Optimal dietary concentration of vitamin E for alleviating the effect of heat stress on performance, thyroid status, ACTH and some serum metabolite and mineral concentrations in broilers

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ABSTRACT: An experiment utilizing Cobb-500 male broilers was conducted to evaluate the effects of vitamin E (d1-α-tocopheryl acetate) supplementation at various concentrations (0, 62.5, 125, 250, or 500 mg/kg of diet) on performance and serum concentrations of Triiodothyronine (T_3), Thyroxin (T_4), Adrenocorticotropine Hormone (ACTH), and some metabolites and minerals in broilers reared under heat stress (32°C). One day-old 150 male broilers were randomly assigned to 5 treatment groups, 3 replicates of 10 birds each. The birds received either a basal diet or basal diet supplemented with vitamin E at 62.5, 125, 250, or 500 mg/kg of diet. Increased supplemental vitamin E linearly increased feed intake (P = 0.01), live weight gain (P = 0.01), and improved feed efficiency linearly (P = 0.001). Increasing dietary vitamin E supplementation also resulted in linear increases in serum T_3 and T_4 concentrations (P = 0.01) but, linear decreases in ACTH concentration (P = 0.01). Serum glucose, uric acid, triglycerides, and cholesterol concentrations decreased linearly (P = 0.001) while, protein and albumin concentrations increased linearly (P = 0.001) when dietary vitamin E supplementation increased. Serum activities of Serum Glutamic Oxalate Transaminase (SGOT) and Serum Glutamic Pyruvate Transaminase (SGPT) were not influenced by dietary vitamin E supplementation (P > 0.10). However, serum activity of Alkaline Phosphatase (AP) increased linearly (P = 0.001) with increasing dietary vitamin E supplementation. Increasing dietary vitamin E supplementation also caused linear increases (P = 0.001) in serum concentrations of Ca and P. Results of the present study conclude that a 250 mg/kg of vitamin E provides an optimal performance in broiler chicks reared under heat stress, and vitamin E supplementation at such a level can be considered as a protective management practice in a broiler diet, reducing the negative effects of heat stress.

Keywords: thyroxin; triiodothyronine; chicken, vitamin E; nutrition; heat stress

High ambient temperature reduces feed intake, live weight gain, and feed efficiency (Donkoh, 1989), thus negatively influencing the performance of broilers. Hurwitz *et al.* (1980) suggested that decrease in growth rate was due partly to the decrease in feed intake. High ambient temperature also reduces thyroid activity in poultry (Evans and Ingram, 1977; Bowen and Washburn, 1985). Plasma T_3 and T_4, important growth promoters in animals, are associated with ambient temperature (McNabb and King, 1993). The circulating concentrations of T_3 and T_4 are reduced at high temperatures (Heninger *et al.*, 1960; Bowen *et al.*, 1984; Hilman *et al.*, 1985). Plasma corticosterone concentration also increases during heat stress (Edens and Siegel, 1975). In addition, Donkoh (1989) reported reduced plasma protein and markedly increased blood glucose concentrations during heat stress.

Several methods are available to alleviate the effect of high environmental temperature on performance of poultry. Since it is expensive to cool animal buildings, such methods are focused mostly on the dietary manipulation. In this respect, vitamin E is used in the poultry diet because of the reported benefits of vitamin E supplementation to laying hens during heat stress (Whitehead *et al.*, 1998; Bollengier-Lee *et al.*, 1998, 1999; Sahin *et al.*, 2001), also because of the fact that vitamin E levels is reduced during heat stress (Feenster, 1985; Whitehead *et al.*, 1998; Boliengier-Lee *et al.*, 1999; Sahin *et al.*, 2001, 2002). One of the most important properties of vitamin E is its antioxidant

function. When animals fed diets rich in unsaturated fatty acids which are susceptible to peroxidation the vitamin E deficiency is augmented (McDowell, 1989). Supplementation of animal diets with tocopherols increases the content of this natural antioxidant in animal food products and prevents lipid peroxidation in broiler meat (Ajuyah *et al.*, 1993). Vitamin E is known to be a lipid component of biological membranes and is considered a major chain-breaking antioxidant (Halliwell and Gutteridge, 1989). Vitamin E is 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). Vitamin E, therefore, protects cells and tissues from oxidative damage induced by free radicals (Gallo-Torres, 1980). In a previous study, we observed that supplemental vitamin E significantly alleviated the heat stress-related decrease in performance suggesting additional vitamin E supplementation into diets may be necessary under heat stress conditions in Japanese quails (Sahin and Kucuk, 2001). Therefore, the objective of this study was to evaluate the effects of optimal dose of vitamin E supplementation on performance and serum concentrations of T_3, T_4, ACTH, and some metabolite and minerals in broilers reared under heat stress (32°C). Supplementing vitamin E to broilers is also important to human health in terms of consuming healthier poultry meat products.

MATERIAL AND METHODS

One hundred and fifty 1 day-old Cobb-500 male chicks provided from Koy-Tür Company, Elazig, Turkey, were used in the study. The birds were randomly assigned, according to their initial body weights, to 5 treatment groups, 3 replicates of 10 birds each. All pens were bedded with a wood-shavings litter and equipped with feeders and waterers in environmental chambers with 24.4 cm^2 per bird. The birds randomly received either a basal diet or basal diet supplemented with vitamin E (d1-α-tocopheryl acetate) either 62.5, 125, 250, or 500 mg/kg of diet in addition to the vitamin E content in starter and grower. Vitamin E contents of the experimental diets were ascertained. Vitamin E (ROVIMIX® E-50 SD, 50%; fairly stable source of vitamin E in feed) were provided by a commercial company (Roche, Levent-Istanbul). The birds were fed a starter diet until 21 d of age followed by a finishing diet from day 21 to day 42. Ingredients and chemical composition of the starter and grower diets are shown in Table 1. The basal diets were formulated using NRC (1994) guideline and contained 23–20% (starter-grower) protein and 13.37 MJ/kg ME. The diets and fresh water were offered *ad libitum*. During the experiment, light was provided continuously (24 hours) and average room temperature was 32 ± 3°C. Average relative humidity inside the hen house was 42 ± 6%. At weekly intervals, feed intake and body weight were determined. Weight gain and feed efficiency of birds were then calculated.

Table 1. Ingredients and chemical composition of the starter and grower diets fed to broilers reared under heat stress (32°C)

	Starter	Grower
	(% of DM)	
Ingredients		
Ground Corn	53.75	61.73
Soybean meal (48% CP)	37.28	30.25
Animal fat	5.51	4.65
Dicalcium phosphate	1.37	1.33
Sodium chloride	0.10	0.20
Limestone, ground	0.53	0.58
Calcium carbonate	0.10	0.16
DL-methionine	0.24	0.25
Lysine	–	0.12
Avilamycine	0.10	0.10
Ca-propionate	0.10	0.10
Clinacox	0.10	0.10
Toxinil	0.30	–
Vitamin premix*	0.27	0.27
Trace mineral premix**	0.16	0.16
Dry matter, %	89.65	89.68
Chemical Analyses (% DM)		
ME, MJ/kg	13.37	13.37
Crude protein (CP)	23.00	20.03
Crude Fat	7.04	6.35
Organic matter	80.12	80.46
Ash	9.53	9.22
Crude fiber	3.32	3.38

*Premix (Rovimix 124/V) supplied for 1 kg: vitamin A, 7.500 IU; cholecalciferol, 1500 IU; vitamin E, 7.5 IU; menadione, 1.25 mg; vitamin B_1, 0.5 mg; vitamin B_2, 5 mg; niacin, 35 mg; d-pantothenic acid, 10 mg; vitaminB_{12}, 0.1 mg; folic acid, 1 mg; biotin, 0.025 mg.

**Premix (Remineral CH) supplied for 1 kg: Mn, 40 mg; Fe, 12.5 mg; Zn, 25 mg; Cu, 3.5 mg; Iodine, 0.15 mg; Se, 0.75 mg; Choline chloride, 175 mg

At the end of day 42, 9 birds randomly chosen from each treatment (3 birds per each replicate) were slaughtered and blood was collected. Blood samples were centrifuged at 3 000 × *g* for 10 min and serum was collected and stored at –20°C for later analysis. Sera were thawed at room temperature and T_3, T_4, and ACTH concentrations were determined using commercially available radioimmunoassay kits (Liaison® T_3, and T_4 Byk-Sangtec Diagnostica, Dietzenbach-Germany; Immulite 2000 ACTH, L2 KAC2, DPC, LA). Intra- and interassay coefficients of variation were 7.35 and 9.26% for T_3, 15.52 and 8.15% for T_4 and 10.84 and 8.16% for ACTH, respectively. Serum glucose, total protein, uric acid, albumin, triglyceride, cholesterol, Ca, and P concentrations, and activities of SGOT, SGPT, and AP were measured using a biochemical analyzer kit (Olympus AU-600 System). Chemical analysis of starter and grower diets were run using international procedures of AOAC (1990).

The data were analyzed using the GLM procedure of SAS (1988). Linear, quadratic and cubic polynomial contrasts were used to evaluate treatment effects.

RESULTS

The effects of supplemental dietary vitamin E during heat stress on performance of broilers are shown in Table 2. Increased supplemental vitamin E linearly increased feed intake ($P = 0.01$), live weight gain ($P = 0.01$), and improved feed efficiency linearly ($P = 0.001$). Live weight gain ($P = 0.04$) and feed intake ($P = 0.05$) also had quadratic responses to increasing vitamin E supplementation. Feed intake and live weight gain increased as dietary vitamin E increased up to 250 mg/kg of diet, but did not increased further as dietary vitamin E supplementation increased to 500 mg/kg of diet. Increasing dietary vitamin E supplementation resulted in linear increases in serum T_3 and T_4 concentrations ($P = 0.01$) whereas, linear decreases in ACTH concentration ($P = 0.01$) (Table 3). Serum T_3 ($P = 0.01$), T_4 ($P = 0.05$), and ACTH ($P = 0.04$) concentrations also had quadratic responses to increasing vitamin E supplementation. Serum concentrations of T_3 and T_4 increased whereas, ACTH concentration decreased as dietary vitamin E increased up to 250 mg/kg of diet, but further increases in dietary vitamin E supplementation up to 500 mg/kg of diet did not change the concentrations of T_3, T_4, and ACTH. Dietary vitamin E had significant effects on most blood parameters measured at the present study (Tables 4 and 5). Serum glucose, uric acid, triglycerides, and cholesterol concentrations decreased linearly ($P = 0.001$) while, protein and albumin concentrations increased linearly ($P = 0.001$) when dietary vitamin E supplementation increased. Serum glucose ($P = 0.01$), protein ($P = 0.04$), uric acid ($P = 0.01$), and albumin ($P = 0.02$) concentrations also had quadratic responses to increasing dietary vitamin E supplementation. Serum concentrations of protein and albumin increased whereas, glucose and uric acid concentrations decreased as dietary vitamin E increased up to 250 mg/kg of diet, but further in-

Table 2. Effects of vitamin E supplementation on performance of broiler chicks reared under heat stress (32°C)

Treatments			
Vitamin E (mg/kg)	Feed intake (g)	Body weight gain (g)	Feed efficiency*
0	3 414	1 899	1.79
62.5	3 425	1 919	1.77
125	3 437	1 949	1.76
250	3 481	2 057	1.69
500	3 465	2 033	1.70
Pooled SEM	6.04	16.23	0.06
		Probabilities	
Polynomial contrasts			
Linear	0.01	0.01	0.001
Quadratic	0.04	0.05	0.15
Cubic	0.16	0.15	0.19

*Gram of feed consumed: gram of gain

Table 3. Effects of vitamin E supplementation on serum concentrations of T_3, T_4, and ACTH (ng/ml) in broiler chicks reared under heat stress (32°C) ($n = 9$)

Treatments			
Vitamin E (mg/kg)	T_3	T_4	ACTH
0	2.13	11.75	18.43
62.5	2.14	11.84	18.17
125	2.22	12.06	17.50
250	2.30	12.97	16.70
500	2.28	12.92	16.75
Pooled SEM	0.10	0.55	1.13
		Probabilities	
Polynomial contrasts			
Linear	0.01	0.01	0.01
Quadratic	0.01	0.05	0.04
Cubic	0.53	0.61	0.75

Table 4. Effects of vitamin E supplementation on some blood metabolites in broiler chicks reared under heat stress (32°C) (n = 9)

Vitamin E (mg/kg)	Glucose(mg/dl)	Protein	Uric acid	Albumin	Triglyceride	Cholesterol
			(g/dl)		(mmol/l)	
0	214	4.37	4.61	2.47	1.58	2.38
62.5	216	4.35	4.66	2.49	1.56	2.37
125	207	4.55	4.31	2.62	1.32	2.24
250	189	4.68	4.08	2.70	1.18	2.15
500	188	4.65	4.11	2.68	1.19	2.16
Pooled SEM	3.7	0.4	0.05	0.02	0.03	0.2
			Probabilities			
Polynomial contrasts						
Linear	0.001	0.001	0.001	0.001	0.001	0.001
Quadratic	0.01	0.04	0.01	0.02	0.08	0.06
Cubic	0.89	0.42	0.21	0.11	0.09	0.13

Table 5. Effects of vitamin E supplementation on some serum enzymes and mineral concentrations in broiler chicks reared under heat stress (32 °C) (n = 9)

Vitamin E (mg/kg)	SGOT	SGPT	AP	Ca	P
		(U/l)		(mg/dl)	
0	166	16.81	260	17.12	5.92
62.5	168	16.65	262	16.86	6.08
125	164	14.63	280	18.36	6.68
250	166	16.00	295	20.90	7.04
500	162	15.40	290	20.95	7.09
Pooled SEM	13.69	4.58	8.24	1.36	0.11
Polynomial contrasts			Probabilities		
Linear	0.87	0.48	0.001	0.001	0.001
Quadratic	0.96	0.57	0.07	0.42	0.48
Cubic	0.51	0.41	0.16	0.51	0.29

creases in dietary vitamin E supplementation up to 500 mg/kg of diet did not change the concentrations of protein, albumin, glucose, or uric acid. Serum activities of SGOT and SGPT were not influenced by dietary vitamin E supplementation ($P > 0.10$). However, serum activity of AP increased linearly ($P = 0.001$) with increasing dietary vitamin E supplementation. Increasing dietary vitamin E supplementation also caused linear increases ($P = 0.001$) in serum concentrations of Ca and P.

DISCUSSION

In the present study, dietary vitamin E supplementation, at 250 mg/kg of diet in particular, resulted in a maximal economic benefit in performance of broilers reared under heat stress (32°C). Similar to our results, Bollengier-Lee *et al.* (1998) have shown that dietary supplementation of vitamin E (d1-α-tocopheryl acetate) can alleviate the negative effects of chronic heat stress in laying hens. A supplement of 500 mg vitamin

E/kg increased egg production by an average of 7% in hens stressed for 4-week periods at 2 different ages and stage of lay compared to birds fed basal diet (Bollengier-Lee *et al.*, 1998). In contrast, a dietary supplement of 125 mg/kg gave only a 4% increase in egg production (Bollengier-Lee *et al.*, 1998). In addition, Bollengier-Lee *et al.* (1999) reported dietary supplement of 250 mg/kg as optimum provided before, during, and after heat stress in laying hens for partially alleviating the adverse effects of chronic heat stress. In addition, Kennedy *et al.* (1992) examined the productivity of 168 broiler flocks fed diets containing either 50 mg/kg or 180 mg/kg dietary vitamin E. The authors reported that at the grater level of vitamin E supplement, productivity was 8.4% greater as a result of improvements in both feed conversion efficiency and higher average weight gain. Similarly, Sahin and Kucuk (2001) found that dietary vitamin E inclusions resulted in a greater performance in Japanese quails reared under heat stress (34°C). It was shown that dietary supplements can modify gene expression induced by heat shock *in vivo* and have a protective role against oxidative stress by enhancing the level of endogenous antioxidants and inducing hsp-70 gene expression (Ushakova *et al.*, 1996). Organisms respond to elevated temperatures and to chemical and physiological stresses by an increase in the synthesis of heat shock proteins (Hsp) or stress proteins. Under such high environmental temperature of the present study, the expression of genes for Hsp must have also enhanced and the proteins must have accumulated in cells. The cells with increased Hsp exhibit tolerance against the additional stress. Hsp29 was identified in broiler chickens' heart muscle and lungs following an *in vivo* heat stress. At the cellular level, when cells are subjected to different stresses such as hyperthermic shock, radiation, toxins, viral infections, ethanol, arsenite, oxygenation after anoxia, or gene transfer, Hsp are overexpressed. In this way, they exert a cytoprotective effect, protecting the cells against harmful insults, thus making the cells resistant to apoptosis (Coronato *et al.*, 1999). Although the mechanisms by which Hsp protect cells are not clearly understood, their expression can be modulated by cell signal transducers, such as changes in intracellular pH, cyclic AMP, Ca^{2+}, Na^{+}, inositol trisphosphate, protein kinase C, and protein phosphatases. (Kiang and Tsokos, 1998). Moreover, it was found that constitutive expression of a major heat shock protein, Hsp70, mediates the protection against toxic effects (i.e., cell lysis) of nitric oxide, a reactive oxygen intermediate created through oxygen-derived free radical action (Bellmann *et al.*, 1996). Herein the results of the present study provide some evidence that under the chosen temperature, somehow vitamin E supplementation might have helped broilers to responded to heat stress; yielding a better performance, perhaps due to increased Hsp synthesis.

Serum concentrations of T_3 and T_4 were greater with greater dietary vitamin E supplements. These results could have been due to the positive effects of vitamin E, alleviating the negative effects of heat stress. Several researchers reported reduced concentrations of T_3 and T_4 in heat-stressed chickens (Heninger *et al.*, 1960; Johnson, 1981; Bowen *et al.*, 1984). The inverse relationship between plasma concentration of T_3 and environmental temperature has been also well-known (Hilman *et al.*, 1985; May *et al.*, 1986; Iqbal *et al.*, 1990). In addition, Yahav (1999) reported a positive linear correlation between plasma T_3 concentration and feed intake and weight gain in turkeys at a constant ambient temperature. In accordance with the results of the performance data of the present study, greater T_3 concentrations with greater dietary vitamin E supplement supported a greater performance. It has been suggested that thyroid activity is affected by environmental temperature (McNabb and King, 1993; Yahav *et al.*, 1997). Huston and Carmon (1962) reported thyroid size and thyroid secretion rate decreased at high temperatures and increased at low temperatures. Jonier and Huston (1957) also reported smaller thyroid sizes at high environmental temperatures and suggested that at high temperatures thyroid activity and subsequently metabolic rate might be reduced. Lower serum concentrations of T_3 and T_4 with lower dietary vitamin E were probably an indication of a greater effect of heat stress (i.e., high temperature) in broiler chickens of the present study.

Serum concentration of ACTH was lower with greater dietary vitamin E, probably indicating lowered effects of heat stress with greater supplemental vitamin E. Similarly, Sahin *et al.* (2002) found that heat stress tended to elevate plasma corticosterone concentrations which were significantly reduced with vitamin E supplementation in a diet of Japanese quails. In addition, increasing concentrations of ACTH was parallel to increases in serum glucose, uric acid, and triglycerides concentrations. This result was probably due to the greater catabolic effect (or concentration) of ACTH, yielding more of glucose, uric acid, and triglycerides in the serum. Similar to results of the present study, Sahin *et al.* (2002) found that vitamin E supplementation increased plasma protein concentration while markedly decreased blood glucose and cholesterol concentrations in Japanese quails under heat stress (34°C).

Although serum activities of SGOT and SGPT remained unchanged, increasing dietary vitamin E caused an increase in serum activity of AP as well as serum concentrations of Ca and P. In agreement with our results, Sahin *et al.* (2002) found increased plasma Ca and P concentrations in heat-stressed Japanese quails fed a diet supplemented with vitamin E.

It is apparent from the results of the present study that a dietary α-tocopherol acetate supplementation offers a feasible way to reduce the losses in performance of broilers due to the negative effects of heat stress. Vitamin E is the major chain-breaking antioxidant in lipid phases such as cellular membrane or low density lipoproteins (Tappel, 1968; Gey, 1998). Vitamin E, therefore, acts as the primary antioxidant by quenching lipid peroxyl radicals.

Results of the present study conclude that a 250 mg per kg of diet of vitamin E provides an optimal performance in broiler chicks reared under heat stress. Vitamin E supplementation at such a level can be considered as a protective management practice in a broiler diet, reducing the negative effects of heat stress. The results of the study also apply to the human nutrition and health. Dietary supplementation of vitamin E in poultry not only can increase the efficiency of animal production but also can provide a healthier poultry meat to human consumption. By having a greater degree of deposited vitamin E, upon supplementation, poultry meat consumption can have health benefits to humans in terms of consuming a natural vitamin E source. The health benefits of vitamin E has been proven for a long time in terms of fighting against cancer.

Acknowledgements

The authors thank Veterinary Control and Research Institute of Ministry of Agriculture, Elazig for providing the research facility, Köy-Tür Company, Elazig for providing the research animals, also the Roche Company, Levent-Istanbul for providing the vitamin E.

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Received: 02–02–11
Accepted after corrections: 02–04–17

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