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# Effect of Vitamin E Supplementation and Stocking Density on Broiler Performance, Carcass Traits and Histological Responses of Lymphoid Organs

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## ABSTRACT

This study was undertaken to evaluate the effect of dietary supplementation with Vitamin E (VE) and stocking density on growth performance and histological responses of broiler chicks. Cobb-500 3 day old chicks were randomly distributed to eight equal groups, each of which contained four replications. An experiment with a factorial arrangement of treatments (4×2), 4 levels of VE (0.0, 200, 300 and 400 mg $kg^{-1}$ diet) and 2 levels of stocking density (11.9 birds $m^{-2}$ as the normal stocking density or 16.66 birds $m^{-2}$ as the high stocking density). Apart from the effect of stocking density, dietary supplementation with VE did not affect Live Body Weight (LBW), Body Weight gain (BW), Feed Intake (FI), Feed Conversion Ratio (FCR), carcass traits or lymphoid organs examined. The high stocking density had a negative effect on LBW, BWG and FI but FCR and carcass traits were not affected compared with the normal stocking density, irrespective of the effect of added VE. Dietary supplementation with VE enhanced the activity of spleen, bursa and thymus to produce many lymphocytes that help improving the immunity of birds. The high stocking density, applied herein, exerted a negative effect on the histology of lymphoid organs which may cause low immune responses. The results of the present study shown that supplemental dietary VE could improve the histological responses of lymphoid organs in broiler chickens.

**Key words:** Vitamin E, stocking density, carcass yield, lymphoid organs, histology, broiler

## INTRODUCTION

The lymphoid organs plays an important role in the defense mechanisms against microorganisms. The chicken has central lymphoid tissues (thymus and bursa of Fabricius) and spleen (Getty, 1975; Akter *et al.*, 2006). The lymphoid system of chicken consists of unique organs and divided into two morphologically and functionally distinct components (Cooper *et al.*, 1966; Akter *et al.*, 2006). The thymus dependent component is represented by the smaller lymphocytes and is responsible for cell mediated immunity, including immune surveillance (Janeway *et al.*, 1988), whereas, the bursa-dependent component is represented by the larger lymphocytes which play an important role in humoral immunity. Concerning this immunological point of view, the histology of the lymphoid tissues of the chicken is very important.

Vitamin E plays an important biological role as a chain-breaking lipid antioxidant and free radical scavenger in the membranes of cells and subcellular organs. Apart from its protective effect on lipid peroxidation, the immuno-regulatory effects of vitamin E on humoral and cell-mediated immunity are well known (Raza *et al.*, 1997; Gu *et al.*, 1999; Rajak, 1999; Niu *et al.*, 2009).

Stocking density has major economic implications for the broiler industry as higher profits can be obtained when more animals are housed under one roof. However, many producers around the world need to increase stocking density to maximize profitability. A high stocking density negatively affects performance, welfare, immunity and gut health (Heckert *et al*., 2002; Thaxton *et al*., 2006; Estevez, 2007). The present study was designed to evaluate the effects of dietary supplementation with Vitamin E as one of major antioxidants on performance, carcass traits and histological observation of lymphoid organs of broiler chicks kept at two stocking densities.

## MATERIALS AND METHODS

The experimental study of the present study was carried out in the Poultry Production Farm; Kalabsho Center of Agricultural Research and Experiments, Faculty of Agriculture, Mansoura University, Egypt from May to June.

**Birds, management and experimental design:** Cobb-500 broiler chickens (n = 192), 3-day-old, were divided into eight treatments, each of which included four replicates. An 4×2 experiment with factorial arrangement of treatments, 4 levels of Vitamin E (VE) supplementation and 2 stocking densities (5 or 7 birds/cage). The VE was added to the basal diet at levels of 0.0, 200, 300 and 400 mg $kg^{-1}$ diet to obtain dietary VE of 18.84, 218.84, 318.84 and 418.84 mg $kg^{-1}$ diet for the starter period, respectively. The dietary supplemental levels of VE used during the grower period were 19.15, 219.15, 319.15 and 419.15 mg of VE $kg^{-1}$ diet, respectively. Birds were reared in battery cages with cage dimensions of 70 cm length, 60 cm width and 40 cm height. Thus, the cage floor area was 0.42 $m^2$. The stocking density of 11.90 birds $m^{-2}$ (5 birds per cage) was considered the normal density and 16.66 birds $m^{-2}$ (7 birds per cage) as the high density. The daily ambient temperature inside the farm was 32°C in the first week and then gradually reduced to a range of 28-30°C in the 2nd week and maintained at 18-24°C from 3rd week until the end of the experiment. All birds were subjected to a daily photoperiod of 23 h throughout the experiment.

Chickens were reared to 42 days of age and fed a starter ration (3127 kcal of ME/kg of diet and 22.51% CP) from 3-17 days of age and grower ration (3141 kcal of ME/kg of diet 19.09% CP) from 18-42 days of age. Diets were formulated to cover or exceed the recommended nutrient requirements of broiler chicks according to NRC (1994). Feed in mash form and water (via nipple drinkers) were provided freely. The composition and calculated analysis of the basal diets are shown in Table 1.

**Performance of broiler chickens:** Live Body Weight (BW), Feed Intake (FI) and Body Weight Gain (BWG) were measured weekly on a replicate group basis throughout the experimental period, then Feed Conversion Ratio (FCR) was calculated (g feed: g gain). Birds were weighed to the nearest gram in the early morning before receiving any feed or water at weekly intervals during the experimental period. Live body weights of broilers were recorded at the beginning of the experiment and at weekly basis thereafter. Weekly records on FI and BWG of broilers were also maintained on a replicate group basis. Accordingly, FCR was calculated as the amount of feed consumed per unit of BWG. Mortality and health status of chicks were visually observed and recorded daily throughout the entire experimental period.

Table 1: Composition and calculated analysis of the basal diets fed to broiler chicks

| Ingredients | Starter (%) | Grower (%) |
|---|---|---|
| Yellow corn (%) | 64.70 | 72.23 |
| Soybean meal (44%) | 13.00 | 11.50 |
| Corn gluten meal (60.2%) | 18.00 | 12.50 |
| Di calcium phosphate (%) | 1.80 | 1.31 |
| Limestone (%) | 1.45 | 1.49 |
| DL-methionine (%) | 0.05 | 0.00 |
| L-Lysine (%) | 0.40 | 0.37 |
| Sodium chloride (%) | 0.30 | 0.30 |
| Vit.+min premix[1] (%) | 0.30 | 0.30 |
| Total | 100.00 | 100.00 |
| VE (mg kg$^{-1}$) | 0.00 | 0.00 |
| Calculated analysis ME (kcal kg$^{-1}$) | 3127.00 | 3141.00 |
| CP (%) | 22.51 | 19.09 |
| Crude fiber (%) | 2.60 | 2.60 |
| Ether extract (%) | 3.00 | 3.10 |
| Calcium (%) | 1.00 | 0.90 |
| Av-phosphorus (%) | 0.45 | 0.35 |
| Methionine (%) | 0.52 | 0.39 |
| Meth+Cys (TSAA, %) | 0.92 | 0.73 |
| Lysine (%) | 1.10 | 1.00 |
| VE (mg kg$^{-1}$) | 18.84 | 19.15 |

[1]Premix provided the following per kilogram of diet, Vitamin A (retinyl acetate): 2654 µg, Vitamin $D_3$ (cholecalciferol): 125 µg, Vitamin E (dl-α-tocopheryl acetate): 9.9 mg, Vitamin $K_3$ (menadione dimethylpyrimidinol): 1.7 mg, Vitamin $B_1$ (thiamin mononitrate): 1.6 mg, Vitamin $B_{12}$ (cyanocobalamin): 16.7 µg, Vitamin $B_2$ (riboflavin): 5.3 mg, Niacin (niacinamide): 36 mg, Calcium pantothenate: 13 mg, Folic acid: 0.8 mg, Biotin: 0.1 mg, Choline chloride: 270 g, BHT: 5.8 g, Fe (iron sulphate monohydrate): 50 mg, Cu (copper sulphate pentahydrate): 12 mg, I (calcium iodate): 0.9 mg, Zn (zinc oxide): 50 mg, Mn (manganous oxide): 60 mg, Se (sodium selenite): 0.2 mg and Co (cobalt sulphate): 0.2 mg, 2 Calculated from data provided by NRC (1994)

**Slaughter test:** At the conclusion of the feeding trial (42 day), three birds from each group; whose body weights were near the average of their respective group, were selected for slaughter test. Just prior to slaughter and again after complete bleeding, birds were individually weighed. Records on individual weights of eviscerated carcass, giblets (including heart, liver and gizzard) were maintained. Carcass yield was calculated as eviscerated carcass plus giblets. Lymphoid organs (spleen and Bursa of Fabricius) of each bird were separated and weighed.

**Tissues specimens and histological procedures:** During slaughtering, representative tissue samples were taken from thymus, spleen and bursa of Fabricius and immediately fixed in 10% formalin-saline solution and then dehydrated in ascending concentrations of alcohol solutions ranged from 70% to absolute ethanol alcohol. Samples were cleared in xylene and then embedded in melted paraffin wax, to obtain tissue blocks. They were then sectioned and stained with hematoxylin and eosin stain (Junqueira *et al.*, 1971). Sections were examined under light microscope and photographed by using a digital Camera.

**Statistical analysis:** Statistical analysis for the obtained data was performed by two-way analysis of variance using the method of least square analysis of Co-variance (SAS., 2006). Duncan's multiple range tests was used to separate significant differences among means (Duncan, 1955). Result are significant at $p>0.05$.

# RESULTS AND DISCUSSION

## Growth performance of broiler chicks

**Live body weight:** Table 2 shows the effects of added dietary VE and stocking density on Live Body Weight (LBW) of broiler chicks during the whole experimental period (3-42 days of age). Neither dietary level of added VE nor stocking density had an effect (p>0.05) on LBW of broiler chicks at 3, 10, 17, 38 or 42 days of age. Apart from the effect of stocking density, added VE at a level of 200 mg kg$^{-1}$ diet produced a slight positive effect ($p\leq 0.05$) on LBW of chicks at 24 days of age. Similarly, LBW of 31 day old chicks was significantly increased ($p\leq 0.05$) due to dietary supplementation with 200 or 300 mg kg$^{-1}$ compared with the control birds. However, increasing stocking density from 11.90-16.66 birds m$^{-2}$ led to significant reductions ($p\leq 0.05$) in LBW of broiler chicks at 24, 31, 38 and 42 days of age, regardless of the effect of dietary VE supplementation. But stocking density did not affect (p>0.05) LBW of chicks at 10 days of age. Dietary VE supplementation by stocking density interactions had no effect (p>0.05) on LBW at different ages.

The lack of effect of supplemental VE on final LBW of chicks, observed in the present study, is in agreement with the findings obtained by other investigators (Niu *et al*., 2009; El-Habbak *et al*., 2011; Habibian *et al*., 2014), who observed no significant effects of supplemental dietary VE on live body weight of broiler chickens. On the other hand, Bobade (2006) and Rajput *et al*. (2009) reported that dietary supplementation of VE achieved significantly higher live body weight of broiler chicks compared with the control group. In addition, Rashidi *et al*. (2010) found that addition of VE to the fish oil ration positively affected live body weight of broilers.

Table 2: Effects of added dietary VE and stocking density on live body weight of broiler chicks from 3-42 days of age

| | LBW (day old) | | | | | | |
|---|---|---|---|---|---|---|---|
| Main effects | 3 | 10 | 17 | 24 | 31 | 38 | 42 |
| **Added VE (A)** | | | | | | | |
| 0.00 mg kg$^{-1}$ (A1) | 0.067 | 0.168 | 0.369 | 0.654$^{b}$ | 1.002$^{b}$ | 1.302 | 1.782 |
| 200 mg kg$^{-1}$ (A2) | 0.067 | 0.171 | 0.380 | 0.676$^{a}$ | 1.030$^{a}$ | 1.308 | 1.781 |
| 300 mg kg$^{-1}$ (A3) | 0.067 | 0.169 | 0.370 | 0.658$^{b}$ | 1.034$^{a}$ | 1.323 | 1.826 |
| 400 mg kg$^{-1}$ (A4) | 0.067 | 0.170 | 0.365 | 0.663$^{ab}$ | 1.013$^{b}$ | 1.310 | 1.785 |
| Pooled SEM | 0.000 | 0.002 | 0.005 | 0.005 | 0.005 | 0.020 | 0.034 |
| Significance | NS | NS | NS | * | * | NS | NS |
| **Cage density (B)** | | | | | | | |
| 11.90 birds m$^{-2}$ (B1) | 0.067 | 0.171 | 0.379$^{a}$ | 0.690$^{a}$ | 1.056$^{a}$ | 1.360$^{a}$ | 1.865$^{a}$ |
| 16.66 birds m$^{-2}$ (B2) | 0.067 | 0.168 | 0.363$^{b}$ | 0.636$^{b}$ | 0.984$^{b}$ | 1.262$^{b}$ | 1.722$^{b}$ |
| Pooled SEM | 0.000 | 0.001 | 0.003 | 0.003 | 0.003 | 0.014 | 0.024 |
| Significance | NS | NS | * | * | * | * | * |
| **AB interaction** | | | | | | | |
| A1×B1 | 0.067 | 0.171 | 0.389 | 0.685 | 1.055 | 1.387 | 1.895 |
| A1×B2 | 0.067 | 0.165 | 0.350 | 0.623 | 0.950 | 1.218 | 1.669 |
| A2×B1 | 0.066 | 0.168 | 0.375 | 0.698 | 1.047 | 1.307 | 1.782 |
| A2×B2 | 0.067 | 0.174 | 0.385 | 0.654 | 1.014 | 1.309 | 1.780 |
| A3×B1 | 0.067 | 0.167 | 0.373 | 0.688 | 1.062 | 1.365 | 1.902 |
| A3×B2 | 0.067 | 0.171 | 0.368 | 0.627 | 1.005 | 1.281 | 1.749 |
| A4×B1 | 0.068 | 0.177 | 0.380 | 0.687 | 1.060 | 1.382 | 1.882 |
| A4×B2 | 0.067 | 0.163 | 0.351 | 0.639 | 0.967 | 1.239 | 1.689 |
| Pooled SEM | 0.000 | 0.003 | 0.007 | 0.007 | 0.007 | 0.029 | 0.048 |
| Significance | NS | NS | NS | NS | NS | NS | NS |

$^{a,b}$In each of the main effects, means in the same column with different superscripts differ significantly ($p\leq 0.05$), NS: Not significant, *Significant at $p\leq 0.05$, LBW: Live body weight

Table 3: Effects of added dietary VE and stocking density on body weight gain of broiler chicks from 3-42 days of age

| | BWG (day) | | | | | | |
|---|---|---|---|---|---|---|---|
| Main effects | 3-10 | 10-17 | 17-24 | 24-31 | 31-38 | 38-42 | TBWG 3-42 day |
| **Added VE (A)** | | | | | | | |
| 0.00 mg kg$^{-1}$ (A1) | 0.101 | 0.201$^{ab}$ | 0.284$^{b}$ | 0.348$^{b}$ | 0.300 | 0.479 | 1.715 |
| 200 mg kg$^{-1}$ (A2) | 0.104 | 0.208$^{a}$ | 0.296$^{ab}$ | 0.354$^{ab}$ | 0.277 | 0.473 | 1.714 |
| 300 mg kg$^{-1}$ (A3) | 0.102 | 0.201$^{ab}$ | 0.287$^{ab}$ | 0.375$^{a}$ | 0.289 | 0.502 | 1.758 |
| 400 mg kg$^{-1}$ (A4) | 0.102 | 0.195$^{b}$ | 0.297$^{a}$ | 0.350$^{b}$ | 0.297 | 0.474 | 1.718 |
| Pooled SEM | 0.002 | 0.002 | 0.004 | 0.007 | 0.017 | 0.020 | 0.034 |
| Significance | NS | * | * | * | NS | NS | NS |
| **Cage density (B)** | | | | | | | |
| 11.90 birds m$^{-2}$ (B1) | 0.103 | 0.208$^{a}$ | 0.310$^{a}$ | 0.366$^{a}$ | 0.304 | 0.505$^{a}$ | 1.798$^{a}$ |
| 16.66 birds m$^{-2}$ (B2) | 0.101 | 0.195$^{b}$ | 0.272$^{b}$ | 0.348$^{b}$ | 0.277 | 0.460$^{b}$ | 1.654$^{b}$ |
| Pooled SEM | 0.001 | 0.002 | 0.002 | 0.005 | 0.012 | 0.014 | 0.024 |
| Significance | NS | * | * | * | NS | * | * |
| **AB interaction** | | | | | | | |
| A1×B1 | 0.104 | 0.217 | 0.296 | 0.369 | 0.332 | 0.507 | 1.827 |
| A1×B2 | 0.097 | 0.185 | 0.273 | 0.326 | 0.268 | 0.451 | 1.602 |
| A2×B1 | 0.101 | 0.206 | 0.323 | 0.349 | 0.260 | 0.475 | 1.715 |
| A2×B2 | 0.107 | 0.211 | 0.268 | 0.360 | 0.294 | 0.471 | 1.713 |
| A3×B1 | 0.100 | 0.206 | 0.315 | 0.373 | 0.302 | 0.537 | 1.835 |
| A3×B2 | 0.104 | 0.196 | 0.259 | 0.377 | 0.276 | 0.467 | 1.682 |
| A4×B1 | 0.109 | 0.202 | 0.307 | 0.372 | 0.322 | 0.500 | 1.814 |
| A4×B2 | 0.096 | 0.188 | 0.287 | 0.328 | 0.271 | 0.449 | 1.621 |
| Pooled SEM | 0.003 | 0.004 | 0.005 | 0.010 | 0.025 | 0.029 | 0.048 |
| Significance | NS | NS | NS | NS | NS | NS | NS |

$^{a-b}$In each of the main effects, means in the same column with different superscripts differ significantly ($p \leq 0.05$), *Significant at $p \leq 0.05$, NS: Not significant, BWG: Body weight gain

The negative effect of high stocking density, reported herein, is in accordance with the results obtained by Mehmood *et al.* (2014), who reported significantly heavier live body weight of chicks kept at the lowest stocking density (0.0650 m$^2$/bird than those of birds stocked at higher densities (0.0557 and 0.0464 m$^2$/bird). In contrast, Massuod *et al.* (2014) showed that cage density had no influence on final live body weight of broiler chicks. Similarly, Buijs *et al.* (2009) found that the 39 day final LBW of broiler chicks was not significantly different among birds reared at different stocking densities (6, 15, 23, 33, 35, 41, 47 and 56 kg m$^{-2}$).

**Body weight gain:** The effects of added dietary VE and stocking density on body weight gain (BWG) of broiler chicks from 3-42 days of age are shown in Table 3. It was observed that added dietary VE did not significantly influence ($p>0.05$) BWG of broiler chicks for the periods of 3-10, 31-38, 38-42 and the whole experimental period (3-42 days of age), irrespective of the effect of stocking density. During the periods of 10-17, 17-24 and 24-31 days of age slight erratic differences ($p \leq 0.05$) were observed in BWG of birds fed the different levels of added VE; such differences may not be related to dietary supplementation with Vitamin E. However, increasing stocking density from 11.90 to 16.66 birds m$^{-2}$ led to significant decreases ($p \leq 0.05$) in BWG of broiler chicks for the periods of 10-17, 17-24, 24-31, 38-42 days of age and the entire experimental period (3-42 days old), regardless of the effect of dietary VE supplementation. But stocking density did not affect

Table 4: Effects of added dietary VE and stocking density on feed intake of broiler chicks from 3-42 days of age

| | F1 (day) | | | | | | |
|---|---|---|---|---|---|---|---|
| Main effect | 3-10 | 10-17 | 17-24 | 24-31 | 31-38 | 38-42 | TF1 3-42 day |
| **Added VE (A)** | | | | | | | |
| 0.00 mg $kg^{-1}$ (A1) | 0.130 | $0.312^{ab}$ | 0.480 | $0.645^{b}$ | 0.768 | 1.044 | 3.382 |
| 200 mg $kg^{-1}$ (A2) | 0.133 | $0.323^{a}$ | 0.493 | $0.655^{ab}$ | 0.727 | 1.023 | 3.356 |
| 300 mg $kg^{-1}$ (A3) | 0.131 | $0.317^{ab}$ | 0.479 | $0.696^{a}$ | 0.735 | 1.067 | 3.427 |
| 400 mg $kg^{-1}$ (A4) | 0.132 | $0.305^{b}$ | 0.497 | $0.648^{b}$ | 0.758 | 1.029 | 3.372 |
| Pooled SEM | 0.002 | 0.004 | 0.006 | 0.011 | 0.033 | 1.037 | 0.055 |
| Significance | NS | * | NS | * | NS | NS | NS |
| **Cage density (B)** | | | | | | | |
| 11.90 birds $m^{-2}$ (B1) | 0.134 | $0.326^{a}$ | $0.521^{a}$ | $0.679^{a}$ | 0.769 | 1.078 | $3.510^{a}$ |
| 16.66 birds $m^{-2}$ (B2) | 0.129 | $0.303^{b}$ | $0.454^{b}$ | $0.643^{b}$ | 0.725 | 1.003 | $3.259^{b}$ |
| Pooled SEM | 0.001 | 0.002 | 0.004 | 0.007 | 0.023 | 0.026 | 0.039 |
| Significance | NS | * | * | * | NS | * | * |
| **AB interaction** | | | | | | | |
| A1×B1 | 0.134 | 0.336 | 0.506 | 0.679 | 0.817 | 1.070 | 3.543 |
| A1×B2 | 0.125 | 0.288 | 0.455 | 0.612 | 0.720 | 1.018 | 3.221 |
| A2×B1 | 0.132 | 0.326 | 0.544 | 0.642 | 0.702 | 1.046 | 3.394 |
| A2×B2 | 0.134 | 0.321 | 0.442 | 0.668 | 0.752 | 1.001 | 3.319 |
| A3×B1 | 0.130 | 0.324 | 0.520 | 0.703 | 0.760 | 1.127 | 3.566 |
| A3×B2 | 0.132 | 0.310 | 0.439 | 0.689 | 0.711 | 1.006 | 3.289 |
| A4×B1 | 0.141 | 0.318 | 0.514 | 0.693 | 0.798 | 1.070 | 3.536 |
| A4×B2 | 0.124 | 0.293 | 0.418 | 0.604 | 0.718 | 0.987 | 3.209 |
| Pooled SEM | 0.003 | 0.005 | 0.008 | 0.015 | 0.046 | 0.052 | 0.078 |
| Significance | NS | NS | NS | NS | NS | NS | NS |

[a,b]In each of the main effects, means in the same column with different superscripts differ significantly ($p \le 0.05$), *Significant at $p \le 0.05$, NS: Not significant, FI: Feed intake

($p>0.05$) BWG of chicks for the periods of 3-10 and 31-38 days of age. The interactions between dietary VE supplementation and stocking density were not significant ($p>0.05$) for all age intervals examined.

The current results are in agreement with those obtained by Coetzee and Hoffman (2001), who observed no differences in BWG of broiler chicks due to dietary supplementation of VE. However, Basmacioglu *et al.* (2004) showed that VE supplementation to diets enriched with n-3 polyunsaturated fatty acid significantly increased BWG of broiler chicks from 22-42 days of age. In harmony with the present results, Beloor *et al.* (2010) found that the BWG of broilers stocked at low density was significantly higher compared to that of the standard and high density groups. In contrast, Asaniyan (2014) found no significant influence of stocking density (6, 12 and 18 birds $m^{-2}$) on cumulative weight gains of the broiler chickens throughout the fattening period.

**Feed intake:** The effects of added dietary VE and stocking density on Feed Intake (FI) of broiler chicks from 3-42 days of age are shown in Table 4. Apart from the effect of stocking density, added VE at a level of 200 mg $kg^{-1}$ diet caused a slight increase ($p \le 0.05$) in FI of chicks at the period of 10-17 days of age. Similarly, chicks fed the diets supplemented with VE at a level of 300 mg $kg^{-1}$ diet consumed significantly more feed ($p \le 0.05$) as compared to the other experimental groups. But added dietary VE did not significantly affect ($p>0.05$) FI of birds during the periods of 3-10, 17-24, 31-38, 38-42 days of age or the whole experimental period, regardless of the effect of stocking density. On the other hand, stocking density had no effect ($p>0.05$) on FI of chicks during the

Table 5: Effects of added dietary VE and stocking density on feed conversion ratio (FCR; kg feed: kg gain) of broiler chicks from 3-42 days of age

| | FCR (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| Main effects | 3-10 | 10-17 | 17-24 | 24-31 | 31-38 | 38- 42 | 3- 42 |
| **Added VE (A)** | | | | | | | |
| 0.00 mg $kg^{-1}$ (A1) | 1.288 | 1.554 | 1.689 | 1.854 | 2.583 | 2.183 | 1.975 |
| 200 mg $kg^{-1}$ (A2) | 1.278 | 1.550 | 1.665 | 1.850 | 2.639 | 2.166 | 1.958 |
| 300 mg $kg^{-1}$ (A3) | 1.288 | 1.576 | 1.669 | 1.856 | 2.566 | 2.134 | 1.950 |
| 400 mg $kg^{-1}$ (A4) | 1.291 | 1.565 | 1.671 | 1.852 | 2.577 | 2.172 | 1.964 |
| Pooled SEM | 0.006 | 0.008 | 0.014 | 0.023 | 0.050 | 0.033 | 0.011 |
| Significance | NS | NS | NS | NS | NS | NS | NS |
| **Cage density (B)** | | | | | | | |
| 11.90 birds $m^{-2}$ (B1) | $1.297^{a}$ | 1.568 | 1.677 | 1.856 | 2.555 | 2.143 | 1.953 |
| 16.66 birds $m^{-2}$ (B2) | $1.275^{b}$ | 1.555 | 1.669 | 1.849 | 2.627 | 2.184 | 1.970 |
| Pooled SEM | 0.004 | 0.005 | 0.009 | 0.016 | 0.035 | 0.023 | 0.008 |
| Significance | * | NS | NS | NS | NS | NS | NS |
| AB Interaction | | | | | | | |
| A1×B1 | 1.290 | 1.547 | 1.709 | 1.835 | 2.474 | 2.112 | 1.939 |
| A1×B2 | 1.285 | 1.561 | 1.668 | 1.873 | 2.692 | 2.255 | 2.011 |
| A2×B1 | 1.300 | 1.579 | 1.683 | 1.843 | 2.708 | 2.205 | 1.978 |
| A2×B2 | 1.257 | 1.522 | 1.646 | 1.858 | 2.570 | 2.128 | 1.938 |
| A3×B1 | 1.307 | 1.576 | 1.647 | 1.886 | 2.550 | 2.112 | 1.946 |
| A3×B2 | 1.269 | 1.576 | 1.692 | 1.825 | 2.582 | 2.155 | 1.955 |
| A4×B1 | 1.292 | 1.570 | 1.671 | 1.862 | 2.488 | 2.145 | 1.949 |
| A4×B2 | 1.290 | 1.559 | 1.672 | 1.841 | 2.666 | 2.200 | 1.979 |
| Pooled SEM | 0.008 | 0.011 | 0.019 | 0.033 | 0.071 | 0.046 | 0.016 |
| Significance | NS | NS | NS | NS | NS | NS | NS |

[a,b]In each of the main effects, means in the same column with different superscripts differ significantly ($p\leq 0.05$)*Significant at $p\leq 0.05$, NS: Not significant, FCR: Feed conversion ratio

periods of 3-10, 31-38 or 38-42 days of age but the high level of stocking density significantly reduced ($p\leq 0.05$) FI of birds during the periods of 10-17, 17-24, 24-31 and 3-42 days of age compared with the normal level of stocking density, independently from the effect of added VE. Meanwhile, dietary VE supplementation by stocking density interactions had no effect ($p>0.05$) on FI for all age intervals examined.

The lack of significant differences in FI of broiler chicks during the whole experimental period (3-42 days of age), reported herein, in response to dietary VE supplementation concurs with the results obtained by Niu *et al.* (2009) and Habibian *et al.* (2014), who failed to find an effect of supplemental dietary on feed intake of broiler chickens. On the contrary, other researchers found a negative effect of added vitamin E of feed intake of broilers (Shaikh *et al.*, 2005; Bobade, 2006; Rajput *et al.*, 2009). On the other hand, the depressed FI of chicks kept at the high stocking density (16.66 birds $m^{-2}$) as compared to those stocked at the normal density (11.9 birds $m^{-2}$) harmonizes with the findings of Shanawany (1988), who found a decline in average FI of broilers when their stocking density was increased from 0.05-0.035 or 0.02 $m^2$/bird. However, Beloor *et al.* (2010) showed that the stocking density had no significant effect on total FI of broiler chicks.

**Feed conversion ratio:** The effects of added dietary VE and stocking density on Feed Conversion Ratio (FCR) of broiler chicks from 3-42 days of age are given in Table 5. Apart from the effect of stocking density, added dietary VE supplementation did not significantly affect ($p>0.05$) FCR of

Table 6: Effects of added dietary VE and stocking density on carcass and lymphoid organs weights of 42-day-old broiler chicks

| Main effects | LBW (kg) | Carcass (kg) | Liver (g) | Heart (g) | Gizzard (g) | Spleen (g) | Bursa(g) |
|---|---|---|---|---|---|---|---|
| **Added VE (A)** | | | | | | | |
| 0.00 mg kg$^{-1}$ (A1) | 1.870 | 1.4043 | 48.266 | 10.3766$^{a}$ | 43.783 | 1.8951 | 1.022 |
| 200 mg kg$^{-1}$ (A2) | 1.951 | 1.4475 | 54.433 | 8.2833$^{b}$ | 40.800 | 2.5731 | 0.753 |
| 300 mg kg$^{-1}$ (A3) | 1.900 | 1.3958 | 48.383 | 9.4500$^{a}$ | 35.645 | 2.5388 | 1.290 |
| 400 mg kg$^{-1}$ (A4) | 1.990 | 1.4618 | 47.666 | 9.7200$^{a}$ | 46.083 | 2.3149 | 1.260 |
| Pooled SEM | 0.044 | 0.0390 | 3.513 | 0.3680 | 2.704 | 0.2400 | 0.125 |
| Significance | NS | NS | NS | NS | NS | NS | NS |
| **Cage density (B)** | | | | | | | |
| 11.90 birds m$^{-2}$ (B1) | 1.972 | 1.463 | 47.825 | 9.139 | 41.205 | 2.242 | 1.199 |
| 16.66 birds m$^{-2}$ (B2) | 1.883 | 1.391 | 51.550 | 9.775 | 41.950 | 2.418 | 0.963 |
| Pooled SEM | 0.031 | 0.027 | 2.484 | 0.260 | 1.912 | 0.170 | 0.088 |
| Significance | NS | NS | NS | NS | NS | NS | NS |
| **AB interaction** | | | | | | | |
| A1×B1 | 1.910 | 1.422 | 45.166 | 10.420 | 42.200 | 1.898 | 0.989 |
| A1×B2 | 1.831 | 1.386 | 51.366 | 10.333 | 45.366 | 1.892 | 1.055 |
| A2×B1 | 1.963 | 1.465 | 50.133 | 7.666 | 43.800 | 2.422 | 0.714 |
| A2×B2 | 1.940 | 1.430 | 58.733 | 8.900 | 37.800 | 2.723 | 0.792 |
| A3×B1 | 1.980 | 1.433 | 51.400 | 8.633 | 36.590 | 1.981 | 1.848 |
| A3×B2 | 1.820 | 1.358 | 45.366 | 10.266 | 34.700 | 3.096 | 0.731 |
| A4×B1 | 2.036 | 1.533 | 44.600 | 9.836 | 42.233 | 2.668 | 1.246 |
| A4×B2 | 1.943 | 1.390 | 50.733 | 9.603 | 49.933 | 1.961 | 1.273 |
| Pooled SEM | 0.062 | 0.055 | 4.969 | 0.521 | 3.825 | 0.340 | 0.177 |
| Significance | NS | NS | NS | NS | NS | NS | NS |

[a,b]In each of the main effects, means in the same column with different superscripts differ significantly ($p \leq 0.05$), NS: Not significant

chicks during all age intervals studied or the whole experimental period (3-42 days of age). Similarly, FCR of broiler chicks was not affected ($p>0.05$) by stocking density, irrespective of the effect of dietary VE supplementation. In addition, insignificant interactions ($p>0.05$) were observed between added dietary VE and stocking density on FCR of broilers for all age intervals investigated.

The absence of significant differences in FCR of broiler chicks due to dietary supplementation with vitamin E, observed in the present study, is in line with the finding of Basmacioglu *et al.* (2009), who suggested that FCR was not affected by antioxidant supplementation. Contrarily, Habibian *et al.* (2014) observed non-linear improvement in FCR of broiler chicks in response to dietary supplementation with vitamin E. The present results are in harmony also with the results of Pettit-Riley and Estevez (2001), who found no effects on FCR of broiler chicks for stocking densities between 0.1 and 0.05 m$^2$/bird. But Bessei (2006) reported a negative effect of high stocking density on FCR of broiler chicks. In addition, Ratsaka *et al.* (2012) noted that FCR of broilers stocked at 0.08 m$^2$/bird was poorer than those of birds kept at higher stocking densities (0.06 or 0.05 m$^2$/bird).

**Carcass traits and lymphoid organs of broiler chicks:** The effects of added dietary VE and stocking density on carcass traits and lymphoid organs weights of broiler chicks 42 day old are given in Table 6 it was observed that neither dietary supplementation with vitamin E nor stocking density had an effect on carcass traits and lymphoid organs weights of broiler chicks. Interactions between added dietary Vitamin E and stocking density were also not significant for all carcass traits studied.

The present results are in agreement with the findings of Konjufca *et al.* (2004), who found that dietary VE (Dl-α-tocopherol) supplementation did not significantly affect the relative weights of organs weight, except for the spleen. Similarly, Niu *et al.* (2009) observed no significant differences in relative weights of lymphoid organs (thymus, bursa and spleen) of broiler chicks due to dietary supplementation with vitamin E. On the other hand, Dozier *et al.* (2005, 2006) found that stocking density did not influence the relative weights of carcass yield or abdominal fat pad in broiler chicks. Similar results were also obtained by Sekeroglu *et al.* (2011), who found no significant differences in weights of liver, heart, kidneys and gizzard of broilers kept at different stocking densities. In contrast, Heckert *et al.* (2002) reported that a significant reduction in absolute and relative weights of bursa in broiler chicks with increasing the stocking density from 0.10-0.05 $m^2$ per bird.

**Histological observations of lymphoid organs:** Histological examination of sections of lymphoid organs in response to added dietary levels of VE and stocking density showed moderate changes associated with the level of VE.

**Thymus gland histology:** The histological structure of thymus gland as influenced by feeding different levels of VE and stocking density are illustrated in Fig. 1a-h. In general, the thymus gland is enclosed by a thin connective tissue capsule composed of coarse collagen fibers and few fine elastic fibrils. There are numerous fine septa which divide the gland to lobules. This structure was observed, to a great extent, in the thymus section of the control group (Fig. 1a). Many thymic lobules with their blood supply could be seen together with numerous lymphocytes. This structure was also observed in the thymus section from the high density group of birds (Fig. 1b), however, many obvious changes could be detected including the presence of many large lymphocytes, large medullary area and thickest collagenous capsule.

Experimental treatments showed considerable changes in response to dietary levels of VE supplementation, especially in the high density treatment groups. It is clear that VE enhanced the histological structure of the thymus gland of the low density group by stimulating the proliferation of lymphocytes within the gland lobules.

This effect was close-dependent with the best results in sections of VE-200 and VE-300 supplemented groups (Fig. 1c and e). On the other hand, VE supplementation was seen to improve the histological feature of the gland from the high density treatment group (Fig. 1d, f and h). However, there were several types of degeneration with many necrotic areas concomitant with an irregular arrangement of thymic lobules along with dispersed lymphocytes within the cortex and medullary regions. Since, the gland undergoes dramatic involution as birds aged, however, dietary inclusion of VE up to 300 mg $kg^{-1}$ could enhance the function of the gland either in low or high stocking density. The high VE dose (400 mg $kg^{-1}$ $diet^{-1}$) did not gave the best, however, it is still better than the control treatment (Fig. 1g and h).

**Spleen histology:** Histological sections of spleen from the control groups (Fig. 2a and b) showed the basic structure of the splenic tissues, where a large White Pulp (WP) area and a dark-stained Red Pulp (RP) area could be seen. There are numerous blood capillaries, sinusoids and lymphocytic cells of different size. The main histological changes were the presence of some lymphoid nodules along with many small-sized lymphocytes, especially in Fig. 2c-g and to a lesser extent in Fig. 2a and 2h.

It appears that the RP area was extended all over the spleen sections of the high-stocking density groups (Fig. 2b, d and f slightly in Fig. 2h). In these sections many large lymphocytes could

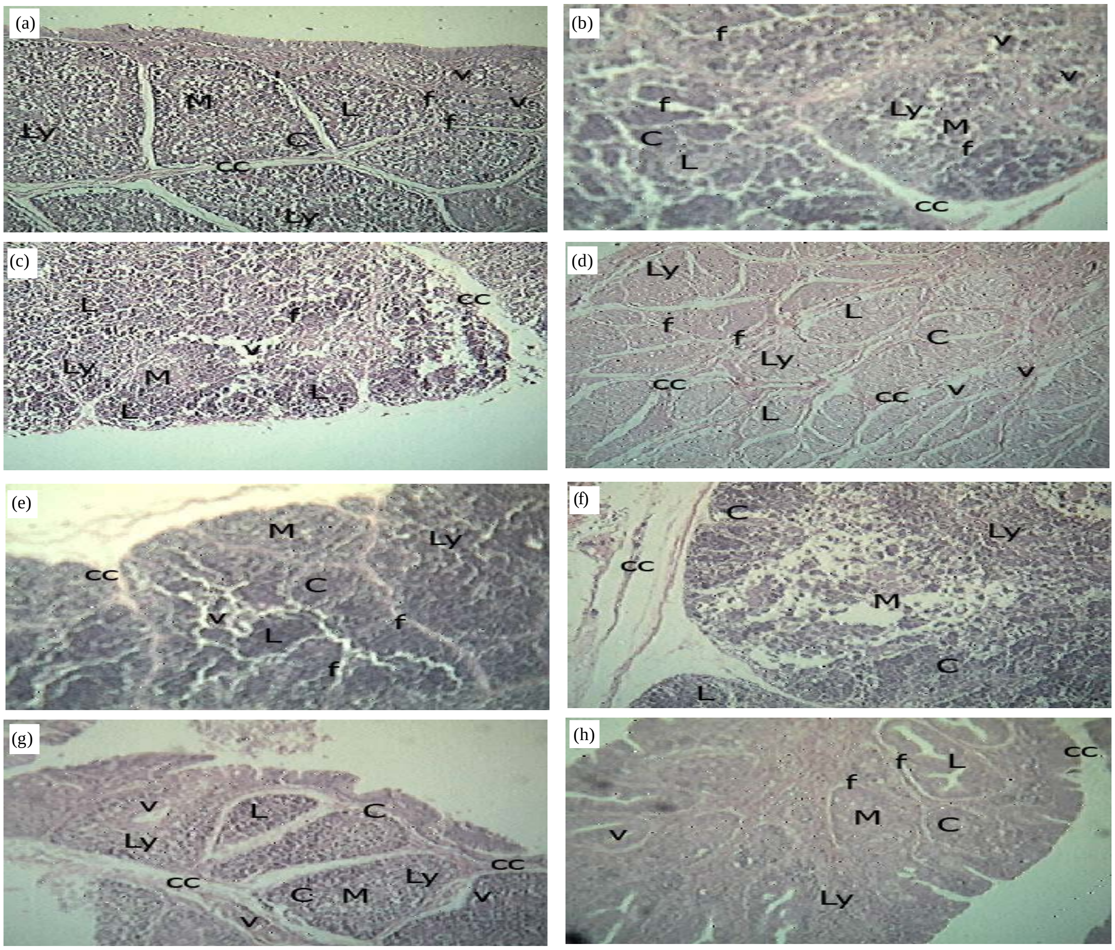


Fig. 1(a-h): T.S. of thymus from (a) Low-stocking density control group, (b) High-stocking density control group, (c) Low-stocking density-VE (200 ppm) treatment group, (d) High-stocking density-VE (200 ppm) treatment group, (e) Low-stocking density-VE (300 ppm) treatment group, (f) High-stocking density-VE (300 ppm) treatment group, (g) Low-stocking density-VE (400 ppm) treatment group and (h) High-stocking density-VE (400 ppm) treatment group of broilers (H and E×40), CC: Collagenous capsule, f: Fine septa, L: Lobule, v: Blood vessels, Ly: Lymphocytes, M: Medulla, C: Cortex

be seen accompanied by basophilic hemosiderin granules in-between blood sinusoids. The effect of VE supplementation was obvious as observed by the increases of splenocytes proliferation and hence the number of lymphocytes increased. The present results showed, however, that VE supplementation improved the histological structure of spleen.

The best result was achieved by VE-300 treatment either in low or high density groups. The fact that some or many lymph nodules were seen in the sections could be explained by the role of applied vaccination programs which lead to lymphoid-organs responses against these vaccines and/or diseases.

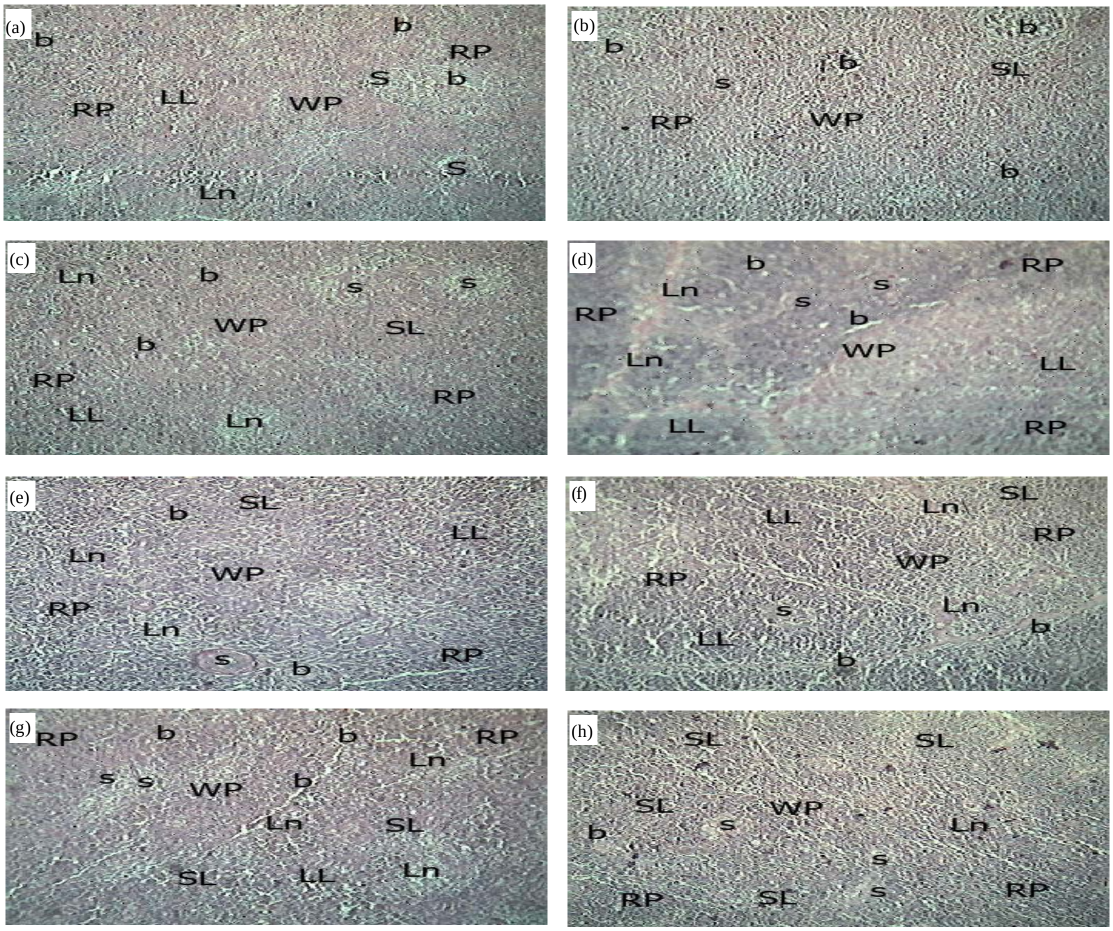


Fig. 2(a-h): T.S. of spleen from (a) Low-stocking density control group, (b) High-stocking density control group, (c) Low-stocking density-VE (200 ppm), (d) High-stocking density-VE (200 ppm) treatment group, (e) Low-stocking density-VE (300 ppm) treatment group, (f) High-stocking density-VE (300 ppm) treatment group, (g) Low-stocking density-VE (400 ppm) treatment group and (h) High-stocking density-VE (400 ppm) treatment group of broilers (H and E×40), WP: White pulp, RP: Red pulp, b: Blood capillaries, s: Blood sinusoids, SL: Small lymphocytes, LL: Large lymphocytes, Ln: Lymph nodule

**Bursa of fabricius histology:** The bursa of Fabricius is a primary lymphoid organ, unique to birds. In general, it is composed of about 15 plicate (folds); each contains numerous Bursal Follicles (BF). These follicles have two distinct areas, cortex and medulla. The present sections showed that the bursal follicles of control treatments are enlarged with many small lymphocytes in the medullary area.

This was clear in the low-stocking density treatment (Fig. 3a) than in the high-density group (Fig. 3b). On the other hand, the follicles lumens appeared quiescent, indicative of hypoactivity. This was not observed in the VE treatment groups where, the lumens were greatly increased in diameter (Fig. 3c and d), irrespective of the stocking density. The same was

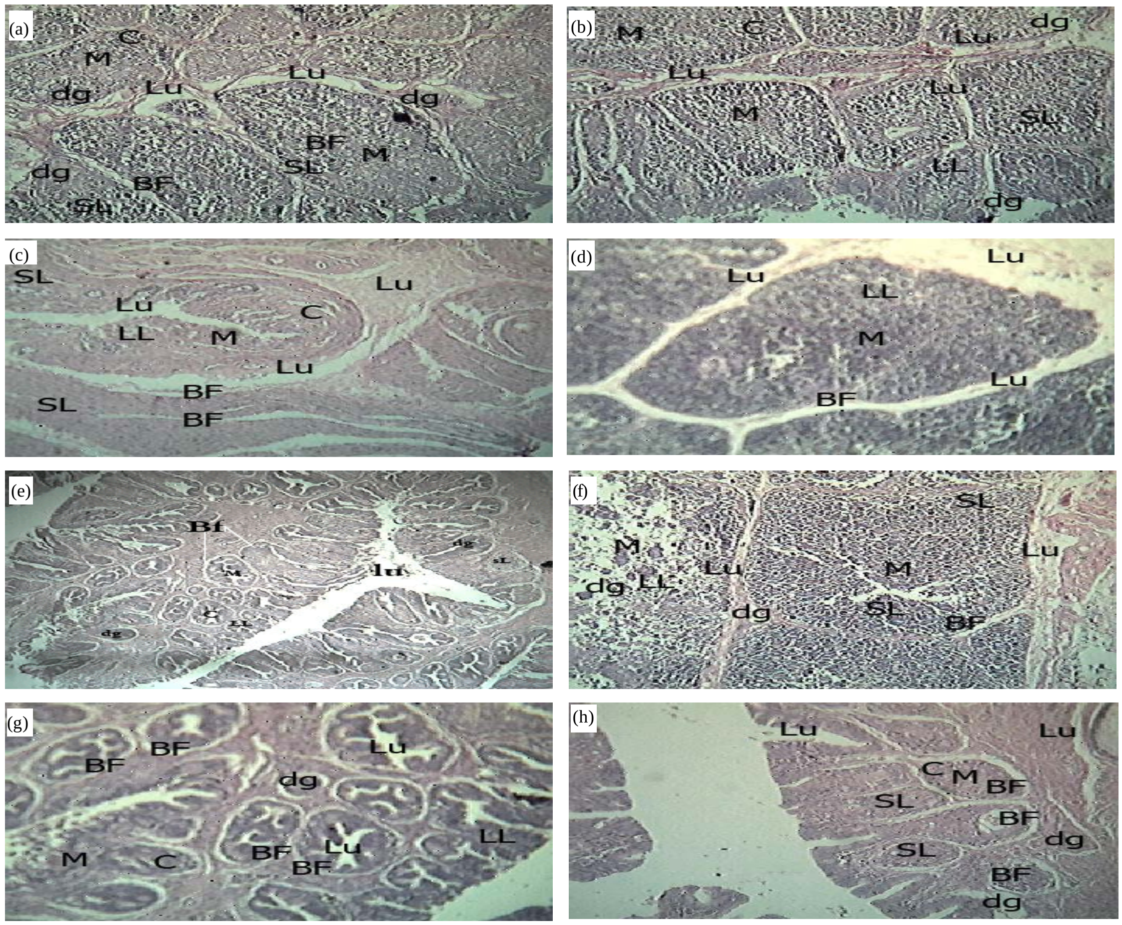


Fig. 3(a-h): T.S. of bursa from (a) Low-stocking density control group, (b) High-stocking density control group, (c) Low-stocking density-VE (200 ppm), (d) High-stocking density-VE (200 ppm) treatment group, (e) Low-stocking density-VE (300 ppm) treatment group, (f) High-stocking density-VE (300 ppm) treatment group, (g) Low-stocking density-VE (400 ppm) treatment group and (h) High-stocking density-VE (400 ppm) treatment group of broilers (H and E×40) BF: Bursal follicles, dg: Degenerative area, Lu: Lumen, M: Medulla, SL: Small lymphocytes, LL: Large lymphocytes, C: Cortex

observed in VE-300 (Fig. 3e and f) treatment groups. However, the higher VE level (400 mg $kg^{-1}$ diet) did not show any response (Fig. 3g and h).

It is clear from the previous results that VE plays an important role in protecting the lymphoid organs tissue from the negative impacts of different stress conditions. In the present study, the high stocking density was known to exert negative effects on lymphoid organs weight and cytology which may cause low immune responses. Dietary supplementation with VE enhanced the activity of spleen, bursa and thymus to produce many lymphocytes that help improving the immunity of birds. This hyperactivity is accompanied by the presence of many large lymphocytes in the cortex area in bursa and thymus or in the RP area of spleen; this concomitant with many phagocytic cells and more enlarged lumen (bursa and thymus) between the follicles. These lumens (abundant in all treatment sections) are responsible for phagocytic process and for maintaining the T and B-cells production.

These observations may be related to the increased relative weights of spleen, bursa and thymus which were obtained in our study. This increase was more obvious in VE-treated groups. In this respect, Konjufca *et al.* (2004) reported that spleen weight (%) increased by VE treatment, also thymus and bursa % were significantly increased (Hussain *et al.*, 2004). The same was also reported by Niu *et al.* (2009), Rashidi *et al.* (2010) and Habibian *et al.* (2014) who observed significant effects of VE on lymphoid organs function which is in close agreement with the present results. Moreover, the negative impact of the high stocking density on lymphoid organs was also in close agreement with other findings by Heckert *et al.* (2002) and Ravindran *et al.* (2006). However, Houshmand *et al.* (2012) reported insignificant effect of stocking density on lymphoid organs activity.

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