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Enhancement of Broiler Performance and Immune Response by α-Tocopherol Supplemented In Diets

[1]Azza A. Attia, [2]A. E. Abou-Zeid, [3]F. F. Mohamed and [4]Haitham M. Yakout
[1]Zoology Department, Faculty of Science, Alexandria University, Egypt
[2]Animal and Poultry Production Department, Faculty of Agriculture, Tanta University, Alexandria Egypt
[3] Department of Nutrition and Clinical Nutrition,
Faculty of Veterinary Medicine, Cairo University, Egypt
[4]Poultry Produciton Department, Faculty of Agriculture, Alexandria University, Egypt

Abstract: The impact of different levels of dietary vitamin-E supplementation on broiler production and immunity was investigated. One hundred and sixty birds were randomly assigned to four equal groups of forty birds each. Birds were fed starter and finisher diets supplemented with different levels of vitamin-E (0, 400, 800 or 1200 mg/kg diet). Statistical differences among treatments were noted for body weight at the sixth week of age, which increased significantly ($P < 0.05$) by elevating vitamin-E levels, while body weight gain, feed consumption and feed conversion were not influenced by dietary treatments. The white blood cells (WBC's) counts increased significantly ($P < 0.001$) with elevating the vitamin-E levels, whereas, the red blood cells (RBC's), hemoglobin (Hb), mean corpuscular volume (MCV) and packed cell volume (PCV%) were not statistically affected by any of the treatments. The immunological parameters were significantly ($P < 0.001$) affected by dietary treatments. Increasing level of vitamin-E caused certain structural alterations in thymus gland rather than bursa of Fabricius comparing to that of low levels.

Key words: Vitamin-E, broiler, immunity, dietary treatments.

Introduction

High intensity poultry production requires fast growing strains, usually at high stocking densities. With this type of husbandry, flocks are highly susceptible to infectious agents, as a result of reduced immune potential (Van der Zijpp, 1983; Lamont and Dietert, 1990). NRC (1994) values for vitamin requirements represent minimum values and do not contain safety margins to protect against deficiency. The relevance of these values to the modern commercial poultry production might be questioned. There is a lack of accurate data on the content and the availability of vitamins from natural ingredients. It has been documented that vitamin-E enhances the performance of broiler under commercial conditions (Kennedy *et al.*, 1992), enhances the immune system in both animal and human studies (Tengerdy and Nockels, 1975, Nockles *et al.*, 1976; Bendich *et al.*, 1986; Bendich, 1993, Meydani *et al.*, 1994, 1997). Furthermore, vitamin-E increases the immune response in chickens (Franchini *et al.*, 1995) and turkeys (Franchini *et al.*, 1990). Vitamin-E (α-tocopherol) acts as an antioxidant in cellular membranes and as a free radical scavenger by blocking the peroxidation of polyunsaturated fatty acids (Osi, 1977).

Research indicated that vitamin level much higher than the requirements have beneficial effects. Higher inclusion of vitamin-E increases the storage life of poultry carcasses (Sheehy *et al.*, 1993). The nutritional and, within it, the antioxidant status of an animal exerts a significant influence on the host defense mechanism system (Finch and Turner, 1996).

As the immunity to pathogenic and non - pathogenic organisms, conferred upon an animal by its immune system, requires the cooperation of many different cell types including B - lymphocytes (bursa derived) and T - lymphocytes (thymus derived). These cells interact during an immune response to form the cellular and hormonal arms of the immune system (Hayek *et al.*, 1996).

The studies reported here have thus sought to assess the effect of different levels of dietary vitamin-E supplementation on broiler's performance, hematological, immunological as well as on the histological and ultrastructural response of immune tissues to these levels.

Materials and Methods

A total of 160 commercial, one-day-old Hubbard chicks were wing banded and randomly allocated into four groups each of forty birds. Chicks were floor reared in four electrically heated rooms (10 birds/m^2). Rooms had a continuous illumination schedule with which feed and water were provided for *ad libitum* consumption. The experimental treatments consisted of four levels of vitamin-E (0, 500, 1000 or 1500 mg/kg diet) of which were added to an unmediated starter and finisher diets serving as basal diets (Table 1). The experimental period lasted for 6 weeks.

Table 1: Composition and calculated analysis of the experimental diets.

Ingredients	(%)	
	Starter (1-21 days)	Finisher (22-2days)
Yellow corn, ground	63.30	74.50
Soybean meal (44%)	31.00	19.80
Meat and Bone meal (60%)	3.00	3.00
Bone Meal	1.60	1.60
Calcium Carbonate	0.38	0.38
Salt (NaCl)	0.35	0.35
Vitamin and mineral Premix[1]	0.30	0.30
DL-Methionine	0.07	0.07
Total	100.00	100.00
Calculated analysis:		
ME. kcal/kg	2910.00	2994.00
Crude Protein	21.00	17.10
C/P ratio	138.00	175.00

[1]Nutrients supplied per kilogram premix: Vitamin-A 7700 IU; Vitamin D_3 1650 ICU; Vitamin-E 5mg; Vitamin K_3 30mg; Vitamin B_2 4.5mg; Niacin 28mg; Pantothenate 6.6mg; Vitamin B_1 15mg; Folic acid 0.44mg; Biotin 75mg; Choline 60mg; Vitamin B_{12} 9mg; Zinc 60mg; Selenium 1mg; Manganese 75mg; Copper 4mg; Iron 40mg; Ethoxyquin 62 mg and Iodine 1 mg.

Performance parameters: Weekly body weights were recorded, from which individual body weight, feed consumption and feed conversion were calculated.

Table 2: Effect of dietary vitamin-E on body weight development in broiler chicks

Vitamin-E (mg/Kg)	Age						
	Day	1st wk	2ed wk	3ed wk	4th wk	5th wk	6th wk
Control	46.08 ± 0.68	120.26 ± 2.26	270.70 ± 1.80	430.30 ± 9.42	710.78 ± 16.76	950.20 ± 12.12	1458.80 ± 2.98 ^{a}
500	46.79 ± 0.56	130.30 ± 1.82	280.92 ± 1.25	435.27 ± 4.51	743.08 ± 9.28	988.82 ± 8.36	1505.80 ± 16.44 ^{b*}
1000	44.66 ± 0.58	136.05 ± 1.64	291.21 ± 2.34	433.33 ± 12.98	755.04 ± 14.56	986.22 ± 22.31	1512.13 ± 9.68 ^{b*}
1500	44.68 ± 0.72	129.34 ± 1.61	294.47 ± 1.84	488.15 ± 6.80	757.28 ± 22.82	964.35 ± 12.98	1520.10 ± 11.56 ^{b*}

Values in the same column with different superscripts vary significantly to control at $P<0.05$

Table 3: Effect of dietary supplementation of vitamin-E on production performance of broiler chicks.

Parameter	Vitamin-E.			
	Control	500 mg/kg	1000 mg/kg	1500 mg/kg
Initial body wt. (g.)	46.08	46.79	44.66	44.68
Final body wt. (g.)	1458.80	1505.80	1512.13	1520.10
Total gain (g.)	1412.72	1459.01	1467.47	1475.42
Feed consumption	3418.78	3209.82	3111.03	3098.38
Feed conversion (g/g)	2.42	2.20	2.12	2.10

Table 4: Effect of dietary supplementation of vitamin-E on HI titer as well as wattle reaction of broiler chicks

	Vitamin-E.			
	Control	500 mg/kg	1000 mg/kg	1500 mg/kg
HI Titer	39.6^{a} ± 14.30	96.0^{b**} ± 33.73	98.4^{b**} ± 30.26	109.6^{b**} ± 31.70
Wattle reaction (mm) 24 hr. after inoculation	2.2^{a} ± 0.66	3.72^{b*} ± 0.58	3.7^{b*} ± 0.80	3.65^{b*} ± 0.90

Table 5: Effect of dietary supplementation of vitamin-E on some hematological values of broiler chicks.

	Vitamin-E.			
	Control	500 mg/kg	1000 mg/kg	1500 mg/kg
RBCs (X 10^{6}/mm^{3})	1.564 ± 0.12	1.726 ± 0.002	1.974 ± 0.18	1.914 ± 0.009
WBCs (X 10^{3}/mm^{3})	56.13^{a} ± 1.1	65.32** ± 2.0	64.18^{b**} ± 1.40	70.72^{b**} ± 1.83
Hgb g/dl	9.4 ± 0.35	10.70 ± 0.34	9.54 ± 0.28	10.0 ± 0.32
MCV u^{3}	124.4 ± 0.84	128.0 ± 1.42	126.0 ± 1.30	128.0 ± 1.25
PCV %	31.66 ± 2.22	32.46 ± 2.46	31.22 ± 2.16	33.26 ± 1.50

Values are means ± SEM

Values in the same row with different superscripts vary significantly compared to the control group at ** $P < 0.01$

Table 6: Effect of dietary supplementation of vitamin-E on lymphoid organs weight index of broiler chicks.

Organ	Vitamin-E.			
	Control	500 mg/kg	1000 mg/kg	1500 mg/kg
Bursa	13.77^{a} ± 1.21	22.26^{b**} ± 1.23	20.84b** ± 0.59	22.12^{b**} ± 1.45
Thymus	20.95^{a} ± 1.81	31.44^{b*} ± 1.72	30.24b* ± 1.45	32.45^{b*} ± 1.75
Spleen	11.28^{a} ± 0.289	20.33^{b*} ± 2.01	21.49b* ± 1.21	19.85^{b*} ± 1.8

Values are means ± SEM

Values in the same row with different superscripts vary significantly compared to the control group at * $P < 0.05$ & ** $P < 0.01$

Vaccination: Birds were vaccinated with NDV LaSota vaccine on the 14th and 28th days by eye drop installation using standard dropper (0.5 ml/ drop containing $10^{5.8}$ EID_{50}) where each bird received two drops; one drop in each eye.

NDV Vaccine: Lentogenic NDV Hichner B1 vaccine (Izo Vac 1000, Izo laboratories, Izo SPA, Italy) in lyopholyzed form with minimum titer of 10^{9} EID_{50} was used.

Antigen used for HI test: The lentogenic LaSota vaccinal strain of NDV was passed two times in the allantoic sac of 9-11 days old chick embryo before being used. Eight HA units suspended in PBS pH 7.2 were used.

Antigen used for dermal reaction: Inactivated NDV vaccine (Inacti/vac ND broiler 1000 dose. Maine Biological Laboratories Inc., USA) was used.

Blood Samples: At the age of 35 days, 2 ml of blood were obtained via cardiac puncture from each bird in a sterile centrifuge tube containing heparin (20 IU/ml) for counting the cells. In addition, 3 ml of blood was also taken from each bird in sterile tubes for the assessing of HI titer.

Hemagglutination inhibition test (HI): The test was performed according to standard procedure described by Majiyabe and Hitchner (1977).

Dermal reaction: At the end of the experiment, 8 birds from each group were injected; each intradermally at the right wattle with 0.1 ml of killed oil adjucant NDV vaccine. The left wattle was considered as control. Birds were kept under observation and were examined for reactivity at different time intervals. The interpretation of the results was based on the difference between thickness of indurated areas injected and the control wattle in each bird.

Hematological parameters: The count of red blood cells (RBC's), white blood cells (WBC's) as well as determination of hemoglobin (Hb), mean corpuscular volume (MCV) and packed cell volume (PCV) were performed according to Schalm *et al.* (1975).

Tissue collection and histological processing: At the end of

6th week of the trial, the primary lymphoid organs (bursa and thymus) along with the spleen of chicks given high level of vitamin-E were collected and weighed (Table 4). These organs were also processed for histological and electron microscopic preparations at the end of 4th and 6th weeks. Regarding the histological studies, small pieces of these organs were fixed in Bouin's fluid and processed to get 5μm thick paraffin sections. These sections were stained with Erhlish hematoxylin and eosin. As for electron microscopic study, very small pieces of these organs were immediately immersed in 5% glutaraldehyde buffered with 0.1M sodium cacodylate at pH 7.3, then post fixed in 1% osmium tetroxide. Semithin 1μm-thick sections were cut, picked up on glass slides, stained with toluidin blue and examined by light microscope. The block was then trimed around for ultrathin sections and were doubly stained with uranyl acetate and lead citrate and examined under a Joel 100s transmission electron microscope at 60 KV accelerating voltage.

Statistical Analysis: Data were analyzed using one way analysis of variance with Newman- Keuls post test using Graph Pad Software (1999).

Results

Data of body weight development throughout the period of 1-day old to 6-weeks of age are presented in Table (2). Results revealed that with elevating vitamin-E supplementation, there was no treatment effect on body weight till the 5th week of age. By the 6th week increasing vitamin-E had a positive significant effect on body weight development ($P<0.01$). Table (3) illustrates the effect of vitamin-E supplementation on production performance parameters. Results indicate no significant effects of vitamin-E supplementation on body weight gain, feed consumption or feed conversion. However, it is worth to note that although it was not statistically illustrated, increasing vitamin- E level resulted in a greater impact on these performance parameters as compared to the control. Body weight gain and feed conversion data were somewhat consistent, 1200 mg/kg vitamin-E gave the most favorable results.

Results of HI titer as well as data of the wattle reaction to injection of inactivated NDV are presented in Table (4). The higher level of vitamin-E (1200 mg/kg diet) had significant ($P<0.01$) effects on both parameters.

Effects of the added vitamin-E on blood hematological values in broiler chicks are posted in Table (5). These findings show that elevating vitamin-E significantly increased ($P< 0.01$) number of WBC's, while other blood hematological parameters showed no significant treatment effect.

Table (6) showed that the lymphoid organ weights of chicks given 1200 mg/kg diet vitamin-E had significantly ($P<0.01$) higher thymus and bursa index as compared to the control group. This illustrates an indirect response of increasing vitamin-E on the immune system.

Histologically, the present results showed that the low level (400 mg/kg diet) supplementation of the vitamin for four weeks revealed a marked medullary hyperplasia in most bursal sections (Fig. 1). However, the high supplemented level for the same period revealed an increase in the mitotic figures and showed the presence of large phagocytic areas (Fig. 2) as compared to the control (Fig. 3). Moreover, the electron micrographs showed rounded shaped nuclei in most bursal lymphocytes (Fig. 4). In addition, large phagocytic area with cellular debris could be seen (Fig. 4) as compared to the control (Fig. 5).

The results also revealed that, chicks given high level (1200 mg/kg diet) supplementation of the vitamin for 6-weeks resulted in an apparent increase in the mitotic figures in most sections of bursa of Fabricius (Fig. 6). Furthermore, many densely stained cells with toluidine blue of both the cytoplasm and the nucleus were distinguished from the bursal lymphocytes (Fig. 6). The electron micrographs revealed large macrophgic cells among the normal shaped lymphocytes (Fig. 7) as compared to the control (Fig. 5).

The most noticeable histological appearance in sections of thymus at 4-weeks old treated chicks with low supplemental level of vitamin-E is that the lymphocytes became densely stained and aggregated into clusters (Fig. 8) when compared to the control (Fig. 9). In addition, the same level of vitamin for 6- weeks showed an increase in both number and size of Hassal's corpuscles (Fig. 10) in most thymic sections. However, the electron micrographs of those given high supplemented vitamin-E showed marked irregularities in the nuclear outlines of most thymic lymphocytes and an increase in size of Hassal's corpuscles (Fig. 11) as compared to the control (Fig. 12).

Furthermore, the light microscopic examination of chicken's spleen sections of the high vitamin-E level revealed an increase in the number of germinal centers (Fig.13) as compared to control (Fig. 14).

Discussion

Data of body weight development throughout the period of 1- day old to 6-weeks of age are compatible with the work of Abawi *et al.* (1985) who observed an enhancing effect of increasing vitamin-E levels on body weight and body weight gain in broiler chicks. Similar results were also reported by Colango *et al.* (1984), as supplementing chicks diets with vitamin-E resulted in an increase in body weight gain and reduction in mortality.

The elevation of vitamin-E (400 to 1200 mg/kg diet) supplementation had a significant increase in body weight and numerically improved feed consumption and feed conversion. These increases could possibly be explained on the basis of enhanced solubility of vitamin-E and nutrients in the mycellar phase (Hollander, 1981), delayed gastric emptying as a result of fat content in the diets (Nishigaki *et al.*, 1976) and also the enhanced efficiency of absorption and transport of vitamins with moderate dietary fat used in the diets.

The histological results suggest that both low (400 mg/kg diet) and high (1200 mg/kg diet) levels of vitamin-E tend to improve the proliferative activities of bursal tissue as a result of the increase in mitotic figures and the relevance of the medullary hyperplasia. Meydani and Beharka (1996) stated that vitamin-E supplementation to retrovirus-infected mice significantly restored T and β cells proliferation.

Furthermore, high dose supplementation of vitamin-E at 6-weeks old chicks was accompanied by an increase in the level of the immunological parameters (HI titer and Wattle reaction), indicating the higher activity of the immune system. Heinzerling *et al.* (1974) found that the mortality of mice infected with *Diplococcus penumonia* Type I decreased from 80 to 20 % after vitamin-E supplementation. They have reported that the protective effect of vitamin-E is associated with higher antibody titer, increased plaque forming units and higher phagocytic activity. Also, Topika *et al.* (1989) found that the addition of α- tocopherol (0.2ìmol/L) to human lymphocytes *In vitro* suppressed lipid peroxidation and oxidant damage to DNA.

At the medullary regions, the results indicated an increase in the number of cells having intensive affinity for toluidine blue stain; referred to as the secretory cells. This was apparent in the bursal tissues of chicks given high level vitamin for 6 weeks. Olah and Glick (1987) suggested that these cells exert

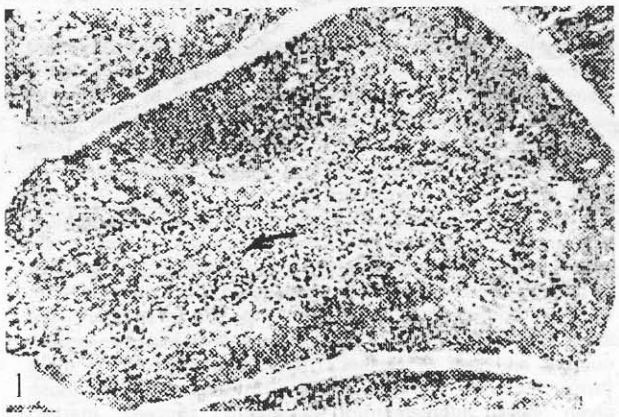

Fig. 1: Section of bursa of 4-weeks old broiler given low level vitamin E, showing a marked medullay hyperplasia (arrow). H & E, (X400).

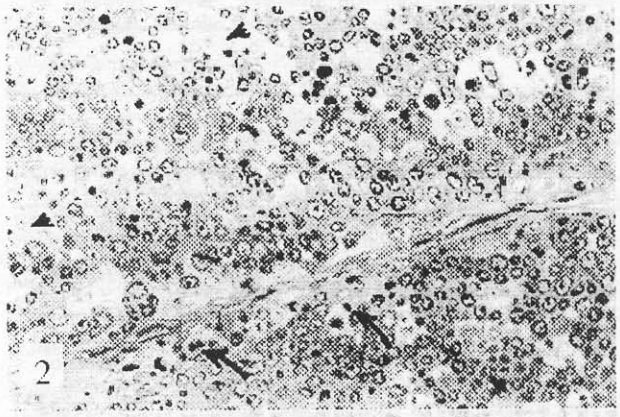

Fig. 2 : Semithin section of bursa of 4 - weeks old chicks fed diet supplemented with high level vitamin E, showing an increase in the mitotic figure (arrows) in the cortical region (c); phagocytic areas (arrowhead). Toluidine blue, (X1000).

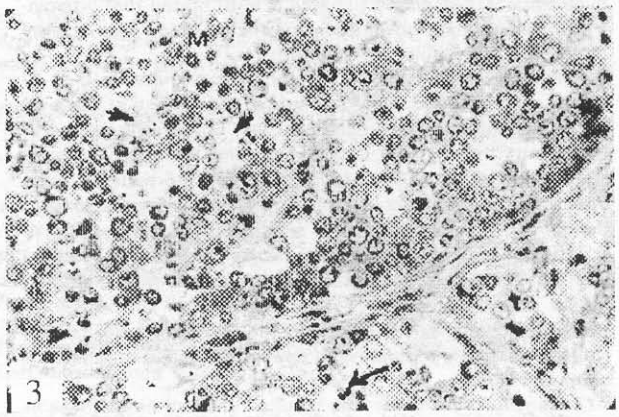

Fig. 3 : Semithin section of control bursa of 6-weeks old chicks, showing certain mitosis (arrow) in cortex (c); medulla (M); arrowheads point at phagocytic areas . Toluidine blue, (X1000).

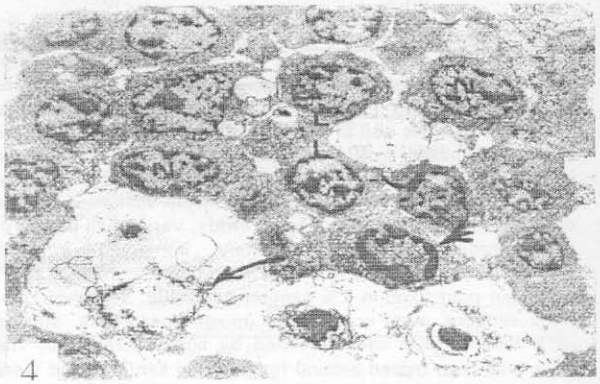

Fig. 4 : Electron micrograph of bursa of 4 - weeks old chicks fed diet supplemented with high dose vitamin-E; Notice: a large phagocytic area with cellular debris (arrow), bursal lymphocytes (L) having round shaped nuclei (N), arrowhead points at a dividing cell (X10000).

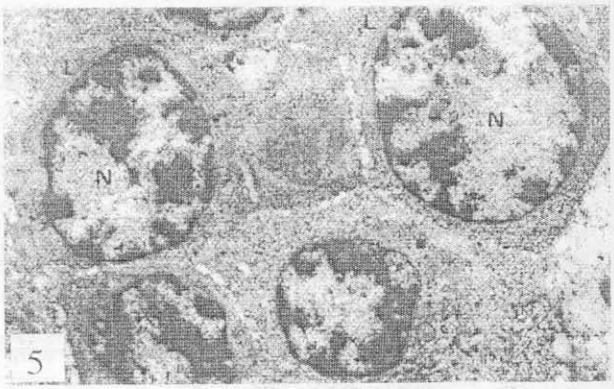

Fig. 5 : Electron micrograph of bursa of 4-weeks old control chicks, showing group of bursal lymphocytes (L) containing round shaped nuclei (N). (X5000).

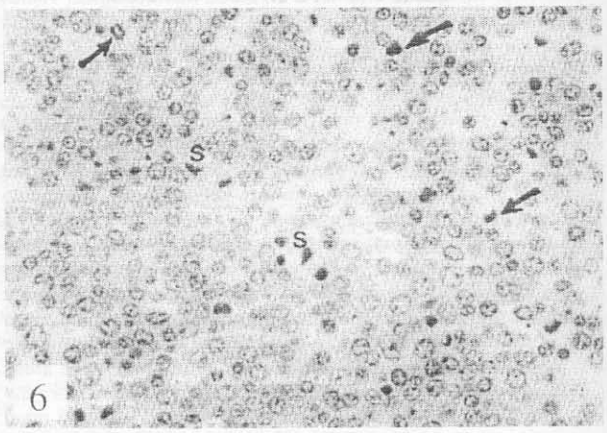

Fig. 6 : Semithin section of bursa of 6 - weeks old chicks fed diet supplemented with high dose vitamin E, showing an increase in mitotic figure (arrows); an intensive affinity of secretory cells (S) to stain. Toluidine blue, (X1000).

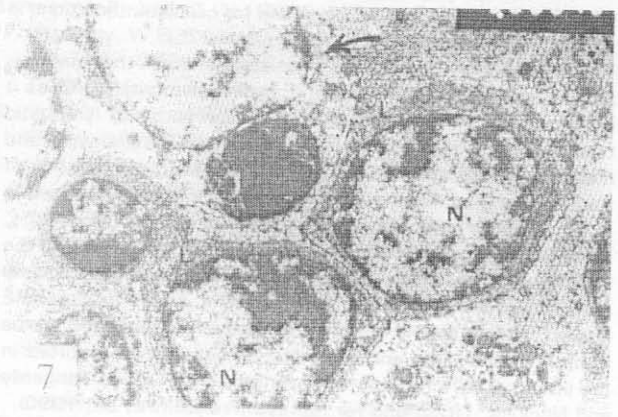

Fig. 7 : Electron micrograph of bursa of 6 - weeks old treated chicks given high level vitamin- E, showing normal rounded shaped appearance of bursal lymphocytes (L) with rounded nuclei (N); phagocytic cell (arrow). (X5000).

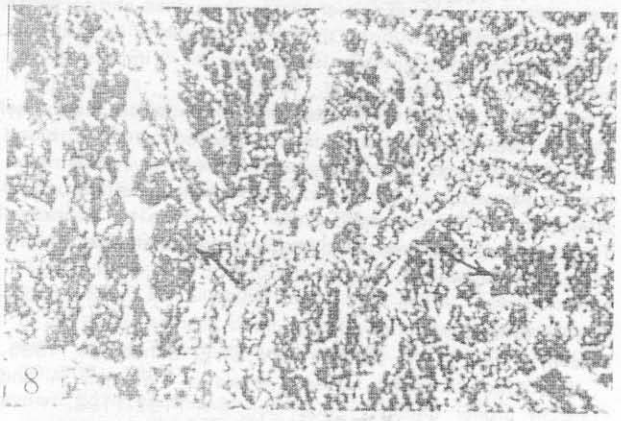

Fig. 8 : Section of thymus of 4- weeks old chicks fed diet supplemented with high level vitamin-E showing cluster of densely stained (arrows) aggregated lymphocytes (arrows) in thymic lobules. H&E, (X600).

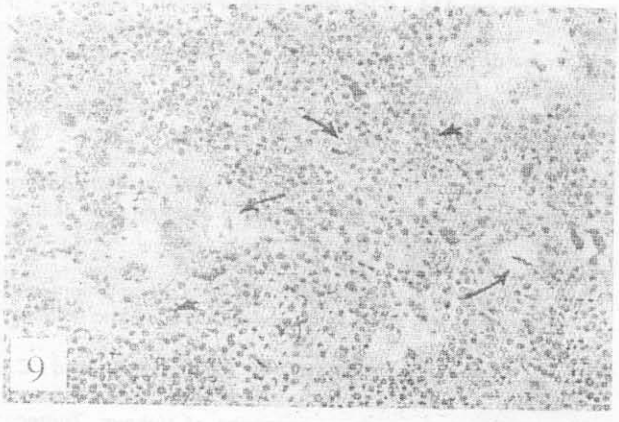

Fig. 9 : Semithin section of thymus of 6 - weeks old broilers fed diet supplemented with high level vitamin-E, showing an increase in number and size of Hassal's corpuscles (arrows); thymic lymphocytes (arrowheads). Toluidine blue, (X400).

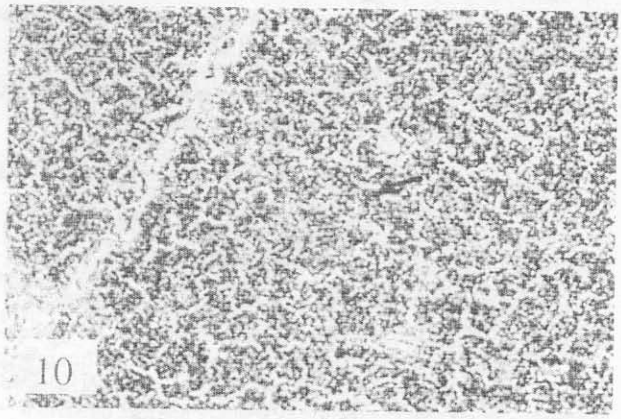

Fig. 10 : Section of the control thymus of 6-weeks old broiler showing, thymic lymphocytes (arrows). H&E, (X600).

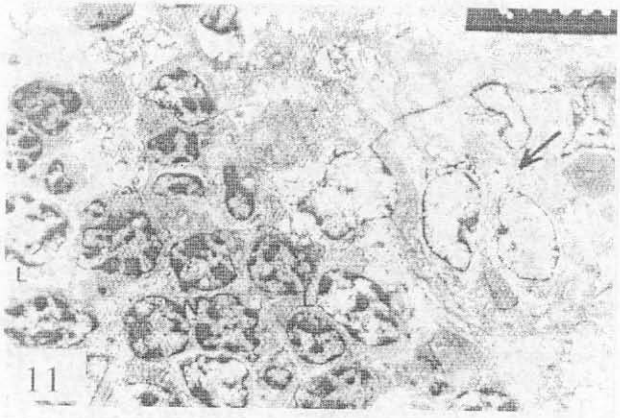

Fig. 11: Electron micrograph of thymus of 6 - weeks old broilers fed diet supplemented with high level of vitamin-E, showing marked irregular outlines of nuclei (N) of thymic lymphocytes (L); large sized Hassal's corpuscle (arrow). (X2700).

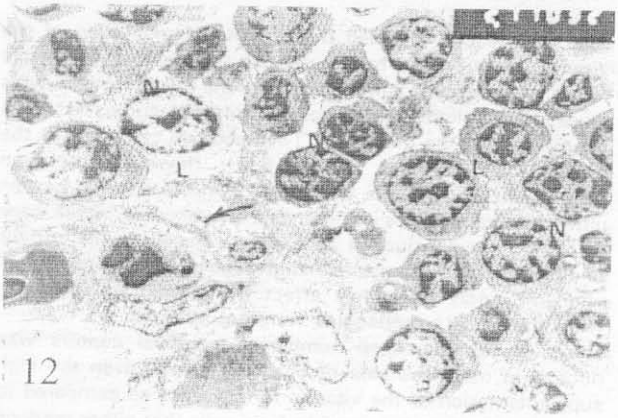

Fig. 12 : Electron micrograph of control thymus of 6 - weeks old broiler, showing the regular rounded shaped nuclei (N) of thymic lymphocytes (L); Hassal's corpuscle (arrow). (X2700).

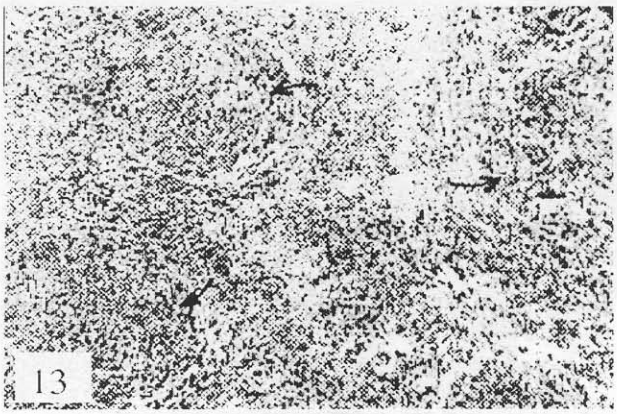

Fig. 13 : Section of spleen of 6-week old broiler fed diet supplemented with high level vitamin-E, showing the presence of germinal centers (arrows). H&E, (X400).

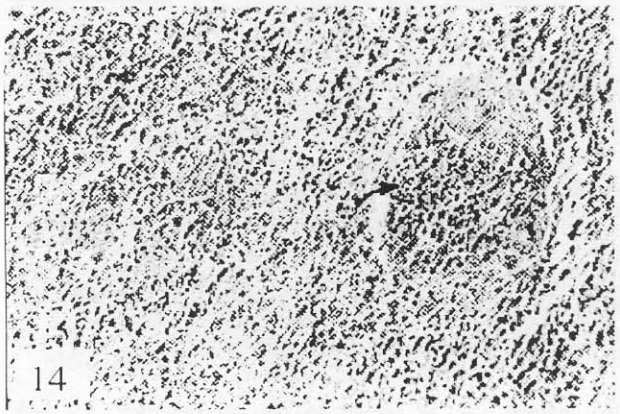

Fig. 14: Section of control spleen of 6 - weeks old chicks showing a germinal center (arrow). H&E, (X400).

an endocrine function in the regulation of induction of α-cells in bursa.

Also, the results revealed that low supplementation of the vitamin for 4-weeks had increased the number of thymic Hassal's corpuscles, although most of the thymic lymphocytes were structurally appeared normal. Hassal's corpuscles have been known to be the site of T-lymphocyte cell death in the medulla (Gartner and Hiatt, 1997). However, the high supplementation of the vitamin at 6-weeks showed certain ultrastructural changes in the thymic lymphocytes, which were of highly irregular nuclear appearance. Based on the current findings, it was suggested that high supplementation of vitamin-E may negatively affect the thymic lymphocytes, while the bursal lymphocytes were not affected.

Moreover, an increased number of germinal centers was noticed in most sections of chick's spleen given the high supplementation of the vitamin for 6 weeks as compared to the control. Spleen is known to form cells that either produce antibodies or activated the cell mediated type of immunological reactions (Gartner and Hiatt, 1997). Zapata (1982) had explained the germinal centers in the spleen of teleost are clusters of macrophages, lymphocytes and plasma cells, where cellular interactions can occur. In mammals, similar clusters were considered the possible regions for cellular interactions in the immune response (Farr and Bruyn, 1975).

The present results are in consistance with the previous studies, suggesting that vitamin-E is similar to vitamin C, as it increases the immune and phagocytic response of lymphatic tissue which is manifested by the action of lymphocytes and macrophages (Krstic, 1991). In this study, supplementation of the diets with increasing levels of dietary vitamin-E resulted in a significant continous increase in the number of WBC's, revealing the highly phagocytic function of WBC's, killing the infectious organisms. These results suggest that plasma constitutes are somewhat sensitive to changes in vitamin-E intake. Thus, it seems feasible to improve the vitamin-E status in broiler especially; and in poultry in general; as reported in the literature for chickens (Heinzerling *et al.*, 1974; Tengerdy and Nockels, 1975) and for turkeys (Franchini *et al.*, 1990).

To that day, many scientists have interest about the mechanism of the immunostimulatory effect of vitamin-E. however, there is a compelling evidence suggesting that vitamin-E exerts its immuno enhancing effect by either decreasing free radical formation (Corwin and Schloss,1980) or stimulating helper T-cell activity, which stimulates the immunity by increasing carrier antigen response and facilitate the immunoglobulin M (IgM) to immunoglobulin G (IgG) shift in antibody synthesis, this ends up by improving the birds immunity (Tanaka *et al.*, 1979).

In conclusion, vitamin-E supplementation in broiler diets may benefits broiler performance, increases body weights, blood hematological and immunological parameters. Furthermore, high supplementation of vitamin-E may cause certain structural alterations in thymus gland rather than bursa of Fabricius. These findings indicate that immune responses are directly affected by vitamin-E.

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