Effects of vitamin E and vitamin A supplementation on performance, thyroid status and serum concentrations of some metabolites and minerals in broilers reared under heat stress (32°C)

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ABSTRACT: An experiment on Cobb-500 male broilers was conducted to evaluate the effects of vitamin E (α-tocopherol-acetate), vitamin A (retinol), and their combination on broiler performance and serum concentrations of triiodothyronine (T_3), thyroxine (T_4), adrenocorticotropine hormone (ACTH) and some metabolite and mineral concentrations in broilers reared under heat stress (32°C). One day-old 120 broilers were randomly assigned to 4 treatment groups, 3 replications of 10 birds each. The birds were fed either a control diet or a control diet supplemented with either vitamin A (15 000 IU retinol/kg diet), vitamin E (250 mg α-tocopherol-acetate/kg diet), or a combination of vitamin A and E (15 000 IU retinol plus 250 mg of α-tocopherol-acetate/kg diet). Considered separately or as a combination, supplemental vitamin A and vitamin E increased feed intake (P = 0.01) and live weight gain (P = 0.03). However, feed efficiency remained similar in all treatments (P = 0.18). Serum T_3 and T_4 concentrations were also higher ($P \leq 0.001$) with vitamin A, vitamin E, and vitamin A plus vitamin E groups than those of the control. However, ACTH concentration in serum was lower ($P \leq 0.001$) in supplemental dietary vitamin groups compared with control. Serum glucose, uric acid, triglyceride, and cholesterol concentrations decreased ($P \leq 0.001$) while protein and albumin concentrations increased ($P \leq 0.001$) when both dietary vitamin E and vitamin A were supplemented. Serum activities of serum glutamic oxalate transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) were not influenced by dietary vitamin E, vitamin A nor by a combination of vitamin A and vitamin E ($P > 0.72$). However, serum activity of alkaline phosphatase (AP) increased ($P \leq 0.001$) with supplemental dietary vitamin E, vitamin A, or a combination of vitamin A and vitamin E. In addition, supplemental dietary vitamin E and vitamin A resulted in an increase in serum concentrations of both Ca and P ($P \leq 0.001$). In general, when a significant effect was found for a parameter, the magnitude of responses to vitamin supplements was greatest with the combination of vitamin A and vitamin E, rather than that of each vitamin supplement separately. The results of the present study show that supplementing a combination of dietary vitamin E and vitamin A offers a good management practice to reduce heat stress-related decreases in broiler performance.

Keywords: heat stress; vitamin E; vitamin A; broilers; performance; blood parameters

INTRODUCTION

High environmental temperatures have deleterious effects, reducing the performance of poultry. A decreased rate of growth was reported in broilers reared at high environmental temperatures (Donkoh, 1989). This negative effect of heat stress on growth rate and production is speculated to be due primarily to reduced feed intake (Hurwitz *et al*., 1980). A high ambient temperature also reduces the 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). In addition, during heat stress the plasma corticosterone concentration increases (Edens and Siegel, 1975). Donkoh (1989) also reported the effects of heat stress as reduced plasma protein and markedly increased blood glucose concentrations.

Several methods are available to alleviate the negative effects of high environmental temperature on the performance of poultry. Since it is expensive to cool animal buildings, such methods are mostly focused on the dietary manipulation. In this respect, vitamin C, vitamin E, and vitamin A are used in the poultry diet because of their anti-stress effects and also because their synthesis is reduced during the heat stress (Sykes, 1978; Hornig *et*

al., 1984; Sahin *et al*., 1999, 2001a; Naziroglu *et al*., 2000). It was observed that serum vitamin E and beta-carotene levels significantly decreased during heat stress in laying hens (Sahin *et al*., 1999). Preliminary studies indicated some benefits of vitamin E supplementation to poultry hens during heat stress (Feenster, 1985; Bollengier-Lee *et al*., 1998, 1999; Whitehead *et al*., 1998; Sahin *et al*., 2001a). Vitamin E is known to be a lipid component of biological membranes and is known to be a major chain-breaking antioxidant (Gallo-Torres, 1980; McDowell, 1989; Gey, 1998). 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 (McDowell, 1989; Packer, 1991). Therefore vitamin E protects cells and tissues from oxidative damage induced by free radicals. Vitamin A is involved in several functions of the body including vision, differentiation of epithelial cells, growth, and reproduction. The relationship between vitamin A and vitamin E has been proposed in such a way that vitamin E appears to have an important effect on the utilization and perhaps absorption of vitamin A, and vitamin E protects vitamin A from oxidative breakdown (Gallo-Tores, 1980). The objective of this study was to evaluate the effects of vitamin E and vitamin A supplementation on performance and serum concentrations of T_3, T_4, ACTH and some metabolite and mineral concentrations in broiler chicks reared under heat stress (32°C).

MATERIAL AND METHOD

A total of 120 one-day-old Cobb-500 chicks supplied by Koy-Tür Company, Elazig, Turkey, were used in the study. The birds were randomly assigned according to their initial body weights to 4 treatment groups, 3 replications of 10 birds each. All pens were bedded with wood-shavings litter and equipped with feeders and waterers in environmental chambers with 24.4 cm^2 per bird. The birds were fed either control diet or control diet supplemented with either 15 000 IU of retinol/kg diet (vitamin A), or 250 mg of α-tocopherol-acetate/kg diet (vitamin E), or a combination of 15 000 IU retinol/kg diet and 250 mg α-tocopherol-acetate/kg diet (vitamin A and E). Vitamin E (ROVIMIX® E-50 SD; fairly stable source of vitamin E in feed) and vitamin A (ROVIMIX® A-500 stable source of vitamin A in feed) were provided from 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 20–23% (starter-grower) protein and 3 200 kcal/kg ME. The diet and fresh water were offered *ad libitum*.

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

	Starter	Grower
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
Ground limestone	0.53	0.58
Calcium carbonate	0.10	0.16
DL-methionine	0.24	0.25
Lysine	–	0.12
Avilamycin	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 (kcal/kg)	3 200	3 200
CP	23.00	20.03
Crude Fat	7.04	6.35
OM	80.12	80.46

*Premix (Rovimix 124/V) supplied per 1 kg: vitamin A, 7 500 IU; cholecalciferol, 1 500 IU; vitamin E, 7 500 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, 50 mg

** Premix (Remineral CH) supplied per 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

During the experiment, light was provided continuously (24 hours) and room temperature was maintained at 32°C using electrical brooders for 24 hours a day. Relative humidity inside the hen house ranged from 42 ± 6%. The experiment was conducted between July 28 and September 7. Feed intake and body weight were determined at weekly intervals. Weight gain and feed efficiency of experimental groups were then calculated.

At the end of day 42, 10 birds randomly chosen from each treatment 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. Serum samples were thawed at room temperature, and T_3, T_4, and ACTH concentrations were determined using commercially available radioimmunoassay kits according to the procedures described by Renden *et al*. (1994). (Liaison® T_3, T_4 and TSH, Byk-Sangtec Diagnostica, Dietzenbach-Germany; Immulite

2000 ACTH, L2 KAC2, DPC, LA). 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 (Technicon RA-XT, New York, USA). Chemical analyses of starter and grower diets were run using international procedures of AOAC (1990).

The data were analyzed by ANOVA using the GLM procedure of SAS (1996). Differences between the means ($P < 0.05$) were determined using Duncan's multiple range test.

RESULTS

The effects of supplemental dietary vitamin A and vitamin E during heat stress on broiler performance are shown in Table 2. Considered separately or as a combination, supplemental vitamin A and vitamin E increased feed intake ($P = 0.01$) and live weight gain ($P = 0.03$). However, feed efficiency remained similar in all treatments ($P = 0.18$). Parallel increases both in feed intake and weight gains resulted in a similar feed efficiency response. Serum T_3 and T_4 concentrations were also higher ($P \leq 0.001$) with vitamin A, vitamin E, and vitamin A plus vitamin E groups than those of the control (Table 3). However, ACTH concentration in serum was lower ($P \leq 0.001$) in supplemental dietary vitamin groups compared with control. Dietary vitamin E and vitamin A had significant effects on most blood parameters measured in the present study (Table 4). Serum glucose, uric acid, triglyceride, and cholesterol concentrations decreased ($P \leq 0.001$) while protein and albumin concentrations increased ($P \leq 0.001$) when both dietary vitamin E and vitamin A were supplemented. Serum activities of SGOT and SGPT were not influenced by dietary vitamin E, vitamin A nor by a combination of vitamin A and vitamin E ($P > 0.72$). However, serum activity of AP increased ($P \leq 0.001$) with supplemental dietary vitamin E, vitamin A, or a combination of vitamin A and vitamin E. In addition, supplemental dietary vitamin E and vitamin A resulted in an increase in serum concentrations of both Ca and P ($P \leq 0.001$). In general, when a significant effect was found for a parameter, the magnitude of responses to vitamin supplements was greatest with the combination of vitamin A and vitamin E rather than that of each vitamin supplement separately.

DISCUSSION

In the present study, inclusion of vitamin E and vitamin A in the diet caused improvements in live weight gain and feed intake, probably alleviating the negative effects of heat stress on broilers under heat stress (32°C). Dietary vitamin E supplementation in laying hens is also common in the literature. Bollengier-Lee *et al*. (1998) showed that dietary supplementation with vitamin E (α-tocopherol acetate) can alleviate the negative effects of chronic heat stress on laying hens. A supplement of 500 mg vitamin E/kg increased egg production by an average of 7% in hens heat-stressed for 4-week periods at 2 different ages and stage of lay compared with birds given 10 mg vitamin E/kg diet (Bollengier-Lee *et al*., 1998). In contrast, in the same study (Bollengier-Lee *et al*., 1998) a dietary supplement of 125 mg/kg gave only a 4% increase in egg production. In addition, Bollengier-Lee *et al*. (1999) reported dietary supplement of 250 mg

Table 2. Effects of vitamin A (15 000 IU/kg) and vitamin E (250 mg/kg) on performance of broilers reared under heat stress (32°C)

Item	Control	Vitamin A	Vitamin E	Vitamin A + Vitamin E	SEM
Feed intake (g)	3116.8[a]	3151.8[b]	3227.1[c]	3314.8[d]	15.21
Body weight (g)	1832.0[a]	1890.14[b]	1900.05[bc]	1985.78[c]	13.10
Feed efficiency*	0.58	0.59	0.59	0.60	0.001

[a,b,c,d] means with no common superscript differ significantly ($P \leq 0.05$)

*feed efficiency: gain in body weight (g)/feed consumed (g)

Table 3. Effects of supplemental vitamin A (15 000 IU/kg) and vitamin E (250 mg/kg) on serum concentrations of T_3, T_4, and ACTH (ng/ml) in broilers reared under heat stress (32°C) ($n = 10$)

Item	Control	Vitamin A	Vitamin E	Vitamin A + Vitamin E	SEM
T_4	4.11[a]	4.42[b]	4.45[b]	4.55[c]	0.02
T_3	0.73[a]	0.82[b]	0.83[b]	0.88[c]	0.003
ACTH	17.90[a]	17.05[b]	16.93[b]	16.13[c]	0.45

[a,b,c,d] means with no common superscript differ significantly ($P \leq 0.05$)

Table 4. Effects of vitamin A (15 000 IU/kg) and vitamin E (250 mg/kg) on some serum metabolites, enzymes, and mineral concentrations in broilers reared under heat stress (32°C)

Item	Control	Vitamin A	Vitamin E	Vitamin A + Vitamin E
Glucose (mg/dl)	229 ± 2.8 [a]	214 ± 3.56 [b]	207 ± 3.3 [c]	191 ± 3.4 [d]
Total protein (g/dl)	4.2 ± 0.004 [d]	4.4 ± 0.003 [c]	4.5 ± 0.003 [b]	4.6 ± 0.002 [a]
Uric acid (g/dl)	4.51 ± 0.03 [a]	4.43 ± 0.05 [b]	4.29 ± 0.03 [c]	4.09 ± 0.04 [d]
Albumin (g/dl)	1.36 ± 0.04 [d]	1.48 ± 0.02 [c]	1.63 ± 0.03 [b]	1.72 ± 0.03 [a]
Triglycerides (mg/dl)	259 ± 3.8 [a]	250 ± 6.3 [b]	231 ± 4.0 [c]	215 ± 4.5 [d]
Cholesterol (mg/dl)	339 ± 3.3 [a]	337 ± 2.4 [a]	324 ± 2.8 [b]	315 ± 2.3 [c]
SGOT (U/L)	166 ± 4.5	166 ± 1.8	162 ± 2.5	164 ± 2.8
SGPT (U/L)	17 ± 1.8	16 ± 1.6	16 ± 1.2	14 ± 0.7
AP (U/L)	248 ± 4.0 [d]	264 ± 4.5 [c]	282 ± 5.7 [b]	298 ± 4.0 [a]
Ca (mg/dl)	14.58 ± 0.6 [d]	16.46 ± 0.4 [c]	17.58 ± 0.3 [b]	20.02 ± 0.5 [a]
P (mg/dl)	5.58 ± 0.3 [d]	6.38 ± 0.6 [c]	6.88 ± 0.3 [b]	7.14 ± 0.8 [a]

[a, b, c, d] means with no common superscript differ significantly ($P \leq 0.05$)

per kg as optimum provided before, during, and after heat stress of laying hens for partially alleviating the adverse effects of chronic heat stress. Sahin and Küçük (2001) also found that dietary vitamin E inclusions, at 250 mg/kg in particular, resulted in higher performance of Japanese quails reared under heat stress. Demir *et al*. (1995) showed that dietary supplementation of vitamin A (15 000 IU retinol per kg) can alleviate the negative effects of heat stress (31°C), resulting in increases in feed intake, egg production, eggshell weight and eggshell thickness in laying hens.

In the present study, serum concentrations of T_3 and T_4 were higher with dietary vitamin E and vitamin A treatments. These results could be due to the positive effects of vitamin E and vitamin A, 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 also been 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 or weight gain in turkeys at a constant ambient temperature. The higher T_3 concentrations of the present study with supplemental vitamin A and vitamin E also supported higher performance of broilers. 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 the thyroid size and thyroid secretion rate decreased at high temperatures and increased at low temperatures. Joiner and Huston (1957) also reported smaller thyroid sizes at high environmental temperatures and suggested that at high temperatures the thyroid activity and subsequently metabolic rate might be reduced.

Serum concentration of ACTH were lower with supplemental dietary vitamin E and vitamin A, probably indicating a lowered response to heat stress by supplementation of these two vitamins. Similarly, Sahin *et al*. (2001b) found that heat stress tended to elevate plasma corticosterone concentrations which were significantly reduced by vitamin E supplementation in Japanese quails.

The effects of vitamin E and vitamin A on blood parameters of broilers under heat stress are not available in the literature. However, the results of the present study showed similar trends for the effects of vitamin A and vitamin E on serum concentrations of T_3, T_4, and ACTH. In the present study, similar effects of vitamin E and vitamin A existed as an evidence that serum glucose, uric acid, tryglyceride, and cholesterol concentrations decreased while protein and albumin concentrations increased by adding vitamin E and vitamin A to the broiler diet. Increases in concentrations of ACTH were parallel to increases in serum glucose, uric acid, and triglyceride concentrations. This result was probably due to the higher catabolic effect (or concentration) of ACTH, yielding more glucose, uric acid, and triglycerides in the serum with supplemental dietary vitamin E and vitamin A. Similarly to the results of the present study, Sahin *et al*. (2001b) found that vitamin E supplementation increased plasma protein concentration while it markedly decreased glucose and cholesterol concentrations in Japanese quails under heat stress (34°C).

Although the serum activities of SGOT and SGPT remained unchanged, dietary vitamin A and vitamin E caused an increase in the serum activity of AP as well as serum concentrations of Ca and P. In agreement with our results, Sahin *et al*. (2001b) found increased plasma Ca and P concentrations in heat-stressed Japanese quails fed a diet supplemented with vitamin E.

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 a high environmental temperature of the present study, the expression of genes for Hsp also must have been enhanced and the proteins must have accumulated in cells. The cells with increased Hsp exhibit tolerance to the additional stress.

The inactivated steroid receptors are chaperoned into a conformation that is optimal for binding hormones by a number of Hsp, including Hsp90, Hsp70, Hsp40, and the immunophilin, FKBP52 (Hsp56). Together with its partner cochaperones, cyclophilin 40 (CyP40) and FKBP51, FKBP52 belong to a distinct group of structurally related immunophilins that modulate the steroid receptor function through their association with Hsp90 (Mark *et al*., 2001). The number of different types of Hsp varies considerably in different organisms and cell types but in all cases proteins of approximately 84 and 70 kDa are among the most prominent (Burdon, 1987). Hps29 was also identified in the heart muscle and lungs of broiler chickens following an *in vivo* heat stress. However, induction of Hps29 occurs after 3 h of heat stress, much later than the induction of Hsp90, Hsp70, and Hsp27; therefore, Hps29 may play a more specific role as a "second stage defense protein" (Einat *et al*., 1996).

Heat shock proteins are a family of constitutive proteins of all pro- and eukaryotic cells that play different physiological roles, including promotion of the folding (acquisition of tertiary structure) assembly, translocation and secretion of newly synthesized polypeptides and participation in the removal or repairing of denatured proteins acting as molecular chaperons (Coronato *et al*., 1999). Most of the Hsp interact with other proteins in cells and alter their function. When the cells are subjected to different stresses such as hyperthermic shock, radiation, toxins, viral infections, ethanol, arsenic, 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). In addition, it has been reported that the presence of Hsp72 increases survival in rat heatstroke by attenuating arterial hypotension, cerebral ischemia and neuronal damage (Yang *et al*., 1998). Moreover, it was found that the constitutive expression of a major heat shock protein, Hsp70, mediates protection against the toxic effects (i.e. cell lysis) of nitric oxide, a reactive oxygen intermediate created through oxygen-derived free radical action (Bellmann *et al*., 1996).

The induction of the heat shock genes in eukaryotes by heat and other forms of stress is mediated by a transcription factor known as heat shock factor 1 (Hsf1). Hsf1 is present in unstressed metazoan cells as a monomer with low affinity for DNA, and upon exposure to stress it is converted to an "active" homotrimer that binds the promoters of heat shock genes with high affinity and induces their transcription. The conversion of Hsf1 to its active form is hypothesized to be a multistep process involving physical changes in the Hsf1 molecule and the possible translocation of Hsf1 from the cytoplasm to the nucleus (Wu, 1995; Mercier *et al*., 1999).

Herein the results of the present study provide an evidence that under the chosen temperature, somehow vitamin E and vitamin A supplementation could helped broilers respond to heat stress better, yielding better growth and serum parameters, perhaps due to increased Hsp synthesis.

The results of the present study showed similar trends for the effects of vitamin E on vitamin A. Similarly, it was also suggested that vitamin E and vitamin A act synergistically (McDowell, 1989). In addition, the overall antioxidant potential was reported to possibly be more efficient and crucial than single antioxidant nutrients (Gallo-Torres, 1980). Therefore, supplement of the combination of vitamin A and vitamin E should offer better results than when supplemented separately despite the fact that vitamin A decreases intestinal vitamin E uptake in chickens (Sklan, 1983).

The results of the present study show that dietary vitamin E and vitamin A have synergistic effects, and as evidences from the results of performance and the blood parameters, supplementing a combination of dietary vitamin E and vitamin A offers a good management practice to reduce heat stress-related decreases in broiler performance.

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