Salam laying hens. A total number of 243 (216 breeder hens and 27 cocks) birds at 25 weeks of age were used in this experiment. Birds were wing banded and randomly distributed into 9 equal treatment groups of 27 birds each (24 breeder hen and 3 cock each). Each group was equally subdivided into three replicates of 11 (eight ♀ and one ♂/replicate) birds each. The experimental treatments were as follows:

1- Birds were fed control diet (unsupplemented with Vit. E or Vit. C (D1)).
2- Birds were fed D1 supplemented with 10 mg/Kg diet Vit. E (D2).
3- Birds were fed D1 supplemented with 20 mg/Kg diet Vit. E (D3).
4- Birds were fed D1 supplemented with 200 mg/Kg diet Vit. C (D4).
5- Birds were fed D1 supplemented with 400mg/Kg diet Vit. C (D5).
6- Birds were fed D1 supplemented with Vit. E 10 mg/Kg diet + Vit. C 200 mg/Kg diet (D6).
7- Birds were fed D1 supplemented with Vit. E 10 mg/Kg diet + Vit. C 400 mg/Kg diet (D7).
8- Birds were fed D1 supplemented with Vit. E 20 mg/Kg diet + Vit. C 200 mg/Kg diet (D8).
9- Birds were fed D1 supplemented with Vit. E 20 mg/Kg diet + Vit. C 400 mg/Kg diet (D9).

Results obtained could be summarized in the following; laying hens fed control diet had higher albumin and AST whereas, those fed diet containing 20 mg Vit. E and 400 mg/kg diet Vit. C had lower albumin and AST during the experimental period. Teeter influenza virus was progressively improved with addition of Vit. E, C and their mixture than the control group. Laying hens diet containing 20 mg Vit. E and 400 mg/kg diet Vit. C had higher hemoglobin, hematocrit, red blood cells count, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration during the experimental period. There were significant decreases in body temperature with addition of Vit. E, C and their mixture than the control group, while, insignificant effects were observed in respiratory rate. Laying hens fed diet containing 20 mg Vit. E and 400 mg/kg diet Vit. C had higher white blood cells count, lymphocyte and heterophils/lymphocyte ratio, while, those fed control diet had higher heterophils. Means of ejaculate volume (ml), sperm concentration (10^6/mm^3), total count, Ph and advanced motility% were not significantly influenced by type of Vit. E or C supplementation or by treatments compared with the control group. In conclusion, feeding El-Salam laying hens on diets containing 20 mg Vit. E and 400 mg/kg diet Vit. C improved the teeter influenza virus and some physiological parameters.

Keywords: *El-Salam laying hen; teeter influenza virus; physiological parameters; semen traits.*

INTRODUCTION

Alpha-tocopherol is the most active natural antioxidant used in animal feeding; it exhibits an antioxidant activity at low concentration and a prooxidant activity at high concentration (Chen *et al.*, 1998). Vitamin E (Vit. E) is a lipid component of biological membranes and is known to be a major chain-breaking antioxidant (Sahin *et al.*, 2002). In nutritional and physiological research with various animal species, Vit. E supplementation has been proven to maintaining immune cell function (Meydani and Beharka, 1998 and Moriguchi and Muraga, 2000), including immunity enhancement (Trushenski and Kohler, 2007), and prevention of inflammatory reactions by the suppression of the activation of the

transcriptional factor, nuclear factor-κB (Calfee-Mason *et al.*, 2004), as well as influencing neuroendocrine function (Khan and Thomas, 2004) and reproduction under environmental toxicity. It is common to include Vit. E in poultry feeds in the form of all-*rac*-α-tocopherol acetate (Villaverde *et al.*, 2008).

On the other hand, chicken cannot synthesize Vit. E, therefore the requirements must be met from dietary source (Chan and Decker, 1994). In addition Vit. E act as a physiological synergist and as a functioning portion of specific enzymes (Franchini *et al.*, 1995).

Ascorbic acid (AA) is a water-soluble vitamin, and it is also an antioxidant by contributing electrons to more oxidized molecules; it can also reduce α-tocopheryl radical (Parcker *et al.*, 1979). Ascorbic acid is involved in a number of biochemical processes. It is necessary for biosynthesis of various vital compounds (i.e. collagen, carnitine, 1, 25-dihydroxy Vit. D, adrenaline etc.) as well as for the regulation of diverse reactions (secretion of corticosterone, regulation of body temperature) and activation of the immune system (McDowell, 1989 and Kutlu, 2001). Vitamin C or polyphenols increased the antioxidant enzymes in red blood cells (Dragsted *et al.*, 2001). In addition concepts of the biochemical functions of Vit. E include its role as a biological free radical scavenger (McCay, 1985), in nucleic acid and protein metabolism (Catignani, 1980) and in mitochondrial metabolism (Corwin, 1980).

Yin *et al.* (1993) reported that a mixture of α-tocopherol and ascorbate delayed myoglobin oxidation, whereas α-tocopherol or ascorbate alone did not delay metmyoglobin formation. Schaefer *et al.* (1995) demonstrated that oxidation of myoglobin is prone to retardation when α-tocopheroxyl radical at the membrane-sarcoplasma interface is reduced by ascorbate. Regarding antioxidant property, there is a positive synergistic effect of Vit. E and C on the immune response. Antioxidant properties of vitamins have been shown to enhance immunity of laying hens, such as lymphocytes, macrophages and plasma cells, against oxidative damage and to enhance the function and proliferation of these cells (Franchini *et al.*, 1991 and Meydani and Blumberg, 1993). In addition to antioxidation, Vit. C has been reported to enhance immune response by modifying corticosteroid synthesis in adrenal glands (Pardue and Thaxton, 1984).

Therefore, the objective of this study was to determine the effects of two dietary levels of Vit. E (10 or 20 mg/Kg diet), Vit. C (200 or 400 mg/Kg diet) separately and their mixtures on immune response against avian influenza, some blood plasma constituents, blood hematology and semen traits of El-Salam laying hens.

MATERIALS AND METHODS

This study was carried out at the Poultry Research Station, El-Azab, Fayoum, to study the effects of two dietary levels of vitamin E (Vit. E) (10 or 20 mg/Kg diet), vitamin C (Vit. C) (200 or 400 mg/Kg diet) and their mixtures on immune response against avian influenza, some blood plasma constituents, blood hematology and semen traits of El-Salam laying hens.

A total number of 243 (216 breeder hens and 27 cocks) birds at 25 weeks of age were used in this experiment. Birds were wing banded and randomly distributed into 9 equal treatment groups of 27 birds (having nearly similar body weight) each (24 breeder hen and 3 cock each). Each group was equally subdivided into three replicates of 11 (eight ♀ and one ♂/replicate) birds each. Birds were reared under the same management conditions in egg production batteries (open system). The experimental period was lasted for 14 weeks from 25 to 39 weeks of age. Treatment groups were fed a commercial layer ration (16% CP and 2703.34 Kcal ME/Kg diet, Table 1), (control group) supplemented with 10 or 20 mg/Kg diet α-tocopherol acetate (Vit. E), 200 or 400 mg/Kg diet of L-ascorbic acid (Vit. C) and their mixtures. Artificial light was used beside the normal day light to provide 16-hour day photoperiod. Feed and water were provided *ad libitum*. Mortality was recorded daily (no mortality of birds were recorded during the study period). The experimental treatments were as follows:

1- Birds were fed control diet (unsupplemented with Vit. E or Vit. C (D1)).
2- Birds were fed D1 supplemented with 10 mg/Kg diet Vit. E (D2).
3- Birds were fed D1 supplemented with 20 mg/Kg diet Vit. E (D3).
4- Birds were fed D1 supplemented with 200 mg/Kg diet Vit. C (D4).
5- Birds were fed D1 supplemented with 400mg/Kg diet Vit. C (D5).
6- Birds were fed D1 supplemented with Vit. E 10 mg/Kg diet + Vit. C 200 mg/Kg diet (D6).
7- Birds were fed D1 supplemented with Vit. E 10 mg/Kg diet + Vit. C 400 mg/Kg diet (D7).

8- Birds were fed D1 supplemented with Vit. E 20 mg/Kg diet + Vit. C 200 mg/Kg diet (D8).
9- Birds were fed D1 supplemented with Vit. E 20 mg/Kg diet + Vit. C 400 mg/Kg diet (D9).

Table (1): Composition of the basal diets.

Item	%
Yellow corn, ground	63.50
Soybean meal (44%CP)	24.57
Wheat bran	2.00
Calcium carbonate	7.77
Sodium chloride	0.30
Vit. and Min. premix[1]	0.30
Di-calcium phosphate	1.50
DL–Methionine	0.06
Total	100.0
Calculated analysis %[2]:	
Crude protein	16.56
Ether extract	2.67
Crude fiber	3.34
Calcium	3.37
Available phosphorus	0.39
Methionine	0.33
Methionine+Cystine	0.61
Lysine	0.84
ME, kcal./Kg	2703
Cost (£.E./ton)[3]	2600.0

[1] *Each 3.0 Kg of the Vit. and Min. premix contains: Vit. A, 10000000 IU; Vit. D_3 2000000 IU; Vit. E, 1000 mg; Vit. K_3, 1000 mg; Vit. B1, 1000 mg; Vit. B2, 500 mg; Vit. B6, 1500 mg; Vit. B12, 10 mg; biotin, 50 mg; folic acid, 1 mg; niacin, 3000 mg; Ca pantothenate, 1000 mg; Zn, 50 g; Cu,4 g; Fe, 30 g; Co, 0.1 g; Se, 0.1 g; I, 0.3 g; Mn, 60 g and anti-oxidant, 10 g, and complete to 3.0 Kg by calcium carbonate.*

[2] *According to NRC. 1994.*

[3] *According to the local market price at the experimental time.*

Avian influenza vaccine was vaccinated at 10, 60 day of age and the last injection was at 190 day of age after determined influenza titer at 160 and 190 day of age. Vaccine was given by injection (0.05 cm at the age of 10 day and 1 cm at 60 and 190 day) under the skin behind the back of the neck. Determination method: Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees) 5th ed. [edited by the OIE Biological Standards Commission and adopted by the International Committee of the OIE]. Published 2004 by Office international des epizooties in Paris.

At the end of the experiment, body temperature was measured as a rectal temperature (°C) by inserting thermometers approximately 1 cm into each chick via the rectum. Respiration rate (breaths/min) was obtained by counting the wave cycles associated with respiratory movement.

At the end of the experiment, individual blood samples were collected from 5 hens per treatment and taken randomly from the brachial vein, then transferred to vial tubes containing EDTA as anticoagulant and immediately centrifuged at 3500 rpm for 15 min. Plasma were harvest after centrifugation of the clotted blood, stored at–20C in the deep freezer until the time of chemical determinations.

Hemoglobin (Wintrobe, 1965); hematocrit concentration (Hb) and red blood cells (RBC's) count were determined. Total (Ritchie *et al.*, 1994) and differential white blood cells (WBC) counts were performed by using standard avian guidelines introduced by Ritchie *et al.* (1994). Leucocyte cells (heterophils (H), lymphocytes (L), eosinophils, monocytes, and basophils) were counted in different microscopic fields in a total of 45 WBC by the same person and the H:L ratios were calculated (Gross and Siegel, 1986).

Plasma constituents were determined commercially using kits, total protein (Weichselbaum, 1946); albumin (Dumas and Biggs, 1972); globulin concentration was calculated as the difference between total protein and albumin; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Reitman and Frankel, 1957); calcium (Lehman and Henry, 1984); Triiodothyronine (T_3) (ng/dl) and thyroxin (T_4) (ng/dl) were determined in plasma by using radioimmunoassay kit.

Semen samples were individually collected twice a week by the massage method from all birds to determine their semen characteristics. Semen volume was measured in graduated tubes. Sperm concentration was determined using the spectrophotometer density meter technique with diluted semen samples (1:250) as described by Lake and Stewart (1978). Eosin-Nigrosine stain was used to determine the percent of morphologically abnormal sperm cells (Lake and Stewart, 1978). Sperm motility percent: A small droplet from each cock's tube was placed on a warm slide, covered with a cover slide and examined for sperm motility microscopically at 100x magnification Observed the edge of the semen to as certain an approximation of the percentage of live active spermatozoa Melrose and Laing (1970).

Semen was collected from trained roosters fed a diet containing supplemental Vit. E and C, all the hens were artificially inseminated according to the method described by Burrows and Quinn (1937).

An ANOVA with the General Linear Models (GLM) procedure of SPSS software (SPSS, 1999) included the effect of type and treatment means. Treatment means indicating significant differences ($P \leq 0.01$ and $P \leq 0.05$) were tested using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Blood hematological and biochemical parameters:

Table (2) shows effect of supplementing laying hens' diets with Vit. E, C and their mixtures on some blood biochemical parameters. Total protein, globulin, albumin/globulin ratio, ALT, T_3 and T_4 were not affected significantly by type of addition (Vit. E, C and their mixtures). Type of addition effect was significant only for blood albumin, AST and calcium (Table 2). laying hens fed control diet had higher albumin, AST and calcium whereas, those fed diet containing mixtures of Vit. E and Vit. C supplementation had lower albumin and AST during the experimental period.

Results presented in Table (2) indicated no significant differences in some blood biochemical parameters among all dietary treatments including the control group except, albumin and AST, laying hens fed control diet had higher albumin and AST whereas, those fed diet containing 20 mg Vit. E and 400 mg/kg diet Vit. C had lower albumin and AST during the experimental period.

Similar results were observed by Gursu *et al.* (2003) who found that serum activities of AST and ALT were not influenced by dietary Vit. E supplementation to Japanese quails diets. While, these results disagree with those of El-Mallah *et al.* (2011) who reported that plasma total protein, albumin and globulin were significantly increased by adding Vit. E. to laying hens diet.

In this respect, Sergeev *et al.* (1990) demonstrated that ascorbic acid plays a critical role in Vit. D metabolism and it is required for the conversion of Vit. D into its metabolite form (calcitrol) which is essential for calcium regulation and the calcification process. Also, it is required for hydroxylation of proline residues necessary for the synthesis of procollagen, which is a precursor to bone formation.

Data in Table (3) indicated that teeter influenza virus was significantly affected by type of addition and all dietary treatments. Teeter influenza virus was progressively improved with addition of Vit. E, C and their mixture than the control group. The obtained results in the present study are in agreement with Tantcheva *et al.* (2003) and Elie *et al.* (2007) who found that Vit. C in combination with Vit. E has stronger effects on reduction of influenza virus infectivity probably through Vit. C's repairing effect on Vit. E's tocopheroxyl compound. Also. Gorton and Jarvis (1999) found that mega-doses of Vit. C had an 85% prevention and relief rate of cold and flu symptoms in students 18 to 30 years of age. The antioxidant properties of Vit. C could play an important role in the antiviral effect against influenza virus. In fact, Hennet *et al.* (1992) demonstrated that oxidant-treated anti-protease is unable to prevent trypsin from cleaving the hem agglutinin protein (HA0) to HA1/HA2, resulting in a 10.000-fold increase in infectious influenza virus.

As a protective effect, anti-proteases are present on the surface of alveoli and are inactivated by reactive oxygen species; consequently, the use of an antioxidant would be of primary importance to reactivate anti-protease to prevent influenza viral infections. Leshchinsky and Klasing (2001) the effect of Vit. E on antibody production depended on the nature of the antigen. After vaccination with killed infectious bronchitis virus.

Table (2): Effects of supplementing laying hens diets with vitamin E, C and their mixtures on some blood biochemical parameters of El-Salam laying hens.

Item	Total protein g/L	Albumin g/L	Globulin g/L	Albumin/ Globulin ratio	AST U/ml	ALT U/ml	Calcium mmol/L	T_3	T_4
Type of addition									
Control	5.50	2.57^{A}	2.93	0.88	38.67^{A}	18.67	34.00^{A}	2.33	3.70
Vitamin (Vit.) E	5.25	2.42^{B}	2.83	0.86	38.33^{A}	17.67	33.50^{A}	2.25	3.78
Vitamin C	5.33	2.35^{B}	2.98	0.80	37.17^{AB}	18.00	30.67^{B}	2.27	3.68
Mixed (Vit. E &Vit. C)	5.32	2.30^{B}	3.02	0.77	35.92^{B}	17.50	33.75^{A}	2.22	3.74
±SEM[1]	0.12	0.05	0.12	0.04	0.61	0.41	0.72	0.05	0.06
Treatments									
Control	5.50	2.57^{a}	2.93	0.88	38.67^{a}	18.67	34.00	2.33	3.70
Vit. E 10 mg/Kg diet	5.37	2.43^{ab}	2.93	0.83	38.67^{a}	18.00	33.00	2.20	3.83
Vit. E 20 mg/Kg diet	5.13	2.40^{ab}	2.73	0.88	38.00^{ab}	17.33	34.00	2.30	3.73
Vit. C 200 mg/Kg diet	5.50	2.40^{ab}	3.10	0.78	36.67^{abc}	17.67	31.00	2.20	3.73
Vit. C 400 mg/Kg diet	5.17	2.30^{bc}	2.87	0.82	37.67^{abc}	18.33	30.33	2.33	3.63
Vit. E 10 mg/Kg diet + Vit. C 200 mg/Kg diet	5.60	2.37^{b}	3.23	0.73	36.00^{abc}	17.67	34.00	2.20	3.80
Vit. E 10 mg/Kg diet + Vit. C 400 mg/Kg diet	5.13	2.37^{b}	2.77	0.86	37.33^{abc}	17.33	33.67	2.30	3.80
Vit. E 20 mg/Kg diet + Vit. C 200 mg/Kg diet	5.20	2.30^{bc}	2.90	0.80	35.33^{bc}	17.67	33.00	2.20	3.73
Vit. E 20 mg/Kg diet + Vit. C 400 mg /Kg diet	5.33	2.17^{c}	3.17	0.70	35.00^{c}	17.33	34.33	2.17	3.63
±SEM	0.13	0.06	0.16	0.06	0.86	0.63	1.11	0.08	0.08

[1] ***Pooled SEM.***

a,....c, and A,... B, values in the same column within the same item followed by different superscripts are significantly different (at P ≤0.05 for a to c; P ≤0.01 for A to B).

Table (3): Effects of supplementing laying hens diets with vitamin E, C and their mixtures on some blood constituents of El-Salam laying hens.

Item	Teeter influenza virus	Hemoglobin (g/dL)	Hematocrit (HCT)%	Red blood cells count (10^6/mm^3)	Mean corpuscular volume (MCV) μ^3	Mean corpuscular hemoglobin (MCH) µµg	Mean corpuscular hemoglobin concentration (MCHC)%
Type of addition							
Control	6.20^{C}	12.80^{C}	30.40^{B}	2.96^{C}	90.10^{C}	37.86^{C}	389.2^{C}
Vitamin (Vit.) E	7.00^{B}	13.35^{BC}	31.10^{B}	3.16^{B}	98.31^{BC}	42.19^{B}	414.5^{BC}
Vitamin C	7.20^{AB}	13.90^{AB}	32.60^{AB}	3.24^{AB}	105.60^{B}	44.99^{AB}	452.9^{B}
Mixed (Vit. E &Vit. C)	7.85^{A}	14.25^{A}	35.45^{A}	3.34^{A}	118.43^{A}	47.59^{A}	505.1^{A}
±SEM[T]	0.23	0.26	0.96	0.04	3.54	0.98	15.51
Treatments							
Control	6.20^{c}	12.80^{c}	30.40^{c}	2.96^{c}	90.10^{E}	37.86^{E}	389.20^{E}
Vit. E 10 mg/Kg diet	7.00^{BC}	13.40^{bc}	30.80^{C}	3.16^{B}	97.38^{DE}	42.36^{CD}	412.30^{DE}
Vit. E 20 mg/Kg diet	7.00^{BC}	13.30^{bc}	31.40^{C}	3.16^{B}	99.24^{DE}	42.01^{D}	416.60^{CDE}
Vit. C 200 mg/Kg diet	7.20^{ABC}	14.00^{abc}	32.00^{C}	3.22^{AB}	103.08^{CDE}	45.01^{BCD}	447.70^{BCDE}
Vit. C 400 mg/Kg diet	7.20^{ABC}	13.80^{abc}	33.20^{BC}	3.26^{AB}	108.12^{BCD}	44.96^{BCD}	458.00^{BCD}
Vit. E 10 mg/Kg diet + Vit. C 200 mg/Kg diet	7.40^{AB}	14.00^{abc}	34.20^{ABC}	3.38^{A}	115.64^{ABC}	47.36^{AB}	479.40^{BC}
Vit. E 10 mg/Kg diet + Vit. C 400 mg/Kg diet	7.80^{AB}	14.10^{ab}	33.60^{ABC}	3.28^{AB}	110.22^{BCD}	46.32^{ABCD}	472.30^{BCD}
Vit. E 20 mg/Kg diet + Vit. C 200 mg/Kg diet	8.00^{AB}	14.10^{ab}	36.40^{AB}	3.32^{AB}	121.06^{AB}	46.74^{ABC}	513.00^{AB}
Vit. E 20 mg/Kg diet + Vit. C 400 mg /Kg diet	8.20^{A}	14.78^{a}	37.60^{A}	3.38^{A}	126.78^{A}	49.94^{A}	555.88^{A}
±SEM	0.33	0.38	1.33	0.06	4.90	1.43	20.61

[T] *Pooled SEM*

a.....c. and A.... E. values in the same column within the same item followed by different superscripts are significantly different (at P ≤0.05 for a to c: P ≤0.01 for A to E).

There was an increase in anti-infectious bronchitis virus titer with increasing Vit. E to 25 IU/kg of added Vit. E. Antibody levels were higher than the positive control provided by the manufacturer (indicating effective vaccination) for diets with 25, 50, 100, and 200 IU added Vit. E /kg and lower for 0 and 10 IU added Vit. E /kg.

Zhang *et al.* (2009) found differences with respect to anti-avian influenza virus antibody titers, where, significant ($P \leq 0.05$) between group alpha-tocopherol treatments at 28 day. Anti-avian influenza virus antibody titers were 4.67, 4.33, 4.67 and 4.83 for control and supplementation at 10 mg/kg, 30 mg/kg and 50 mg/kg respectively at 16 day old chicken and 6.50, 6.33, 6.83 and 7.33 for control and supplementation at 10 mg/kg, 30 mg/kg and 50 mg/kg at 28 day old chicken.

As shown in Table (3) type of vitamins supplementation and all dietary treatments to El-Salam laying hens diets increased ($P \leq 0.01$) hemoglobin (Hb), hematocrit, red blood cells count (RBCs), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Its clear that laying hens fed control diet had lower Hb, hematocrit, RBCs, MCV, MCH and MCHCS, whereas, those fed diet containing 20 mg Vit. E and 400 mg/kg diet Vit. C had higher Hb, hematocrit, RBCs, MCV, MCH and MCHCS, during the experimental period (Table 3).

The obtained results in the present study are in agreement with Ajakaiye *et al.* (2010) who found that Hb, MCV, MCH and MCHC were significantly increased ($P \leq 0.001$) when birds supplemented with Vit. C and E individually or in combination (150 mg Vit. C and 150 mg Vit. E) as compared to the control group.

Data in Table (4) indicated that there were significant ($P \leq 0.05$) decrease in body temperature with addition of Vit. E, C and their mixture than the control group, while, insignificant ($P \geq 0.05$) effects were observed in respiratory rate. Numerically, laying hens fed diet containing Vit. E, C and their mixture had lower respiratory rate value while, those fed control diet had higher respiratory rate (the difference is not significant) during the experimental period.

Table 4 shows that there were significant ($P \leq 0.01$) in blood WBC's count, H, L, H/L ratio and eosinophils ($P \leq 0.05$), while, insignificant ($P \geq 0.05$) effects were observed in the other blood constituents being basophils and monocytes as affected by type of addition. Laying hens fed diet containing mixtures of Vit. E and C had higher WBC's, H and H/L ratio and hens fed control diet had higher L and eosinophils.

The obtained results in the present study are in agreement with El-Sebai (2000); Abaza (2002) and El-Sebai (2005), they found that the Vit. E supplementation to the basal diet improved remarkably their hematological parameters. The increase in lymphocyte may be attributed to the production of specific or non-specific antibodies against different antigens, since lymphocytes and heterophils are responsible for achieving the defense mechanism and immune response introduced into body (El-Sebai, 2005). In addition to beneficial effects of Vit. E on cell proliferation, Vit. C enhances lymphocyte proliferation by improving the responsiveness of T lymphocytes to mitogens (Johnston and Huang, 1991) and its antioxidant activity (Jacob, 1995 and Retsky and Frei, 1995). Also, Meydani and Blumberg (1993) and Haq *et al.* (1996) suggested that Vit. E could enhance lymphocyte activity by protecting lymphocytes from lipid oxidation by its antioxidant activity. In another study, supplementation Vit. E improved cell-mediated response, as assessed by the cutaneous basophil hypersensitivity response (Abdukalykova and Ruiz-Feria., 2006) and found that very high levels of Vit. E (400 IU/kg of feed) consistently reduced both humoral and cellmediated immune responses. Bendich *et al.* (1984) reported that the responses of T and B lymphocytes of guinea pigs were enhanced by diets containing higher-than-standard levels of Vit. C and E supplementation. Moriguchi and Muraga (2000) observed that Vit. E improved the immune system by enhancing host antiviral activity and the production of the antiviral cytokine interferon-γ, which is produced by activated T cells.

Results of blood hematological parameters as affected by treatments for El-Salam laying hens are presented in Table 4. Means of WBC's, H and H/L ratio were significantly ($P \leq 0.01$) increased in vitamins treated groups which fed diet containing 20 mg Vit. E and 400 mg/kg diet Vit. C had higher WBC's, H and H/L ratio as compared with those fed control diet, while, those fed control diet had higher L. While, insignificant ($P \geq 0.05$) effects were observed in the other blood constituents being eosinophils, basophils and monocytes (Table 4).

In this respect, Puthpongsiriporn *et al.* (2001) reported that supplemental Vit. E increased the immune response as measured by cutaneous basophil hypersensitivity assay, for some indices (antibody response) and decreased the response for others (mitogen-driven proliferation, and heterophilia).

Table (4): Effects of supplementing laying hens diets with vitamin E, C and their mixtures on other blood constituents of El-Salam laying hens.

Item	Body temperature (C°)	Respiratory rate	White blood cells count (10^3/mm^3)	Heterophils (H)	Lymphocyte (L)	H / L ratio	Eosinophils	Basophils	Monocytes
Type of addition									
Control	42.94^{a}	60.40	13.16^{C}	19.60^{D}	69.60^{A}	0.282^{D}	2.60^{a}	4.00	4.20
Vitamin (Vit.) E	42.89^{ab}	57.50	13.63^{B}	21.80^{C}	67.10^{B}	0.325^{C}	2.30^{ab}	4.30	4.50
Vitamin C	42.85^{ab}	58.90	13.62^{B}	23.70^{B}	66.00^{B}	0.359^{B}	1.80^{b}	4.20	4.30
Mixed (Vit. E &Vit. C)	42.77^{b}	57.85	14.00^{A}	26.05^{A}	64.05^{C}	0.408^{A}	1.75^{b}	4.15	4.00
±SEM[1]	0.4	1.17	0.11	0.54	0.43	0.01	0.21	0.24	0.23
Treatments									
Control	42.94	60.40	13.16^{C}	19.60^{D}	69.60^{A}	0.282^{E}	2.60	4.00	4.20
Vit. E 10 mg/Kg diet	42.86	57.60	13.52^{BC}	21.80^{C}	67.40^{B}	0.323^{D}	2.60	4.00	4.20
Vit. E 20 mg/Kg diet	42.92	57.40	13.74^{AB}	21.80^{C}	66.80^{BC}	0.326^{D}	2.00	4.60	4.80
Vit. C 200 mg/Kg diet	42.88	59.80	13.68^{AB}	23.40^{BC}	66.40^{BC}	0.353^{CD}	1.80	4.20	4.20
Vit. C 400 mg/Kg diet	42.82	58.00	13.56^{BC}	24.00^{B}	65.60^{CD}	0.366^{BC}	1.80	4.20	4.40
Vit. E 10 mg/Kg diet + Vit. C 200 mg/Kg diet	42.76	59.40	13.92^{AB}	24.20^{B}	66.00^{BC}	0.367^{BC}	1.80	4.40	3.60
Vit. E 10 mg/Kg diet + Vit. C 400 mg/Kg diet	42.86	60.00	14.00^{AB}	25.20^{B}	64.20^{DE}	0.393^{B}	1.80	4.40	4.40
Vit. E 20 mg/Kg diet + Vit. C 200 mg/Kg diet	42.70	56.40	13.98^{AB}	27.40^{A}	63.20^{E}	0.434^{A}	1.80	3.80	3.80
Vit. E 20 mg/Kg diet + Vit. C 400 mg /Kg diet	42.74	55.60	14.10^{A}	27.40^{A}	62.80^{E}	0.437^{A}	1.60	4.00	4.20
±SEM	0.06	1.63	0.16	0.67	0.49	0.01	0.31	0.35	0.32

[1]*Pooled SEM*

a,….b, and A,… E, values in the same column within the same item followed by different superscripts are significantly different (at P ≤0.05 for a to b; P ≤0.01 for A to E).

Vit. E have been shown to play a major role in the development and maintenance of the immune system of birds (Tengerdy and Nockels 1973; Neuzil *et al.*, 2007 and Zingg, 2007). Bollengier *et al.* (1999) stated that nutritional deficiencies in Vit. E caused impaired immune function. Vitamin E appears to stimulate immune responses when fed to levels more than the requirement (Yu, 1994 and Bollengier *et al.* 1999).

Abdukalykova and Ruiz-Feria (2006) reported that Vit. E maintained high antibody levels overtime, Vit. E also improved cell-mediated response. In the same experiment, he found that very high levels of Vit. E (400 IU/ kg of feed) consistently reduced both humoral and cellmediated immune responses.

Also, McCorkle *et al.* (1980) reported that ascorbic acid can modulate the activity of B cells, and addition of dietary ascorbate prior to immunization has been found to increase antibody production. Dietary supplementation with ascorbic acid, therefore, may have beneficial effects on immuno responsiveness in chickens. Ascorbic acid has been demonstrated to improve immuno responsiveness and increase disease resistance in chickens by optimizing the functions of the immune system (Rund, 1989). Ostriches receiving Vit. C showed increase in T_3, RBC and L, a decrease in plasma AST, ALT levels, WBC and H\L%, while, did not significantly effect on H (El-Badry *et al.*, 2011).

Semen traits:

Data presented in Table (5) indicated that means of ejaculate volume (ml), sperm concentration ($10^6/mm^3$), total count, Ph and advanced motility% were not significantly influenced by type of Vit. E or C supplementation or by treatments compared with the control group. Numerically, all dietary treatments had higher (the difference is not significant) sperm concentration ($10^6/mm^3$), total count and advanced motility (%) as compared with the control group (the improvement in these sperm characteristics may be due to the role of antioxidants in suppressing or limiting the damaging effects of lipid peroxidation). In this respect, Fraga *et al.* (1991) and Luck *et al.* (1995) concluded that the antioxidant properties of ascorbic acid are essential to maintain membranes and the genetic integrity of sperm cells by preventing oxidative damage to sperm DNA. White Rock roosters fed 100 mg of ascorbic acid/kg of feed showed improved semen volume and sperm concentration (Perek and Snapir, 1963 and Pardue and Thaxton, 1986).

Table (5): Effects of supplementing laying hens diets with vitamin E, C and their mixtures on semen traits of El-Salam laying hens.

Item	Ejaculate volume (ml)	Sperm concentration ($10^6/mm^3$)	Total count (10^6)	Ph	Advanced motility (%)
Type of addition					
Control	0.30	12.80	38.40	7.33	76.67
Vitamin (Vit.) E	0.30	17.37	51.27	7.35	80.00
Vitamin C	0.40	14.97	59.97	7.22	85.00
Mixed (Vit. E &Vit. C)	0.40	16.68	66.30	7.29	81.67
±SEM[1]	0.04	1.55	7.20	0.13	2.38
Treatments					
Control	0.30	12.80	38.40	7.33	76.67
Vit. E 10 mg/Kg diet	0.30	18.93	54.07	7.37	80.00
Vit. E 20 mg/Kg diet	0.30	15.80	48.47	7.33	80.00
Vit. C 200 mg/Kg diet	0.40	15.00	60.87	7.40	83.33
Vit. C 400 mg/Kg diet	0.40	14.93	59.07	7.03	86.67
Vit. E 10 mg/Kg diet + Vit. C 200 mg/Kg diet	0.37	16.00	60.00	7.33	76.67
Vit. E 10 mg/Kg diet + Vit. C 400 mg/Kg diet	0.43	15.60	66.13	7.00	86.67
Vit. E 20 mg/Kg diet + Vit. C 200 mg/Kg diet	0.47	17.60	80.67	7.40	86.67
Vit. E 20 mg/Kg diet + Vit. C 400 mg /Kg diet	0.33	17.47	58.40	7.43	76.67
±SEM	0.05	2.38	10.69	0.18	2.94

[1] *Pooled SEM*

While, these results disagree with those of previous findings obtained by El-Saadany (2002); Abdel

Galil and Abdel Samad (2004) and El-Sebai (2005) with chicken, they reported that supplementation of Vit. E has been show to significantly increase total sperm output, semen volume, percentages of sperm motility and sperm concentration, while dead spermatozoa and sperm abnormalities were significantly lower in males treated with Vit. E than untreated one. In addition, it's well known that Vit. E deficiency caused male sterility and degeneration of testis. In fact, peroxidative damage to spermatozoa is believed to be a major cause of male sub-fertility (Aitken, 1994 and Sikka *et al*, 1995).

Thus, the viability and fertilizing ability of spermatozoa are highly dependent on the expression of an effective antioxidant capacity by these cells and in the surrounding seminal plasma.

Moreover, Shamberger (1983) found that adding Vit. E has a direct effect on pituitary gland and gonads activity. They protect of these glands against the oxidizing agents which cause denaturation, necrosis and or interfere with lipid transport by modifying the cell membrane permeability (Damron *et al.*, 1981).

According to Tengerdy *et al.* (1984) the improvement in the reproductive performance of buck rabbits fed diet supplemented with Vit. E may be due to the biological effect on enzymatic oxidation and reduction, nucleic acid metabolism and promoting the activity of oxidized substances or to the prevention of the oxidative breakdown of cell membranes associated with hydroperoxides of polyunsaturated fatty acid (Hughes, 1999). The improvement of semen characteristics could be attributed to the effect of Vit. E in maintaining the viability and permeability of cell membrane (Nour El-Din, 2000). Aitken and Clarkson (1988) reported that lipid-soluble antioxidants (such as Vit. E) can be permeate plasma membranes and suppress the free radical damage. The most obvious mechanisms by which Vit. E affect reproduction may be due to their antioxidant role in protecting the reproductive tissue from oxidative degeneration (Freeman and Crapo, 1982 and Khalil *et al*, 2005).

CONCLUSION

The results of this study indicated that feeding El-Salam laying hens on diets containing 20 mg Vit. E and 200 mg/kg diet Vit. C improved immune response against avian influenza and some physiological parameters.

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تأثير إضافة مستويات مختلفة من فيتامين ج ،هـ لعلائق الدجاج البياض على 2- الاستجابة المناعية للتحصين ضد مرض أنفلونزا الطيور وبعض المقاييس الفسيولوجية.

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اجريت هذه الدراسة بمحطة بحوث الدواجن بالعزب-الفيوم- مصر لدراسة تأثير استخدام مستويين من فيتامين هـ (10 او20 ملليجرام/كجم عليقة) و فيتامين ج (200 او400 ملليجرام/كجم عليقة) وخليطهما علي، منحني مرض الانفلوانزا، بعض مكونات بلازما الدم ، خصائص السائل المنوي لدجاج السلام البياض. استخدم عدد 243 (216 أنثي و27 ديك) طائر عمر 25 أسبوع قسمت عشوائيا إلي 9 معاملات متساوية 27 طائر/كل معاملة (24 أنثي و3 ذكر) ثم قسمت كل معاملة إلي 3 مكررات 11 طائر/مكرر(8 إناث و 1 ذكور).

وكانت المعاملات التجريبية كما يلي :

1- تغذية الطيور علي عليقة الكنترول (م1).
2- م1 مضاف إليها 10 ملجم/كجم عليقة فيتامين هـ.
3- م1 مضاف إليها 20 ملجم/كجم عليقة فيتامين هـ.
4- م1 مضاف إليها 200 ملجم/كجم عليقة فيتامين ج.
5- م1 مضاف إليها 400 ملجم/كجم عليقة فيتامين ج.
6- م1 مضاف إليها 10 ملجم/كجم عليقة فيتامين هـ+200 ملجم/كجم عليقة فيتامين ج.
7- م1 مضاف إليها 10 ملجم/كجم عليقة فيتامين هـ+400 ملجم/كجم عليقة فيتامين ج.
8- م1 مضاف إليها 20 ملجم/كجم عليقة فيتامين هـ+200 ملجم/كجم عليقة فيتامين ج.
9- م1 مضاف إليها 10 ملجم/كجم عليقة فيتامين هـ+400 ملجم/كجم عليقة فيتامين ج.

وتتلخص أهم النتائج المتحصل عليها فيما يلى:

1- كان الدجاج المغذي علي عليقة المقارنة أعلي في نسبة الألبيومين وأنزيم AST بينما الدجاج المغذي علي عليقة تحتوي علي20 ملجم/كجم عليقة فيتامين هـ و400 ملجم/كجم عليقة فيتامين ج اقل نسبة الألبيومين وأنزيم AST.

2- تحسنت الاستجابة المناعية للتحصين ضد مرض أنفلونزا الطيور بإضافة فيتامين هـ ، ج أو مخلوطهما عند مقارنتها بالتي تغذت علي العليقة الضابطة.

3- كان للدجاج المغذي علي عليقة تحتوي علي20 ملجم/كجم عليقة فيتامين هـ و400 ملجم/كجم عليقة فيتامين ج اعلي في نسبة الهيموجلوبين ، المكونات الخلوية، عدد كرات الدم الحمراء، MCV, متوسط حجم الخلايا، متوسط وزن الهيموجلوبين فى الكرات الحمراء خلال فترة التجربة.

4- كان هناك انخفاض في درجة حرارة جسم الدجاج نتيجة لإضافة فيتامين هـ ، ج أو مخلوطهما عن عليقة المقارنة، لم يكن هناك أي تأثير معنوي علي معدل التنفس.

5- كان للدجاج المغذي علي عليقة تحتوي علي 20 ملجم/كجم عليقة فيتامين هـ و400 ملجم/كجم عليقة فيتامين ج اعلي في عدد كرات الدم البيضاء، الخلايا اللمفاوية، وHeterophils/Lymphocyte ratio ، بينما كان للدجاج المغذي علي عليقة المقارنة أعلي Heterophils.

6- لم يكن هناك أي تأثير معنوي لنوع الإضافة أو المعاملة علي حجم القذفة وتركيز الحيوانات المنوية، والعدد الكلي، رقم الأس الايدروجيني ، الحيوية عند مقارنتها بمجموعة المقارنة.

ومن ذلك يمكن استنتاج أن تغذية دجاج السلام علي عليقة تحتوي علي 20 ملجم/كجم عليقة فيتامين هـ و400 ملجم/كجم عليقة فيتامين ج أدى إلي تحسين الاستجابة المناعية للتحصين ضد مرض أنفلونزا الطيور.