

The Effects of Vitamin E on the Antioxidant System, Egg Production, and Egg Quality in Heat Stressed Laying Hens

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Received: 12.10.2006

Abstract: An experiment was carried out to investigate the effect of vitamin E on the metabolic impact of heat stress in hens. The study included 150 Leghorn laying hens, which were assigned to 2 groups and initially subjected to the same environmental conditions. Diets were based on standard layer rations with 30, 80, or 105 mg of vitamin E/kg. All birds were kept in 45% relative humidity (RH) and at a room temperature of 21 °C for first 3 weeks for adaptation. In the fourth week the temperature and RH were increased to 35 °C and 65%, respectively, in the experimental group. Before, during, and after exposure to heat stress blood samples were taken from both groups. Vitamin E analyses were determined by HPLC. Biochemical parameters were analyzed spectrophotometrically. Statistically significant ($P \leq 0.05$) increases in plasma malondialdehyde (MDA), erythrocyte MDA, glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD), and egg yolk MDA concentration, and a decrease in plasma vitamin E were seen in the experimental group during heat stress. Egg quality parameters also decreased in the experimental group during heat stress. Dietary supplementation with higher levels of vitamin E alleviated some of the metabolic consequences of heat stress; there was no evidence of a beneficial effect on egg production during heat stress within the dietary range investigated.

Key Words: Vitamin E, laying hens, antioxidant defense system, heat stress

Isı Stresi Oluşturulan Yumurta Tavuklarında Oral Vitamin E'nin Antioksidan Aktivite, Yumurta Verimi ve Yumurta Kalitesine Etkisi

Özet: Çalışmada ısı stresine bağlı azalan savunma mekanizmasını desteklemek amacıyla antioksidan bir ajan olan E vitamini farklı dozlarda yumurtacı diyetlerine ilave edilerek, ısı stresi sonucu gelişen olumsuzluklar üzerine etkisi araştırılmıştır. Çalışmada; aynı ortam koşullarına sahip 2 kümes kullanılmıştır. Tüm hayvanlar adaptasyon açısından, üç hafta süre ile % 45 bağıl nem ve 21 °C oda ısısında tutulmuşlardır. Dördüncü hafta deneme grubunun ısı 35 °C'ye, nem de % 65'e yükseltilmiştir. Tüm hayvanlardan stres öncesi, sırası ve sonrasında kan alınmıştır. E vitamini analizleri HPLC ile yapılmıştır. Biyokimyasal parametreler spektrofotometrik yöntemlerle belirlenmiştir. Deneme gruplarında stres sırasında plazma MDA, eritrosit MDA, GSH-Px, CAT, SOD ve yumurta sarısı MDA konsantrasyonlarındaki artış ve plazma E vitaminindeki düşüş istatistik olarak önemli bulunmuştur ($P \leq 0.05$). Aynı şekilde yumurta kalitesine belirleyen parametrelerde deneme gruplarında stres sırasında düşmüştür. Sonuç olarak, ısı stresinin kanatlılarda antioksidan savunma sistemini olumsuz yönde etkilediği saptanmıştır. Isı stresinin, yem tüketimi ve yumurta verimini azalttığı görülmüştür. Yem tüketimi azalmasının canlı ağırlık kaybının artmasına neden olduğu belirlenmiştir. Diyetlere ilave edilen ekstra E vitamininin ısı stresinden kaynaklanan olumsuzlukları azalttığı gözlemlenmiştir.

Anahtar Sözcükler: E vitamini, yumurta tavukları, antioksidan savunma sistemi, sıcaklık stresi

Introduction

Stress is a factor that has been shown to increase the requirement for vitamins and energy. It is a well-known fact that under good nutritional conditions stress has little negative effect on the immune system; however, in situations of inadequate nutrition, side effects can appear when exposed to intense stress (1). In many regions of

Turkey, including areas in which egg production is common, one of the most important environmental factors is heat stress (2). Elevated environmental temperature causes disorders of the body-heat regulating mechanism in poultry (3). When the temperature exceeds 30 °C, signs of heat stress are likely to appear. It is proposed that heat stress negatively affects egg

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production and egg shell quality, decreases feed consumption and live weight, and disrupts the acid-base balance of the blood, thus causing some changes in metabolism and oxidative damage to cells (2).

The present study aimed to demonstrate that the importance of an antioxidant defense mechanism against free radicals in laying hens increases, depending on the degree of oxidative damage caused by heat stress. With the aim of investigating the antioxidant response against increasing free radicals, 2 different doses of vitamin E were added to the diets of laying hens in order to measure the effects of vitamin E on antioxidant enzyme activity, the defense mechanism (on the cellular level), and egg productivity and quality.

Materials and Methods

The study included Leghorn birds, aged 26 weeks, which were housed in individual cages. Two environmental temperatures (21 °C and 35 °C) × 3 dietary treatments (30, 80, and, 105 IU/kg feed of vitamin E) were utilized. There were 5 replications of 5 hens per pen for each treatment group. Each bird was given 125 g of the respective dietary treatment per day (Table 1). Egg production was monitored daily and feed intake and weight gain were monitored weekly during the 9-week study. Hens were given access to water ad libitum. After 3 weeks of adaptation 50% of the hens (control groups C1, C2, and C3, n = 75) were maintained at normal environmental temperatures of 21 °C and 65%

Table 1. Contents of the standard pullet grower ration.

Ingredients	%	C1	C2	C3	E1	E2	E3
Corn	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Wheat	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Full fat soybean	11.00	11.00	11.00	11.00	11.00	11.00	11.00
Sunflower meal	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Animal or vegetable fat	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Dicalcium Phosphate	2.00	2.00	2.00	2.00	2.00	2.00	2.00
DL-Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Limestone	Eyl. 50	Eyl. 50	Eyl. 50	Eyl. 50	Eyl. 50	Eyl. 50	Eyl. 50
Vitamin+mineral	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Meat and bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Gluten feed or meal (60% CP)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
L-Lysine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin E**	30 m/kg	30 mg/kg	80 mg/kg	105 mg/kg	30 mg/kg	80 mg/kg	105 mg/kg
Antioxidant	10 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg
Dry Matter (%)	89.00	89.00	89.00	89.00	89.00	89.00	89.00
Crude protein (%)	17.50	17.50	17.50	17.50	17.50	17.50	17.50
Crude fiber (%)	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Ash (%)	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Crude fat (%)	May. 20	May. 20	May. 20	May. 20	May. 20	May. 20	May. 20
Ca (%)	Mar. 90	Mar. 90	Mar. 90	Mar. 90	Mar. 90	Mar. 90	Mar. 90
P (%)	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Metabolizable energy (cal/kg)	2750	2750	2750	2750	2750	2750	2750

Control 1 (C1): Fed standard layer feed (30 mg/kg); Exp. 1 (E1): Fed standard layer feed (30 mg/kg); Control 2 (C2): Standard layer feed + 50 mg/kg vitamin E; Exp. 2 (E2): Standard layer feed +50 mg/kg vitamin E; Control 3 (C3): Standard layer feed + 75 mg/kg vitamin E, Exp. 3 (E3): Standard layer feed + 75 mg/kg vitamin E.

RH, while the other 50% (experimental groups E1, E2, and E3, $n = 75$) were maintained at the elevated environmental temperature of 35 °C and 45% RH for 3 weeks; then, all the birds were returned to an environment of 21 °C for the last 3 weeks. The light period was 14 h light and 10 h dark. Blood samples were collected, using heparin as anticoagulant, from wing veins before heat stress, during heat stress, and after heat stress. Plasma and erythrocyte hemolysates were stored at -25 °C until analysis. Erythrocyte GSH-Px concentration was measured using a commercial kit (4). Vitamin E analyses were conducted on feed samples, egg yolk, and plasma using the reversed phase HPLC procedures of Bieri et al. (5). Plasma, erythrocyte, and egg yolk MDA, and erythrocyte CAT and SOD concentrations were analyzed according to previously described methods (6-10). Egg quality parameters were analyzed as described by Hamilton (11). Two-way ANOVA and Duncan's tests were used to test statistical significance (SPSS v. 11.5).

Results

Mean plasma vitamin E and MDA, erythrocyte MDA, GSH-Px, CAT, and SOD, egg quality parameters, and standard deviations of the means, and significant differences between the groups and between the study weeks are presented in Tables 2 and 3. While plasma vitamin E levels of the experimental groups decreased significantly during heat stress, they increased after heat stress. During heat stress plasma MDA, and erythrocyte MDA, GSH-Px, CAT, and SOD levels of the experimental groups increased significantly in comparison to the control groups; however, no significant differences were observed in the plasma MDA, and erythrocyte MDA, GSH-Px, CAT, and SOD levels of the control groups during the entire trial period. Additionally, there were no differences in egg quality parameters between the control groups. During heat stress egg shell thickness in the experimental groups decreased significantly. Egg weight and egg specific gravity levels in groups E1 and E2 also decreased

Table 2. Biochemical parameters in the control and experimental groups.

Parameter	Time	C1	C2	C3	E1	E2	E3
Vitamin E Plasma ($\mu\text{g/ml}$)	Before	3.71 \pm 0.23C	6.67 \pm 0.52B	9.35 \pm 0.86A	3.32 \pm 0.12aC	4.78 \pm 0.19B	7.73 \pm 0.73aA
	During	3.55 \pm 0.33C	7.65 \pm 0.48B	9.41 \pm 0.87A	2.10 \pm 0.10bB	4.44 \pm 0.27A	4.53 \pm 0.13bA
	After	3.13 \pm 0.14C	6.66 \pm 0.63B	9.30 \pm 0.88A	3.25 \pm 0.02aB	4.93 \pm 0.53A	6.21 \pm 0.76abA
Plasma MDA (nmol/ml)	Before	8.60 \pm 1.05A	7.40 \pm 0.60A	5.10 \pm 0.42B	7.05 \pm 0.97b	6.35 \pm 0.61b	5.47 \pm 0.25b
	During	8.86 \pm 1.09A	7.57 \pm 0.44A	5.17 \pm 0.43B	15.40 \pm 1.13a	13.58 \pm 0.44a	13.77 \pm 0.43a
	After	9.00 \pm 0.94A	7.36 \pm 0.48A	5.04 \pm 0.44B	7.06 \pm 0.97b	6.33 \pm 0.64b	5.47 \pm 0.25b
Erythrocytes MDA (n-mol/g Hb)	Before	167.24 \pm 8.91	164.06 \pm 12.94	159.80 \pm 14.32	164.07 \pm 9.11b	153.85 \pm 13.55b	154.48 \pm 15.96b
	During	169.59 \pm 8.70	168.92 \pm 12.31	153.32 \pm 0.17	219.87 \pm 6.58aA	212.28 \pm 6.81abA	189.53 \pm 4.95aB
	After	168.79 \pm 8.33	166.58 \pm 11.72	153.43 \pm 5.78	167.83 \pm 11.59b	160.17 \pm 8.60b	154.39 \pm 5.55b
Erythrocytes GSH-Px (U/l Hemolysate)	Before	393.25 \pm 19.10B	548.70 \pm 37.99A	644.57 \pm 64.32 A	406.05 \pm 42.57bB	466.78 \pm 53.18bB	677.86 \pm 89.50bA
	During	394.06 \pm 18.73B	621.65 \pm 53.37A	657.45 \pm 43.79 A	843.48 \pm 108.59a	1096.60 \pm 147.53a	1145.20 \pm 156.76a
	After	395.18 \pm 17.62B	548.71 \pm 37.10A	657.00 \pm 66.72 A	405.79 \pm 42.65bB	466.78 \pm 58.13bB	677.86 \pm 89.50bA
Erythrocytes CAT (k/g-Hb)	Before	9.83 \pm 0.97B	13.00 \pm 1.04 B	17.27 \pm 1.66A	8.94 \pm 0.52bB	9.38 \pm 1.40 bB	14.35 \pm 0.85bA
	During	9.85 \pm 0.94B	12.18 \pm 0.97 B	17.11 \pm 1.15A	50.93 \pm 5.22a	55.94 \pm 3.12a	58.58 \pm 4.58a
	After	9.77 \pm 0.96B	12.82 \pm 1.10 B	20.05 \pm 0.92A	8.00 \pm 0.85bB	9.38 \pm 1.40bA	12.11 \pm 0.76bA
Erythrocytes SOD (U/g-Hb)	Before	49.46 \pm 4.64	58.51 \pm 4.36	57.24 \pm 2.54	45.36 \pm 5.08b	54.82 \pm 5.73 b	56.81 \pm 5.64b
	During	51.88 \pm 1.87	52.40 \pm 2.29	57.83 \pm 1.84	74.70 \pm 4.98aC	94.90 \pm 2.28aB	165.00 \pm 6.28aA
	After	49.42 \pm 4.66	58.51 \pm 17.47	54.98 \pm 1.98	45.28 \pm 5.11b	54.82 \pm 5.73b	56.81 \pm 5.63b

^{a,b,c}Means within the same column with different letters differ ($P \leq 0.05$). ^{A,B,C}Means within the same line with different letters differ ($P \leq 0.05$).

Control 1 (C1): Fed standard layer feed (30 mg/kg). Exp. 1 (E1): Fed standard layer feed (30 mg/kg).

Control 2 (C2): Standard layer feed + 50 mg/kg vitamin E .

Exp. 2 (E2): Standard layer feed + 50 mg/kg vitamin E

Control 3 (C3): Standard layer feed + 75 mg/kg vitamin E.

Exp. 3 (E3): Standard layer feed + 75 mg/kg vitamin E.

Table 3. The results of egg quality parameters in control and experimental groups during investigation.

Parameter	Time	C1	C2	C3	E1	E2	E3
Haugh unit (mm)	Before stress	4.00 ± 0.23	3.86 ± 0.14	3.83 ± 0.24	4.25 ± 0.26A	3.42 ± 0.15bB	3.84 ± 0.22AB
	During stress	3.70 ± 0.09	3.50 ± 0.12	3.55 ± 0.09	4.20 ± 0.17	4.14 ± 0.18a	4.22 ± 0.18
	After stress	3.91 ± 0.22	3.79 ± 0.13	3.83 ± 0.24	4.25 ± 0.26A	3.42 ± 0.15bB	3.84 ± 0.23AB
Egg shell Thickness (mm)	Before stress	36.86 ± 0.51	40.70 ± 0.59	40.52 ± 0.72	40.94 ± 0.66a	40.32 ± 0.84a	40.13 ± 0.60a
	During stress	40.09 ± 0.38	39.80 ± 0.40	39.43 ± 0.39	32.52 ± 0.96b	34.28 ± 0.84b	34.70 ± 0.63b
	After stress	39.75 ± 0.53	40.64 ± 0.61	40.52 ± 0.71	40.91 ± 0.69 a	40.35 ± 0.82 a	40.13 ± 0.60 a
Egg Shell Weight (g)	Before stress	5.85 ± 0.18B	6.95 ± 0.15A	7.03 ± 0.11Aa	5.77 ± 0.26	5.95 ± 0.19	6.04 ± 0.19
	During stress	5.61 ± 0.16 B	6.60 ± 0.12 A	6.60 ± 0.12Ab	5.24 ± 0.27	5.61 ± 0.14	5.09 ± 0.25
	After stress	5.84 ± 0.17B	6.94 ± 0.16 A	7.12 ± 0.12Aa	6.35 ± 0.15	6.32 ± 0.008	6.17 ± 0.13
Egg Weight (g)	Before stress	62.65 ± 1.18	63.27 ± 1.01	62.87 ± 0.89	61.41 ± 1.02aAB	63.99 ± 1.28aA	59.11 ± 1.70B
	During stress	60.66 ± 1.06	61.10 ± 0.75	60.89 ± 0.77	55.00 ± 1.46b	52.12 ± 1.96b	54.72 ± 0.99
	After stress	62.64 ± 1.17	63.25 ± 1.02	62.87 ± 0.89	61.46 ± 0.99aAB	60.31 ± 1.31aA	59.11 ± 1.70B
Egg Specific Gravity (g)	Before stress	58.47 ± 1.20	58.79 ± 0.95	57.21 ± 0.72	58.77 ± 0.96a	56.86 ± 0.85a	56.03 ± 1.54
	During stress	57.50 ± 1.04	57.91 ± 0.73	55.90 ± 0.39	52.62 ± 1.32b	52.43 ± 1.16b	52.48 ± 0.99
	After stress	58.92 ± 1.17	57.61 ± 0.70	57.21 ± 0.72	58.63 ± 0.99a	56.83 ± 0.86a	56.03 ± 1.54

^{a,b,c}Means within the same column with different letters differ ($P \leq 0.05$). ^{A,B,C}Means within the same line with different letters differ ($P \leq 0.05$).

Control 1 (C1): Fed standard layer feed (30 mg/kg).

Control 2 (C2): Standard layer feed + 50 mg/kg vitamin E .

Control 3 (C3): Standard layer feed + 75 mg/kg vitamin E.

Exp. 1 (E1): Fed standard layer feed (30 mg/kg).

Exp. 2 (E2): Standard layer feed + 50 mg/kg vitamin E

Exp. 3 (E3): Standard layer feed + 75 mg/kg vitamin E.

significantly. There were no significant differences in weight gain and egg production between the control groups before, during, or after heat stress (Figures 1 and 2). Mean weight gain and egg production level decreased significantly during heat stress (Figures 3 and 4).

Discussion

Heat stress can negatively affect the defense mechanism in poultry, which can lead to suppression of the immune system and cause increased production of oxygen-free radicals; this effect is well documented (12,13). During the test period of the present study mean plasma vitamin E level of the animals fed a standard layers diet, (group C1) was 3.46 mg/ml, a value similar to previous reports (13-15). For groups C2 and C3 mean plasma vitamin E level was 7.00 mg/ml and 9.35 mg/ml, respectively, which differed from that of group C1 ($P \leq 0.05$) (Table 3). The variation observed between the groups is the result of adding vitamin E to the diets (14).

In the experimental groups, plasma vitamin E levels during heat stress decreased in comparison to the control groups (Table 3). During heat stress, plasma vitamin E levels declined ($P \leq 0.05$) as feed consumption declined, (14), which suggests that prior to the application of heat stress plasma vitamin E concentration is directly proportional to the amount of vitamin E in the diet. Some researchers reported striking reductions in feed consumption and vitamin E concentrations in poultry exposed to heat stress that are similar to the present study's results (15-17). MDA level increases in groups E2 and E3 were less than those in group E1. Similar to the present study, Puthongsiriporn et al. (18) stated that the level of plasma MDA continued to rise in hens exposed to heat stress (32 °C) and that laying hens fed rations containing the highest dose of vitamin E (65 IU) had the lowest plasma MDA values. As environmental temperature increased so did the hens' respiration and evaporation as they tried to maintain optimal body temperature, which in turn increased their metabolism and energy consumption (19). If increased energy needs

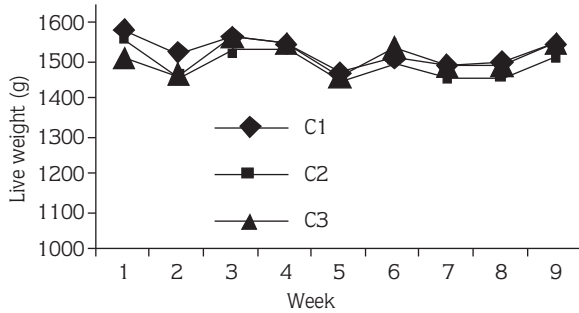


Figure 1. Weight gain in the control groups.

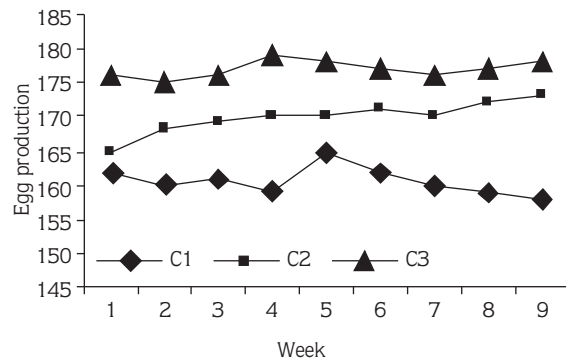


Figure 2. Egg production in the control groups.

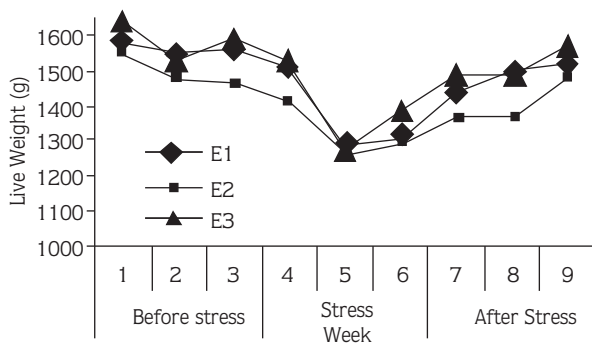


Figure 3. Weight gain in the experimental groups.

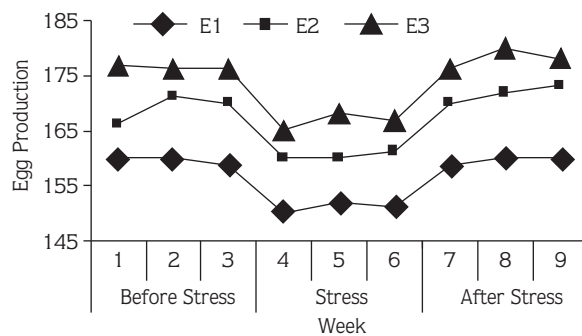


Figure 4. Egg production in the experimental groups.

are not met by feed, mobilization of lipids from stored fat takes place. With regards to lipid mobilization, the malondialdehyde level, an indicator of lipid peroxidation, increases (20). In the present study feed consumption decreased in accordance with the degree of heat stress; thus, the rising energy requirement of the animals could not be supplied by the feed; consequently, MDA levels were high as a result of increased lipid peroxidation due to the mobilization of fat, and the generation of free radicals increased. Higher levels of free radicals increase the level of MDA. The smaller rise in plasma malondialdehyde levels in the animals that were given a higher dose of vitamin E, as compared to the control groups, suggests that this is due to the fact that vitamin E is an antioxidant agent (21-23) and reduces lipid peroxidation caused by heat stress (21). The increase in erythrocyte MDA level in group E3 was less than that in groups E1 and E2 ($P \leq 0.05$) (Table 3). Similarly, Öztürk-Ürek et al. (24) reported that hens fed diets

supplemented with 0.07 mg of Se, 70 mg of vitamin E, and 500 mg of vitamin C had an increase in erythrocyte MDA that was clearly less than that in the control groups. The observed increase in erythrocyte MDA levels in the groups that were given vitamin E might have been lower than that in group E1 because of the inhibition of lipid peroxidation in erythrocyte membranes due to the antioxidant effect of vitamin E. Groups C2 and C3 were fed extra vitamin E in their diets and their erythrocyte GSH-Px activity was higher than that of group C1 ($P \leq 0.05$) (Table 3). For the experimental group subjected to heat stress, erythrocyte GSH-Px levels exhibited a rising trend in comparison to the control groups ($P \leq 0.05$) (Table 3). Among the groups whose diets were supplemented with vitamin E, GSH-Px activity was high while under heat stress, which is similar to the findings of Öztürk-Ürek et al. (24) who reported that in hens given 0.07 mg of selenium and 70 mg of vitamin E, erythrocyte GSH-Px increased more dramatically than in the control

group. Erythrocyte CAT activity in group C3, which was fed a high dose of vitamin E, was higher than that of group C1 ($P \leq 0.05$) (Table 3). Erythrocyte CAT levels in the experimental group under heat stress demonstrated an increasing trend, as compared to the control groups ($P \leq 0.05$). Avanzo et al. (25) on the other hand, in their study of the effects of vitamin E and selenium against oxidative stress, reported that CAT activity increases in the muscles of animals fed a diet lacking vitamin E and selenium. Among the experimental groups fed vitamin E, SOD activity was high during heat stress and reached levels similar to those reported by Öztürk-Ürek et al. (24). In terms of the defense mechanism, it was determined that GSH-Px, CAT, and SOD enzyme levels in the antioxidant enzyme group analyzed demonstrated an upward trend under heat stress, as compared to the control groups ($P \leq 0.05$). With lipid peroxidation, the free radicals that develop in connection with heat stress in poultry damage cells (13). Free radicals extending along the membrane demolish lipids, enzymes, sugars, protein thiols, and nucleic acids. As it is the most abundant antioxidant in membranes, vitamin E increases antioxidant enzyme activity during stress and so removes free radicals in the early phases of lipid peroxidation (12). The high antioxidant enzyme activity observed in groups E2 and E3, which were given extra vitamin E, is possibly indicative of this fact. As a result of decreased feed consumption due to heat stress, inadequate intake of minerals, particularly calcium, may occur. As calcium is important to egg shell structure, a decrease may cause a reduction in egg shell quality. It is suggested that an increase in bicarbonate ion concentration in blood and decreased calcium carrying capacity due to heat stress results in calcium loss (2). Moreover, in the animals exposed to heat stress, together with increased

respiration, increasing carbon dioxide loss may cause egg shells to become thinner, resulting in decreased egg shell weight (26). In the experimental groups weekly live weights during the heat stress period fell in comparison to the control groups (Figure 3). Similarly to the present study, Deaton et al. (27) compared the live weights of broilers and stated that live weight loss in broilers exposed to heat reaching 35 °C was greater than that in those exposed to 21 °C. In other studies (28,29) it was similarly stated that heat stress decreases live weight. In the experimental groups, weekly egg productivity levels fell dramatically in comparison to the control groups (Figure 4). Similarly, for the animals whose diets were supplemented with 10 mg of vitamin E, a significant decrease was observed in egg productivity, while in the group given 500 mg of vitamin E an increase 9.20% greater than that in the control group was observed (12). Bollengier-Lee et al. (14) reported that during the first 4 weeks of their study, when the temperature was 22 °C, egg productivity was similar among all the groups and that vitamin E application did not have any significant effect. It was reported that together with heat stress, productivity fell in those fed control diets and that optimum productivity was noted in those given 250 mg/kg of vitamin E. Previous studies (14,30) reported that heat stress blocks the synthesis of egg yolk proteins in circulation, thus negatively affecting egg productivity. It is stated that vitamin E increases egg productivity by preventing liver cell damage, which is important for egg yolk protein synthesis.

Acknowledgments

The present work was supported by the Research Fund of İstanbul University, Project no: T-22/23072002.

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