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Influence of Vitamin E Supplementation and Stocking Density on Performance, Thyroid Status, Some Blood Parameters, Immunity and Antioxidant Status in Broiler Chickens

F.S.A. Ismail, M.R. El-Gogary and M.I. El-Nadi
Department of Poultry Production, Faculty of Agriculture, Mansoura University, 35516, Egypt

Corresponding Author: M.R. El-Gogary, Department of Poultry Production, Faculty of Agriculture, Mansoura University, 35516, Egypt

ABSTRACT

The present study was carried out to investigate the effects of dietary supplementation with vitamin E and stocking density on performance, thyroid function, plasma concentration of corticosterone (Cort), some blood parameters, (antibody (Ab) titers against Newcastle Disease Virus (NDV) and Avian Flu virus (AF)) and antioxidant status in broiler chickens. In all 192; 3-day-old commercial broiler chickens (cobb 500) were randomly divided into 8 treatments groups, each of which include 4 replicates. Experimental treatments consisted of a 4×2 factorial arrangement design with 4 levels of vitamin E and 2 levels of stocking density (11.90 birds m^{-2} as the normal stocking density or 16.66 birds m^{-2} as the high stocking density). Vitamin E levels were 0.0, 200, 300 and 400 mg kg^{-1} diet. Increasing vitamin E level in the diet did not affect Live Body Weight (LBW), Body Weight Gain (BWG), feed intake and FCR. Also, increasing vitamin E levels had no effects on T4, Total Lipids (TL), total cholesterol (Chol) and high density lipoprotein cholesterol (HDL). However, supplementing vitamin E had a significant effect on SOD, NDV and AF titers but decreasing effect on MDA. In addition, plasma Cort and LDL levels were lower at 300 mg kg^{-1} vitamin E supplementation but plasma Total Protein (TP) and albumin (Alb) levels were higher at 300 mg kg^{-1} vitamin E supplementation. The normal stocking density has significant effects on LBW, BWG, FI, Cort, TP, TL and chol compared with the high stocking density. However, stocking density did not affect FCR, T3, T4, Alb, LDL, HDL and SOD. The results of the present study show that supplementing of vitamin E at 300 mg kg^{-1} of diet has a positive effect on productivity, immunity and blood parameters in broiler chickens.

Key words: Malondialdehyde, superoxide dismutases, thyroid, corticosterone, cholesterol, vitamin E, stocking density, broiler

INTRODUCTION

Vitamin E (α-Tocopherol) is an important lipid-soluble antioxidant. It performs its functions as antioxidant in the glutathione peroxidase pathway (Wefers and Sies, 1988) and it protects cell membranes from oxidation by reacting with lipid radicals produced in the peroxidation chain reaction (Herrera and Barbas, 2001; Traber and Atkinson, 2007). This would remove the free radical intermediates and prevent the oxidation reaction from continuing. Supplementation of animal diets with vitamin E increases the content of this natural antioxidant in animal food products and prevents lipid peroxidation in broiler meat (Ajuyah *et al*., 1993). Tocopherols are

mainly found in the hydrocarbon part of membrane lipid bilayer towards the membrane interface and in close proximity to oxidase enzymes which initiate the production of free radicals (Putnam and Comben, 1987; McDowell, 1989; Packer, 1991).

Malondialdehyde (MDA) is one of the most frequently used indicators of lipid peroxidation (a marker of radical induced damage). MDA is a three-carbon low molecular weight aldehyde and spontaneous breakdown product of peroxides that can be produced from free radical attack on poly unsaturated fatty acids (Cordis *et al*., 1998; Pilz *et al*., 2000). Superoxide dismutases (SOD) are enzymes that function to catalytically convert superoxide radical to oxygen and hydrogen peroxide. These enzymes carry out catalysis at near diffusion controlled rate constants via a general mechanism that involves the sequential reduction and oxidation of the metal center, with the concomitant oxidation and reduction of superoxide radical (Abreu and Cabelli, 2010). Oxidative stress is known to be an important contributing factor in many chronic diseases (Mahima *et al*., 2013). The combination of vitamin E is more effective in ensuring adequate stability of the diet and in protecting the bird's immune system and thereby improving performance (Bou *et al*., 2004).

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, as profits increase, the welfare of the animals may start to decline. Furthermore, the effects of density are multidimensional, because it affects performance, health, welfare of the birds in many different ways (Estevez, 2007). Several morphological anomalies have also been presented, indicating that increased stocking density can compromise immunity (Proudfoot *et al*., 1979; Greene *et al*., 1985; McIlroy *et al*., 1987; Heckert *et al*., 2002; Thaxton *et al*., 2006). Therefore, the present study was designed to evaluate supplementation of vitamin E as one of major antioxidants on performance, immunity response, lipid peroxidation and stress indicators of broilers chickens at different stocking densities.

MATERIALS AND METHODS

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

Birds and management: Cobb 500 broiler chickens (n = 192), 3-day-old, were divided into 8 treatments groups, each of which included 4 replicates (cages). Experiments consisted of a 4×2 factorial arrangement with 4 levels of vitamin E supplementation and 2 stocking densities. Vitamin E (VE) was supplemented to the basal diet at 0, 200, 300, 400 mg kg^{-1} diet to obtain the dietary VE of 18.84, 218.84, 318.84 and 418.84 mg kg^{-1} of starter diet, respectively. These levels were 19.15, 219.15, 319.15 and 419.15 mg of VE kg^{-1} of the grower diet, respectively. Birds were reared in battery cages and the length, width and height of each cage were 70, 60 and 40 cm, respectively. Thus the cage floor area was 0.42 m^2 (70×60 cm). The number of birds located in each was 5 or 7 birds per cage density. The stocking density was 11.90 birds m^{-2} as the normal density (5 birds per cage) and 16.66 birds m^{-2} as the high density (7 birds per cage). The daily temperature inside the farm in first week was 32°C and then gradually reduced to be ranged from 30-28°C in the 2nd week and maintained at 18-24°C from 3rd week until the end of the experiment. The photoperiod was 23L: The 1 D throughout the experiment. Chickens were reared to 42 days of age and fed a starter ration from three to 17 days of age (3127 kcal of ME kg^{-1} of diet and 22.51% CP) and grower ration from 18-42 days of age (3.141 kcal of ME kg^{-1} of diet 19.09% CP). Diets were,

formulated to cover or exceed the recommended requirements of broiler chicks according to NRC (1994). Feed in mash form and water (via nipple drinkers) were provided freely. The composition and chemical analysis of the experimental 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 throughout the experimental period, then Feed Conversion Ratio (FCR) was calculated (feed: Gain g). Mortalities and health status were visually observed and recorded daily throughout the entire experimental period.

Blood sampling and biochemal analysis: Three birds of each treatment were randomly chosen, slaughtered and blood samples were collected in heparinized tubes then centrifuged at 4000 rpm for 15 min and the plasma obtained was stored at -20°C until analysis. Plasma samples were tested calorimetrically using commercial kits according to the procedures outlined by the manufactures, for determination of total protein (Doumas *et al*., 1981), albumin (Doumas *et al*., 1971), total lipids (Frings and Dunn, 1970), cholesterol (Allain *et al*., 1974) high density lipoprotein and low density lipoprotein (Myers *et al*., 1994). Concentrations of thyroid hormones triiodothyronine (T3) and thyroxine (T4) and corticosterone in blood plasma were measured by RIA techniques according to

Table 1: Composition and chemical analysis of basal diet fed to the experimental chickens

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
Vitamin E (mg kg^{-1})	0.00	0.00
Analyzed chemical composition (g kg^{-1})		
ME (kcal kg^{-1})	3127.00	3141.00
CP	22.51	19.09
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
Vitamin E (mg kg^{-1})	18.84	19.15

[1]Premix provided the following per kilogram of diet: Vitamin A (retinyl acetate), 2654 µg, vitamin D3 (cholecalciferol), 125 µg, vitamin E (dl-α-tocopheryl acetate), 9.9 mg, vitamin K3 (menadionedimethylpyrimidinol), 1.7 mg, vitamin B1 (thiamin mononitrate), 1.6 mg, vitamin B12 (cyanocobalamin), 16.7 µg, riboflavin, 5.3 mg, niacin (niacinamide), 36 mg, calcium pantothenate, 13 mg, folic acid, 0.8 mg, d-biotin, 0.1 mg, choline chloride, 270, BHT, 5.8, 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, Co (cobalt sulphate), 0.2 mg, [2]Calculated from data provided by NRC (1994), [3]The respective diet formulated to contain 18.84, 218.84, 318.84 and 418.84 mg kg^{-1} vitamin E and the dose titrations were achieved by addition of vitamin E at the expense of soybean meal

the methods of Britton *et al.* (1975) and Satterlee *et al.* (1980), respectively. Superoxide dismutase (SOD) was determined by available kit according to Misra and Fridovich (1972) and malondialdehyde (MAD) determined by available kits according to Uchiyama and Mihara (1978). Antibody titers against Newcastle Disease Virus (NDV) and Avian Flu Virus (AF) were determined by hem agglutination inhibition technique using U-Bottonmicrotiter plates (96 wells) as reported by Wegmann and Smithies (1966) and Van der Zijpp *et al.* (1983). Antigens were prepared manually in incubated chicken eggs, kept frozen as allantois fluid, till used within a month.

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., 1996). Duncan's multiple range test was used to separate significant differences among means (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance: The results relating to the influence of vitamin E levels and stocking density on body weight and feed consumption at 42 days of age are shown in Table 2. In present study, increasing vitamin E level in the diet did not affect LBW, BWG, feed intake and FCR ($p<0.05$). This result is in agreement with Coetzee and Hoffman (2001) who reported that there was no difference ($p>0.05$) in weight gain or feed conversion ratio between levels of dietary vitamin E supplementation. This observation differs from those of other authors. Hosseini-Mansoub *et al.* (2010) found that diet enrichment with vitamin E resulted in the better performance of the birds ($p<0.05$) in comparison with those fed the control diet. The lowest Feed Conversion Ratio (FCR)

Table 2: Effect of vitamin E levels and stocking density on growth performance of broilers at 42 days of age

Parameters	LBW (kg)	BWG (kg)	Feed intake/bird (kg)	FCR
Diet (mg)				
0.0	1.782	1.715	3.382	1.975
200	1.781	1.714	3.356	1.958
300	1.826	1.758	3.427	1.950
400	1.785	1.718	3.372	1.964
SEM	0.034	0.034	0.055	0.011
Significance	NS	NS	NS	NS
Density				
5b	1.865[a]	1.798[a]	3.510[a]	1.953
7b	1.722[b]	1.654[b]	3.259[b]	1.970
SEM	0.024	0.024	0.039	0.008
Significance	*	*	*	NS
Interaction				
0×5	1.895	1.827	3.543	1.939
0×7	1.669	1.602	3.221	2.011
200×5	1.782	1.715	3.394	1.978
200×7	1.780	1.713	3.319	1.938
300×5	1.902	1.835	3.566	1.946
300×7	1.749	1.682	3.289	1.955
400×5	1.882	1.814	3.536	1.949
400×7	1.689	1.621	3.209	1.979
SEM	0.048	0.048	0.078	0.016
Significance	NS	NS	NS	NS

[a-b]Means in the same colum with different superscripts differ significantly ($p\leq0.05$)

Table 3: Effect of dietary supplementation with vitamin E and stocking density on plasma concentrations of T3, T4 and corticostrone (μg mL^{-1}) in broilers chickens

Parameters	T3	T4	Cort
Diet (mg)			
0.0	2.570[b]	14.990	2.3683[a]
200	2.806[ab]	14.240	2.0833[ab]
300	3.021[a]	15.051	2.0133[b]
400	3.048[a]	15.558	2.0933[ab]
SEM	0.112	0.512	0.1050
Significance	*	NS	*
Density			
5b	2.962	15.086	1.955[b]
7b	2.760	14.833	2.323[a]
SEM	0.079	0.362	0.074
Significance	NS	NS	*
Interaction			
0×5	2.610	15.493	2.013
0×7	2.530	14.486	2.723
200×5	2.963	14.51	1.786
200×7	2.650	13.97	2.380
300×5	3.166	15.14	1.953
300×7	2.876	14.963	2.073
400×5	3.110	15.203	2.070
400×7	2.986	15.913	2.116
SEM	0.158	0.724	0.148
Significance	NS	NS	NS

[a-b]Means in the same colum with different superscripts differ significantly ($p \leq 0.05$)

and the highest Body Weight (BW) were observed in those fed the enriched diet and exposed to normal conditions. Birds at normal stocking density (11.9 birds m^{-2}) had significantly better performance compared with the high stocking density (16.66 birds m^{-2}). This indicates greater role of stocking density as a stress factor on performance. Broiler LBW, BWG and feed intake were affected negatively by high stocking density. Similar, results were reported Elwinger (1995), Thomas *et al.* (2004), Muniz *et al.* (2006) and El-Gogary and Azzam (2014) indicated that increasing the number of birds per unit depresses growth rate and feed intake. In contrast, Buijs *et al.* (2009) found that LBW was not significantly different between birds reared at different stocking densities at 39 days of age. Also, FCR was not affected adversely by high stocking density. Similarly, other researchers (Feddes *et al.*, 2002; Galobart and Moran Jr., 2005) concluded that there was no significant effect of stocking density on FCR of broilers. In contrast, Houshmand *et al.* (2012) and El-Gogary and Azzam (2014) found that during the grower phase, broilers raised at a high density had an inferior FCR compared with birds housed at a normal density.

Blood parameters: The effect of dietary VE levels and stocking density on plasma T3, T4 and corticosterone concentrations are presented in Table 3. There was no a significant effect of VE levels on plasma T4 but high levels on VE increase than other groups. However, plasma T3 level increased significantly ($p \leq 0.05$) in response to VE level and the highest levels occurred at 400 mg kg^{-1} VE. Thiese results were in agreement with previous studies which reported that serum concentration of T3 and T4 were higher with dietary vitamin E treatment (Sahin *et al.*, 2001,

Table 4: Effect of dietary supplementation with vitamin E and stocking density on some blood plasma constituents in broilers chickens

Parameters	TP	Alb	TL	Chol	LDL	HDL
	----------(g dL^{-1})----------		----------(mg dL^{-1})----------			
Diet (mg)						
0.0	4.025^{b}	2.220^{b}	901.966	228.116	62.400^{a}	52.333
200	4.118^{b}	2.386^{ab}	912.566	216.300	53.533^{b}	57.400
300	4.401^{a}	2.523^{a}	905.183	213.933	55.000^{b}	56.866
400	4.078^{b}	2.318^{b}	905.866	212.15	58.150^{ab}	59.500
SEM	0.078	0.059	14.366	4.995	1.9140	2.6590
Significance	*	*	NS	NS	*	NS
Density						
5b	4.283^{a}	2.38	880.550^{b}	210.458^{b}	55.833	55.825
7b	4.028^{b}	2.344	932.241^{a}	224.791^{a}	58.708	57.225
SEM	0.055	0.042	10.158	3.532	1.353	1.880
Significance	*	NS	*	*	NS	NS
Interaction						
0×5	4.156	2.29	863.100	230.733	63.433	53.066
0×7	3.893	2.15	940.833	225.500	61.366	51.600
200×5	4.253	2.39	874.600	203.666	49.900	55.700
200×7	3.983	2.383	950.533	228.933	57.166	59.100
300×5	4.586	2.513	900.500	208.633	51.433	53.266
300×7	4.216	2.533	909.866	219.233	58.566	60.466
400×5	4.136	2.326	884	198.80	58.566	61.266
400×7	4.020	2.310	927.733	225.50	57.733	57.733
SEM	0.111	0.084	20.317	7.064	2.707	3.76
Significance	NS	NS	NS	NS	NS	NS

^{a,b}Means in the same Colum with different superscripts differ significantly ($p \leq 0.05$)

2002). Also, Gruzauskas *et al.* (2014) showed that triiodothyronine level in blood of broiler chickens in experimental groups contained VE increased compared with the control group ($p>0.05$). No significant effects of stocking density on plasma thyroid hormones. This result is in agreement with other studies reported that stocking density did not affect plasma levels triiodothyronine and thyroxine (Tong *et al.*, 2012; El-Gogary and Azzam, 2014). Plasma corticosterone decreased significantly in response to dietary VE and the lowest level occurred at 300 mg kg^{-1}. A reduction in ACTH concentration in plasma from antioxidant supplemented animals was observed by Butterweck *et al.* (2004) in rats. Taniguchi *et al.* (2001) found that broiler chickens supplemented with vitamin E had reduced corticosterone content in the adrenal glands. Also, ACTH concentration in serum was lower in supplemental dietary vitamin E groups compared with control. These results are in agreement with the findings by Lin *et al.* (2006) who showed that corticosterone concentration on day 42 increase ofas stocking density (Lin *et al.*, 2006). However, disagreement with the current result, Türkyilmaz (2008) found that stocking density had no significant effect on blood corticosterone concentration in broiler chickens. There was a trend toward increasing corticosterone concentration with higher stocking density with a statistical significant increase in blood corticosterone concentration in the high stocking density groups.

The effects of vitamin E and stocking density on the plasma concentrations of total protein, albumin, total lipids, cholesterol, high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL) are presented in Table 4. Blood plasma concentrations of total lipids, cholesterol and HDL did not differ significantly between all treatment groups. These results are in agreement with those by Arslan *et al.* (2001) who showed that no significant difference in plasma

Table 5: Effect of dietary supplementation with vitamin E and stocking density on plasma concentrations of SOD, MDA, NDV and AF in broiler chickens

Parameters	SOD (U mL^{-1})	MDA (nmol mL^{-1})	NDV (log_2)	AF (log_2)
Diet (mg)				
0.0	11.518^{d}	53.866^{a}	5.866^{c}	4.033^{b}
200	14.086^{c}	50.016^{ab}	6.016^{bc}	4.600^{a}
300	16.521^{b}	47.133^{b}	6.550^{ab}	4.766^{a}
400	18.413^{a}	46.433^{b}	6.733^{a}	4.716^{a}
SEM	0.516	2.025	0.201	0.165
Significance	**	*	*	*
Density				
5b	15.264	42.5583^{b}	6.675^{a}	4.858^{a}
7b	15.005	56.1666^{a}	5.908^{b}	4.200^{b}
SEM	0.364	1.432	0.142	0.117
Significance	NS	*	*	*
Interaction				
0×5	11.750	47.033	6.1	4.266
0×7	11.286	60.700	5.633	3.800
200×5	15.080	43.666	6.300	4.833
200×7	13.093	56.366	5.733	4.366
300×5	16.136	41.800	7.133	5.133
300×7	16.906	52.466	5.966	4.400
400×5	18.090	37.733	7.166	5.200
400×7	18.736	55.133	6.300	4.233
SEM	0.729	2.864	0.284	0.234
Significance	NS	NS	NS	NS

$^{a\text{-}d}$Means with different superscripts differ significantly

cholesterol level between control and vitamin E groups. In addition, plasma LDL level was lower at 300 mg kg^{-1} vitamin E supplementation but plasma Total Protein (TP) and albumin (Alb) levels were higher at 300 mg kg^{-1} vitamin E supplementation. These results are in agreement with other studies (Sahin *et al*., 2002) found that plasma protein and albumin concentrations increased linearly with dietary vitamin E supplementation. However, Arslan *et al*. (2001) found insignificant effect in plasma total protein between control and supplemental vitamin E levels. Results showed also that stocking density had a significant effect on plasma TP, TL and Chol, while plasma albumin, LDL and HDL was not influenced. It appears that plasma lipids were influenced positively by stocking density which may be related to the increased concentration of corticosterone indicative of an adverse effect of stocking density on plasma lipids profile. It is well known that high plasma corticosterone level enhances gluconeogenesis and lipolysis (Whittow, 2000) which may explain the higher levels of TL and Chol ($p \leq 0.05$) and also LDL and HDL although they lacked the significant level.

The results relating to the influence of dietary supplementation of vitamin E and stock density on superoxide dismutase (SOD), malondialdehyde (MDA), antibody titers against Newcastle Disease Virus (NDV) and Avian Flu virus (AF) in plasma are shown in Table 5. In this study, the supplementation of vitamin E levels significantly increased SOD compared to the control group. These results are in agreement with other studies (Tras *et al*., 2000) which demonstrated that vitamin E plus selenium supplementation increased the serum superoxide dismutase level. The plasma MDA was found to be significantly lower in the groups fed enriched diets with vitamin E compared to the control diet. Sahin *et al*. (2001) and Hosseini-Mansoub *et al*. (2010) showed that

serummal on dialdehyde was significantly lower in groups fed vitamin E. The vitamin E diminishes the peroxidation of polyunsaturated fatty acids via scavenging free radicals (McDowell, 1989; Packer and Landvik, 1990). The present results showed that the antibody titer against NDV and AF in blood plasma of vitamin E treated broiler chickens were significantly higher compared with that of the control group. Lin and Chang (2006) findings suggest that moderate supplementation of vitamin E may enhance immune responses to selective antigens in cockerels but excessive vitamin E may depress specific immune response which is the case in our study. No significant effects of stocking density on plasma SOD but levels of MDA increased by increasing stocking density. Physiological stress (as a result of increasing stocking density) if the stocking density is too high, the temperature may rise dangerously since there will be more metabolic heat being added to the house air than was planned for. It is well known that heat causes an increased production of MDA (Sahin *et al*., 2001; Naziroglu *et al*., 2000; Halliwell and Gutteridge, 1989). There were significant lowering effect of stocking density on Ab titers against NDV and AF. Erisir and Erisir (2002) reported that there was a significant decrease in immune response with an increase in stocking density in Japanese quails. Tufft and Nockels (1991) also reported that a decrease in space allowance made broilers more susceptible to infections. However, other disagreements result (Türkyilmaz, 2008) demonstrated that stocking density had no effect on immune response in broilers which is similar to those reported by Hocking *et al*. (2002) who found that immune function was not affected by food restriction inbroiler breeders.

CONCLUSION

The findings of present study suggest that addition of vitamin E at a level of 300 mg kg^{-1} diet has a positive effect on productive performance, immune responses and antioxidative status of broiler chickens.

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