Protective role of supplemental vitamin E on lipid peroxidation, vitamins E, A and some mineral concentrations of broilers reared under heat stress

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ABSTRACT: An experiment utilizing Cobb-500 male broilers was conducted to evaluate the effects of vitamin E supplementation at various concentrations on malonyldialdehyde (MDA) as an indicator of lipid peroxidation, serum and liver concentrations of antioxidant vitamins and some minerals of 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 (dl-α-tocopherol acetate) at 62.5, 125, 250, or 500 mg/kg of diet. Increased supplemental vitamin E linearly increased serum vitamin E and A, but decreased ($P = 0.001$) MDA concentrations. Increasing dietary vitamin E supplementation also resulted in linear increases in liver vitamin E and A concentrations, but linear decreases in MDA concentrations ($P = 0.01$). Increasing dietary vitamin E caused a linear increase in serum concentrations of Fe and Zn ($P = 0.001$), but a decrease in serum concentration of Cu ($P = 0.001$). Results of the present study conclude that in broiler chicks reared under heat stress a 250 mg of vitamin E supplementation can be considered as a protective management practice in a broiler diet, reducing the negative effects of heat stress. ***For free full paper in pdf format see***
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Keywords: malonyldialdehyde; vitamin A; vitamin E; Fe, Zn, Cu

INTRODUCTION

High ambient temperature reduces feed intake, live weight gain, feed efficiency (Donkoh, 1989), thus negatively influences the performance of broilers. Hurwitz *et al.* (1980) suggested that decrease in growth rate was due partly to the decrease in feed intake. Animals stressed under improper environmental conditions or subjected to an artificial stress via ACTH and epinephrine injections are found to have reduced α-tocopherol, retinol, and ascorbic acid concentrations in plasma and blood cells (McDowell, 1989), whereas lipid peroxidation levels were found to be high in plasma and tissues due to increased production of free radicals (Naziroğlu *et al.*, 2000). Moreover, heat stress impairs absorption of vitamins A, E and C, and thus, increases the requirement of these vitamins (Naziroğlu *et al.*, 2000; Sahin *et al.*, 1999; Klasing, 1998) On the other hand, stress causes reduction in plasma and tissue concentrations of minerals such as Fe, Zn and Cu which are related to immune system (Beisel, 1982).

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), also because of the fact that vitamin E synthesis is reduced during the heat stress (Feenster, 1985; Whitehead *et al.*, 1998; Sahin *et al.*, 1999; Boliengier-Lee *et al.*, 1999; Naziroğlu *et al.*, 2000). 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). Determination of some blood parameters has a substantial merit in understanding metabolic changes in heat-stressed poultry fed with dietary vitamin E. Therefore, the objective of this study was to evaluate the effects of dietary vitamin E supplementation on serum and liver MDA, vitamin E, and vitamin A status and se-

rum Fe, Zn, and Cu concentrations in broilers under heat stress (32°C).

MATERIAL AND METHODS

Animals and experimental design

One hundred and fifty 1 day-old Cobb-500 male chicks obtained from Koy-Tur Company, Elaziğ, 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 received either a basal diet or basal diet supplemented with vitamin E (dl-α-tocopherol acetate) either 62.5, 125, 250, or 500 mg/kg of diet. Vitamin E (ROVIMIX® E-50 SD; fairly stable source of vitamin E in feed) were provided by a commercial company (Roche, Levent-Istanbul, Turkey). 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% (grower-starter) protein and 3200 kcal/kg ME. The diets and fresh water were offered ad libitum. During the experiment, light was provided continuously (24 hours). Average room temperature was 32°C. Relative humidity inside the hen house was 42%. The experiment was conducted between July 28 and September 7.

At the end of day 42, 10 birds randomly chosen from each treatment were slaughtered and blood was collected. Blood samples were centrifuged at 3000 × g for 10 min and sera were collected and stored at –20°C.

Chemical analysis

Chemical analysis of the diet were run using international procedures of AOAC (1990). Lipid peroxidation as thiobarbituric acid-reactive substances (TBARS) were determined in serum and liver samples by method of Placer *et al.* (1966) as modified by Matkovics *et al.* (1989). The values of TBARS material were expressed in terms of malondialdehyde (μmol/ml serum or μmol/g liver). Vitamins E and A concentrations in serum and liver samples were determined via a modification of the method described by McMurray *et al.* (1980). Fluorimetric detection of vitamin A used excitation and emission wavelengths of 330 and 480 nm, respectively. The relevant wavelengths for vitamin E detection were 292 and 330 nm. Calibration was performed using standard solutions of all trans retinal and α-tocopherol in methanol. Fe, Zn, and Cu concentrations of serum were measured

Table 1. Ingredients and chemical analyses of the starter and grower diets for broiler

	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
Chemical Analyses		
Dry matter (%) 89.65	89.68	
MJ (kg)	13.37	13.37
Crude protein (%)	23.00	20.03
Crude fat (%)	7.04	6.35
Crude fiber, %	3.74	3.56
Ash (%)	9.53	9.22
Organic matter (%)	80.12	80.46

* Ingredients in 2 kg of Premix (Rovimix 124/V): vitamin A, 15 000 000 IU; cholecalciferol, 3 000 IU, vitamin E, 15 000 IU; menadione, 2500 mg; vitamin B_1, 1 000 mg; vitamin B_2, 10 000 mg; niacin, 70 000 mg; d-pantothenic acid, 20 000 mg; vitaminB_{12}, 4 000 mg; folic acid, 2 000 mg; biotin, 100 mg

** Premix (Remineral CH) supplied for 2 kg : Mn, 80 000 mg; Fe, 25 000 mg; Zn, 50 000 mg; Cu, 7 000 mg; Iodine, 300 mg; Se, 150 mg; Choline cloride, 350 000 mg

at specific wavelengths for each element by using atomic absorption spectrometer (Shimadzu AA-660). Calibrations for the mineral assays were conducted with a series of mixtures containing graded concentrations of standard solutions of each element.

The data were analyzed using the GLM procedure of SAS (1989). Linear, quadratic and cubic polynomial contrasts were used to evaluate treatment effects.

RESULTS

The effects of supplemental dietary vitamin E during heat stress on serum and liver MDA, vitamins E and A of broilers are shown in Tables 2 and 3. Increased sup-

Table 2. Effects of vitamin E supplementation on serum concentrations of MDA, vitamins E and A of broiler chicks reared under heat stress (32°C) (*n* = 10)

Vitamin E (mg/kg)	MDA (μmol/ml)	Vitamin E (μg/ml)	Vitamin A (μg/ml)
Treatments			
0	2.03	3.37	0.35
62.5	1.45	7.30	0.43
125	1.16	10.5	0.48
250	0.98	13.24	0.51
500	0.93	16.83	0.52
Pooled SEM	0.08	0.33	0.13
Probabilities			
Polynomial contrasts			
Linear	0.001	0.001	0.001
Quadratic	0.331	0.542	0.680
Cubic	0.450	0.368	0.286

Table 3. Effects of vitamin E supplementation on liver concentrations of MDA, vitamins E and A of broiler chicks reared under heat stress (32°C) (*n* = 10)

Vitamin E (mg/kg)	MDA (μmol/ml)	Vitamin E (μg/ml)	Vitamin A (μg/ml)
Treatments			
0	5.98	3.20	0.32
62.5	4.63	7.30	0.43
125	4.23	8.15	0.48
250	3.56	13.82	0.51
500	3.38	16.00	0.52
Pooled SEM	0.46	0.42	0.03
Probabilities			
Polynomial contrasts			
Linear	0.001	0.001	0.001
Quadratic	0.856	0.542	0.680
Cubic	0.653	0.368	0.286

Table 4. Effects of vitamin E on serum concentrations of Fe, Zn and Cu in broilers reared under heat stress (32°C) (*n* = 10)

Vitamin E (mg/kg diet)	Fe (μg/dl)	Zn (μg/dl)	Cu (μg/dl)
Treatments			
0	292	105	25
62.5	305	108	23
125	328	122	21
250	356	131	18
500	378	138	16
Pooled SEM	22.6	6.5	1.5
Probabilities			
Polynomial contrasts			
Linear	0.001	0.001	0.001
Quadratic	0.550	0.350	0.750
Cubic	0.842	0.480	0.530

plemental vitamin E linearly increased serum and liver vitamins E and A, but decreased ($P = 0.001$) MDA concentrations ($P = 0.001$). Dietary vitamin E had significant effects on minerals measured at the present study (Table 4). Serum copper concentration decreased linearly ($P = 0.001$) while, iron and zinc concentrations increased linearly ($P = 0.001$) when dietary vitamin E supplementation increased.

DISCUSSION

In the present study, effects of dietary vitamin E supplementation on serum and liver MDA, vitamins E, A and serum Fe, Zn, and Cu concentrations in broilers reared under heat stress (32°C) were investigated. This study suggests that lipid peroxides formed under heat stress conditions can be partially counteracted by dietary inclusion of antioxidants such as vitamin E. To the authors' knowledge, this is the first study carried out on the ability of vitamin E to reduce the prooxidative effects of heat stress in broilers. It is known that vitamin E diminishes the peroxidation of polyunsaturated lipids via scavenging free radicals (McDowell, 1989; Packer and Landvik, 1990). Vitamin E is also an important structural component of biological membranes, contributing to their stability (Gallo-Torres, 1980). The methyl groups of tocopherol interact with the cis double bounds of the fatty acids to form a stable complex in membrane phospholipids (McDowell, 1989; Packer and Landvik, 1990).

It is well known that heat stress causes an increased production of MDA (Naziroğlu *et al.*, 2000; Halliwell and Gutteridge, 1989). On the other hand, heat stress reduces the concentrations of vitamins E, C, A, and some minerals such as Fe and Zn in serum and liver (Klasing, 1998; Feenster, 1985). In the present study, serum and liver MDA level decreased, while vitamins E and A concentrations increased when dietary vitamin E was increased. It has been speculated that vitamin E supplementation may have reduced synthesis of MDA in liver by protecting liver from lipid peroxidation and cell membranes from damage. Vitamin E is the first line of defense against lipid peroxidation (McDowell, 1989). By its free radical quenching activity, it breaks chain propagation and thus terminates free radical attack at an early stage; such an effect of vitamin E is on polyunsaturated fatty acids of biomembranes (McDowell, 1989) The concentration of tocopherol in tissues is inversely correlated to lipid peroxidation (Kornburst and Mavis, 1980). Similar to the results of the present study, Morrissey *et al.* (1997) reported that dietary supplementation of chicken diets with α-tocopherol increased tissues α-tocopherol concentrations, while markedly decreased MDA concentration. Liver damage is detected by the measurement of the activities of plasma enzymes that have been released in to blood from the cells. According to antioxi-

dant theory (McDowell, 1989; Gallo-Torres, 1980), when the concentrations of antioxidant vitamins decreases, lipid peroxidation increases in the plasma and tissues leading to damage of cell membranes. Bollengier-Lee *et al.* (1999) have shown that dietary supplementation of vitamin E (250 mg/kg) can alleviate the negative effects of heat stress by increasing serum and liver vitamin E levels.

Vitamin A is the precursors of beta-carotene and can function as an effective radical-trapping antioxidant (McDowell, 1989). Vitamin A represents a previously unknown class of biological antioxidants. There is ample evidence that vitamin A is a very effective quencher of singlet oxygen (McDowell, 1989; Burton and Ingold, 1984). Sahin *et al.* (1999) reported that serum vitamin A concentration decreases upon stress conditions. Similarly, McDowell (1989) indicated that stress conditions cause reduction in conversion of carotene to vitamin A. Serum and liver vitamin A concentrations increased by dietary vitamin E in the present study. Similar results were reported in a previous study (Sunder *et al.*, 1997). These results confirm the thesis that vitamin E supply and vitamin A concentration in the liver are correlated positively (Brubacher *et al.*, 1964). Overall antioxidant potential has been reported to possibly be more efficient and crucial than single antioxidant nutrients (Tufft and Nockles, 1991). Dietary vitamin E appear to have an important effect on the utilization and perhaps absorption of cartotenoids. It is uncertain whether the vitamin E contribute directly to efficient absorption of or whether protect both carotene and vitamin A from oxidative breakdown.

It has been reported that stress conditions decrease Fe and Zn but increase Cu concentrations of serum and tissue in poultry (Butler and Curtis, 1973; Beisel, 1982; Tufft and Nockles, 1991; Klasing, 1998). In the present study, increasing dietary vitamin E caused an increase in serum concentrations of Fe and Zn, but a decrease in serum Cu concentrations (Table 4). It is known that dietary ascorbic acid causes iron release into the serum, thus increasing the serum iron concentration. Vitamin E is also thought to have similar effects to those of ascorbic acid (Linder, 1991). It has been reported that zinc stabilizes the red cell membrane against cellular changes caused by peroxidations and that zinc plays a role similar to that of vitamin E in reducing peroxidative damage on cellular membrane (Pond *et al.*, 1995). Frank *et al.* (1986) reported that dietary vitamin E did not cause any change in serum and liver concentrations of zinc under thermo-neutral conditions. However, our results showed that dietary vitamin E increased serum zinc and iron concentrations under heat stress. On the other hand, in agreement with results of present study, an opposite relation has been reported between dietary Cu and serum and liver concentrations of vitamin E (Kutsky, 1981; Van Saun, 1990).

Vitamin E protect the cell from the detrimental effects of peroxidation. Vitamin E is present in the membrane components of the cell and prevents peroxide formation (Mc Dowell, 1989). The absorption of vitamin E is facilitated by the formation of micelles which are then solubilized by the action of bile salts and pancreatic juices in the intestines (Gallo-Torres, 1980; Putnam and Comben, 1987). 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.

Results of the present study conclude that a 250 mg 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.

Acknowledgements

REFERENCES

A.O.A.C. (1990): Official Methods of Analysis Association of Agricultural Chemists Virginia. D. C., U.S.A.

Beisel W.R. (1982): Single nutrients and immunity. Am. J. Clin. Nutr., *35*, 442–451.

Bollengier-Lee S., Mitchell M.A., Utomo D.B., Williams P.E.V., Whitehead C.C. (1998): Influence of high dietary vitamin E supplementation on egg production and plasma characteristics in hens subjected to heat stress. Brit. Poultry Sci., *39*, 106–112.

Bollengier-Lee S., Williams P.E.V., Whitehead C.C. (1999): Optimal dietary concentration of vitamin E for alleviating the effect of heat stress on egg production in laying hens. Brit. Poultry Sci., *40*, 102–107.

Brubacher G., Scharer K., Studer A., Wiss O. (1964): Uber die gegensetiege Beeinflussung von vitamin E, vitamin A and carotinoiden. Z. Ernahrungswiss., *5*, 190–202.

Burton G., Ingold K.U. (1984): β-Carotene: An unusual type of lipid antioxidant. Science, *224*, 569–573.

Butler E.J., Curtis M.J. (1973): The effect of *Escherichia coli* endotoxin and ACTH on the plasma zinc concentration of the domestic fowl. Res. Vet. Sci., *15*, 367–269.

Donkoh A. (1989): Ambient temperature: a factor affecting performance and physiological response of broiler chickens. Int. J. Biometeorol., *33*, 259–265.

Feenster R. (1985): High temperatures decrease vitamin utilization. Mis. Poult., 38–41.

Frank A., Pehrson B., Petersson L.R. (1986): Concentration of some important elements in liver of young cattle supplemented with selenite enriched feed. J. Vet. Med. A., *33*, 4212–4225.

Gallo-Torres D.C. (1980): Absorption, blood transport and metabolism of vitamin E. In: Maclin L.J. (ed.): A Comprehensive Treatise. Marcel Dekker, New York. 170–267.

Halliwell B., Gutteridge J.M.C. (1989): Free Radicals in Biology and Medicine. 2nd ed. Oxford University Press, New York.

Hurwitz S., Weiselberg M., Eisner U., Bartov I., Riesenfeld G., Sharvit M., Niv A., Bornstein S. (1980): The energy requirements and performance of growing chickens and turkeys, as affected by environmental temperature. Poultry Sci., *59*, 2290–2299.

Klasing K.C. (1998): Comparative Avian Nutrition. University Press, Cambridge. 277–299.

Kornburst D. J., Mavis R.D. (1980): Relative susceptibility of microsomes from lung, heart, liver, kidney, brain and testes to lipid peroxidation: Correlation with vitamin E content. Lipids, *15*, 315–320.

Kutsky R J. (1981): Handbook of Vitamins, Minerals and Hormones. 2nd ed. VNR, New York. 157–207.

Linder M.C. (1991): Nutrition and metabolism of the trace elements. In: Linder M.C. (ed.): Nutritional Biochemistry and Metabolism with Clinical Applications. Elsevier, New York. 215–276.

Matkovics B., Szabo I., Varga I.S. (1989): Determination of enzyme activities in lipid peroxidation and glutathione pathways. Lab. Diagn., *15*, 248–249.

McDowell L.R. (1989): Vitamins in Animal Nutrition – Comparative Aspects to Human Nutrition. Vitamin A and E. Academic Press, London. 10–52, 93–131.

McMurry C.H., Blanchflower W.J., Rice D.A. (1980): Influence of extraction techniques on the determination of α-tocopherol in animal feedstuffs, J. Assoc. Off. Anal. Chem., *63*, 1258–1261.

Morrissey P.A., Brandon S., Buckley D.J., Sheehy P.J.A., Frigg M. (1997): Tissue content of α-tocopherol and oxidative stability of broilers receiving dietary α-tocoperyl acetate supplement for various periods post-slaughter. Brit. Poultry Sci., *38*, 84–88.

National Research Council (1994): Nutrient requirements of poultry National Academy Press. Washington, D.C. Ninth Revised Edition.

Naziroğlu M., Şahin K., Şimşek H., Aydilek N., Ertaş O.N. (2000): The effect of food withdrawal and darkening on lipid peroxidation of laying hens in high ambient temperatures. Dtsch. Tierarztl. Wschr., *107*, 199–202.

Packer L. (1991): Protective role of vitamin E in biological systems. Am. J. Clin. Nutr., *53*, 1050–1055.

Packer L., Landvik S. (1990): Vitamin E in biological systems. In: Emerit I., Packer L., Auclair (eds.): Antioxidants in Therapy and Preventive Medicine. Plenum, New York. 93–103.

Placer Z.A., Cushmann L.L., Johnson B.C. (1966): Estimation of products of lipid peroxidation in biochemical systems. Anal. Biochem., *16*, 359–364.

Pond W.G., Church D.C., Pond K.R. (1995): Zinc. In: Basic Animal Nutrition and Feeding. John Wiley & Sons. Fourth Ed. New York. 190–193.

Putnam M.E., Comben N. (1987): Vitamin E. Vet. Rec., *121*, 541–545.

SAS Institute (1989): SAS® User's Guide: Statistics. SAS Institute Inc., Cary, NC.

Sunder A., Richter G., Flachowsky G. (1997): Influence of different concentrations of vitamin E in the feed of laying hens on the vitamin E-transfer into the egg. Proc. Soc. Nutr. Physiol., *6*, 14–152.

Sahin K., Ertaş O.N., Güler T. (1999): Sicaklik stresi altindaki yumurta tavuklarinda farkli yemleme yöntemlerinin vitamin A, vitamin E ile bazi kan parametreleri üzerine etkileri. In: Serbest Radikaller ve Antioksidanlar Araştirma Dernegi II, Ulusal Kongresi. P-24 58.

Tufft L.S., Nockles C.F. (1991): The effects of stress, *Escherichia coli*, dietary Ethylenediamintetraacetic acid, and their interaction on tissue trace elements in chicks. Poultry Sci., *70*, 2439–2449.

Van Saun R.J. (1990): Rational approach to selenium supplementation essential. Feedstuffs, *15*, 15–18.

Whitehead C.C., Bollengier-Lee S., Mitchell M.A., Williams P.E.V. (1998): Vitamin E can alleviate the depression in egg production in heat stressed laying hens. In: Proc. of Spring meeting, WPSA-UK Branch. 55–56. Scarborough.

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