

## Vitamin C and E can alleviate negative effects of heat stress in Japanese quails

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### Abstract

The present study investigates the effects of vitamin C (L-ascorbic acid) and E ( $\alpha$ -tocopherol acetate) supplementation on egg production, egg quality, apparent digestibility, serum metabolites, and antioxidant status of Japanese quails (*Coturnix coturnix japonica*) exposed to a high ambient temperature (33 °C). One hundred and twenty laying Japanese quails (45-d-old) were divided into four groups, 30 birds per group. Quails were fed a basal diet or the basal diet supplemented with either 250 mg of L-ascorbic acid/kg of diet, 250 mg of  $\alpha$ -tocopherol-acetate/kg of diet, or 250 mg of L-ascorbic acid plus 250 mg  $\alpha$ -tocopherol-acetate/kg of diet. Separately or as a combination, supplemental vitamin C and E significantly increased live weight, feed intake and egg production ( $P < 0.05$ ). Separately, dietary supplementation of vitamin C and E improved the egg quality, and the combination of vitamins C and E resulted in a greatest egg weight, specific gravity and Haugh unit value, thickest egg shell, and heaviest egg shell weight ( $P < 0.05$ ). Apparent digestibility of nutrients (DM, OM, CP, and EE) was increased by supplementation of vitamin C and E ( $P < 0.05$ ). Separately or as a combination, supplemental vitamin C and E increased serum concentration of total protein but decreased corticosterone, glucose, and cholesterol concentrations ( $P < 0.05$ ). Supplemental vitamin C and E also increased serum vitamin C and E but decreased MDA (malondialdehyde) concentrations ( $P < 0.05$ ). Results of the present study show that dietary supplementation of vitamin C and E, particularly as a combination, improved the performance, egg quality and antioxidant status of laying Japanese quails exposed to heat stress. Such a combination of supplement can offer a potential protective management practice in preventing heat stress-related losses in performance of laying Japanese quails.

**Key words:** Heat stress, vitamin C, vitamin E, egg, digestibility, MDA, quail.

### Introduction

High ambient temperature reduces feed intake, live weight gain, egg production, and feed efficiency<sup>9,50</sup> in poultry. Heat stress causes the release of corticosterone and catecholamines and initiates lipid peroxidation in cell membranes<sup>11,17,46,48</sup>. Donkoh<sup>9</sup> reported that heat stress significantly reduced plasma protein level and increased blood glucose concentrations. There are numerous methods to alleviate the negative effects of high environmental temperature on performance of poultry. Due to the fact that it is expensive to cool animal buildings, such methods are focused mostly on dietary manipulations. For this purpose, vitamin C and E are used in the poultry diet because of their anti-stress effects and because their levels is reduced during the heat stress<sup>16,46,51</sup>. Many studies have indicated benefit of dietary vitamin E supplementation to laying hens during heat stress<sup>3,4,15,43,48,56</sup>. Positive effects of vitamin C supplementation in broiler chickens and laying hens have been documented<sup>25,27,37,40,47</sup>. Vitamin C and vitamin E are primary antioxidants in biological systems and break the chain of lipid peroxidation in cell membranes. Overall antioxidant potential has been reported to possibly be more efficient and crucial than single antioxidant nutrients<sup>21,22,57</sup>. In this respect, vitamin C and vitamin E work together such that vitamin E is the major chain-breaking antioxidant in lipid phases such as cellular membrane or low density lipoproteins, and the oxidizing free radical chain reactions are terminated in aqueous compartments, with vitamin C as terminal reductant<sup>23,52</sup>. Regarding antioxidant property, there is a positive synergistic effect of vitamins C and E on the immune response<sup>2,24</sup>. Vitamin E is mainly found in the hydrocarbon part of membrane lipid bilayer towards the membrane interface and in close prox-

imity to oxidase enzymes which initiate the production of free radicals<sup>30,32,58</sup>. The objective of this study was to evaluate the effects of vitamin C and E supplementation on performance, egg quality, apparent digestibility, serum metabolites, and antioxidant status of laying Japanese quails reared under heat stress (33°C).

### Materials and Methods

**Animals, diets and experimental design:** Forty five-d-old 120 Japanese quails (*Coturnix coturnix japonica*) obtained from a commercial company were used in the study. The birds were fed either a basal diet containing 17.65% CP and 2780 kcal/kg ME or the basal diet supplemented with either 250 mg of L-ascorbic acid/kg of diet, 250 mg of  $\alpha$ -tocopherol acetate/kg of diet, or 250 mg of L-ascorbic acid plus 250 mg of  $\alpha$ -tocopherol acetate /kg of diet. Vitamin C (ROVIMIX® STAY-C® 35; specifically produced for use as a stabilized source of vitamin C in feed) and 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 basal diet was formulated using NRC guidelines<sup>36</sup>. Ingredients and chemical composition of the basal diet are shown in Table 1. Small amounts of the basal diet were first mixed with the respective amounts of vitamin C and E as a small batch, then with a larger amount of the basal diet until the total amount of the respective diets were homogeneously mixed. The birds were randomly assigned to caging units, 30 birds each. Each subcage unit (19 cm x19 cm x19 cm) contained two birds. Water and the diets were offered ad libitum throughout the experiment. The bird house was lit for 17 h per day. During the experiment, hen house's temperature and humidity were measured four times a day (06.00, 12.00, 18.00, and 24.00). Average ambient relative humidity inside the hen house was  $41 \pm 6\%$ . The mean value of daily tempera-

ture in the hen house was  $33 \pm 4^\circ\text{C}$ . The experiment was carried out between the June 30 to September 20.

**Performance variables and egg quality:** Body weights were recorded at the beginning and at the end of the study to determine body weight changes. Feed consumption was measured weekly. The number of eggs and egg weights were recorded daily. Egg quality measurements were conducted monthly using all eggs of one day from all treatments. Parameters included for egg quality measurement were specific gravity, egg shell thickness, egg shell weight, and Haugh unit. Specific gravity of eggs was determined by using the saline flotation method of Hempe et al.<sup>20</sup>. Salt solutions were made in incremental concentrations of 0.005 in the range from 1.065 to 1.120. Haugh units were calculated using the HU formula<sup>12</sup> based on the height of albumen determined by a micrometer and egg weight (Saginomiya, TLM-N1010, Japan). Shell thickness was determined measuring the thickness mean values taken at three locations on the egg (air cell, equator, and sharp end) by using a dial pipe gauge (Mitutoyo, 0.01-20 mm, Japan).

**Sample collection and laboratory analysis:** From day 55 to 60, forty birds were placed into individual battery cages and distributed into four treatments, 10 birds each, for apparent digestibility [Dry matter (DM), organic matter (OM), crude protein (CP), and ether extract (EE)]. Apparent digestibility of nutrients was measured by collecting excrement samples twice a day. The composited excrement samples were oven-dried at  $60^\circ\text{C}$  for 48 h then were ground and subsampled (1 g) for chemical analysis. Apparent digestibility of nutrients was measured using  $\text{Cr}_2\text{O}_3$  described by Petry and Rapp<sup>41</sup>. At the end of the study, 10 birds randomly chosen from each treatments were slaughtered and blood samples were collected, centrifuged at 3000 x rpm for 10 min and sera was collected and stored at  $-20^\circ\text{C}$ . Sera samples were thawed at room temperature, and corticosterone concentration were determined. Radioimmunoassay for corticosterone concentration was performed using a commercially available kit (IMMULITE 2000, No. L2KAC2). Serum glucose, total protein, and cholesterol concentrations were measured using biochemical analyzer (Technicon RA-XT, New York, USA). Chemical analysis of the diets and excrement samples were run using international procedures of AOAC<sup>1</sup>. In order to estimate protein digestibility, excrement N was chemically analyzed according to the method of Terpstra and de Hart<sup>53</sup>. Lipid peroxidation as thiobarbituric acid-reactive substances (TBARS) were determined in serum samples by method of Placer et al.<sup>42</sup> as modified by Matkovic et al.<sup>31</sup>. The values of TBARS material were expressed in terms of malondialdehyde (MDA, nmol/ml serum). Serum vitamin E concentration was determined by a modification of the method described by McMurray et al.<sup>34</sup>. The relevant wavelengths for vitamin E detection were 292 and 330 nm. Calibration was performed using standard solutions of all trans-retinol and  $\beta$ -tocopherol in methanol. Serum ascorbic acid concentration was measured as described by Kway<sup>28</sup> method (Fosfotungstat) with spectrophotometer.

**Statistical analyses:** The data were analyzed using the GLM procedure of SAS<sup>45</sup>. Significant differences ( $P < 0.05$ ) among treatment means were determined using Duncan's new multiple range test.

## Results

The effects of supplemental dietary vitamin C and E during heat stress on performance of laying Japanese quails are shown in Table

2. Combination of vitamin C and E, rather than each separately, provided the highest results. Supplemental vitamin C and E significantly increased live weight and feed intake ( $P < 0.05$ ). Egg production was also highest ( $P < 0.05$ ) with each supplemental compared with the control, but was the highest with the combination of supplemental vitamin C and E. Egg quality of laying Japanese quails is shown in Table 3. Egg weights were significantly different among treatments ( $P < 0.05$ ), being highest with the combination of vitamin C and E and being lowest with the control diet. Separately, dietary supplement of vitamin C and E improved the egg quality, but a combination of supplemental vitamin C and E resulted in a higher specific gravity, thickest egg shell, and heaviest egg shell weight ( $P < 0.05$ ). Haugh unit was also higher ( $P < 0.05$ ) with each supplemental group compared with the control, but was the highest with the combination of vitamin C and E. Separately or as a combination, supplemental vitamin C and E increased apparent digestibility of nutrients (DM, OM, CP, and EE) ( $P < 0.05$ ) (Table 4). The effects of vitamin C and E supplementation on serum corticosterone, glucose, cholesterol, and total protein are shown in Table 5. Separately or as a combination, supplemental vitamin C and E increased serum concentration of total protein but decreased corticosterone, glucose, and cholesterol concentrations ( $P < 0.05$ ). Supplemental vitamin C and E also increased serum vitamin C and E concentrations (Table 3). Serum MDA concentrations, however, decreased ( $P < 0.05$ ) upon vitamin C and E supplementation.

## Discussion

In the present study, vitamin C and E supplementation improved the performance namely live weight, egg production as well as egg quality in laying Japanese quails reared under high ambient temperature. With respect to dietary ascorbic acid supplementation under heat stress, in terms of a better performance of poultry, results of the present study are in agreement with the findings of many researchers<sup>25,37,27,38</sup>. It is well known that growth rate and egg production decrease when ambient temperature goes above thermo-neutral zone<sup>14</sup>. At temperatures above or below thermoneutral zone, corticosteroid secretion increases as a response to stress<sup>5</sup>. Kutlu and Forbes<sup>27</sup> reported that ascorbic acid reduces the synthesis of corticosteroid hormones in birds. By decreasing synthesis and secretion of corticosteroids, vitamin C alleviates the negative effects of stress<sup>32</sup>. It has been also postulated that the improved performance of poultry results from a decrease in protein-derived gluconeogenesis<sup>40</sup>. Similar to results of the present study, El-Boushy et al.<sup>13</sup> reported that dietary vitamin C supplementation increased egg production, egg shell strength, and interior egg quality in stressed-laying hens. Ascorbic acid plays a role in bone maturation by improving hydroxyproline production which is required for collagen formation. Accordingly, in birds, it was postulated that ascorbic acid stimulates 1,2,5 dihydrox-cholecalciferol and together with it increases calcium mobilization from bone, suggesting that vitamin C has an important role in egg shell formation<sup>8,10</sup>. In the present study, dietary vitamin E inclusions resulted in a better performance, egg quality, and apparent digestibility. Dietary vitamin E supplementation is common in laying hens in the literature. Causing oxidative damage on membrane of hepatic cells, heat stress has been shown to decrease plasma egg yolk precursor proteins, vitellogenin and tryglyceride<sup>3</sup>. This kind of negative effect of heat stress was alleviated by dietary vitamin E supplementation<sup>56</sup>. It was speculated that the positive influence of vitamin E supplementation on egg

**Table 1.** Ingredients and chemical composition of the basal diet fed to laying Japanese quails.

Ingredients	g kg <sup>-1</sup>
Ground Corn	58.62
Soybean Meal	26.72
Vegetable Oil	3.80
Limestone	8.60
Dicalcium Phosphate	1.60
Vitamin+Mineral Premix <sup>1</sup>	0.25
DL-methionine	0.10
Sodium Chloride	0.35
ME, MJ/kg <sup>2</sup>	12.4

**Chemical Analyses, Dry matter (DM) basis**

Crude Protein <sup>3</sup> , %	17.00
Calcium <sup>3</sup> , %	3.53
Phosphorus <sup>3</sup> , %	0.65

<sup>1</sup>Mix supplied per kg of diet: retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; dl-β-tocopheryl acetate, 1.25 mg; menadione sodium bisulfite 2.5 mg; thiamine-hydrochloride, 1.5 mg; riboflavin, 3 mg; d-pantothenic acid, 5 mg; pyridoxine hydrochloride, 2.5 mg; vitamin B-12, 0.0075 mg; folic acid, 0.25 mg; niacin, 12.5 mg; Mn (MnSO<sub>4</sub>·H<sub>2</sub>O), 50 mg; Fe (FeSO<sub>4</sub>·7H<sub>2</sub>O), 30 mg; Zn (ZnO), 30 mg; Cu (CuSO<sub>4</sub>·5H<sub>2</sub>O), 5 mg; I (KI), 0.5 mg; Se (Na<sub>2</sub>SeO<sub>3</sub>), 0.15 mg; Co (CoCl<sub>2</sub>·6H<sub>2</sub>O), 0.1 mg; choline chloride, 125 mg.

<sup>2</sup>ME : Metabolizable energy, Calculated from the tabular values (22).

<sup>3</sup>Analyzed value.

**Table 2.** Effects of supplemental vitamin C and E on performance of laying Japanese quails reared at high ambient temperature (n=30).

Item	T r e a t m e n t s *				SEM
	C	Vit C	Vit E	Vit C + E	
Live wt, g					
Final	218 <sup>a</sup>	228 <sup>b</sup>	226 <sup>b</sup>	237 <sup>c</sup>	2.4
DMI <sup>**</sup> , g/d	25.1 <sup>a</sup>	29.5 <sup>b</sup>	28.3 <sup>b</sup>	33.0 <sup>c</sup>	1.5
Hen-day egg production, %	68.3 <sup>a</sup>	75.3 <sup>b</sup>	74.1 <sup>b</sup>	81.6 <sup>c</sup>	1.6

a,b,c,d:Mean values within a row with no common superscript differ significantly ( $P < 0.05$ ).

\*C: control (basal) diet, Vit C: control diet + 250 mg of L-ascorbic acid/kg of diet, Vit E: control diet +250 mg of β-tocopherol acetate /kg of diet, Vit C+E: control diet + 250 mg of L-ascorbic acid/kg + 250 mg of β-tocopherol acetate /kg of diet ; \*\* : Dry matter intake ; \*\*\* : kg of feed consumed for egg production/kg of egg production

**Table 3.** Effects of supplemental vitamin C and E on egg quality of of laying Japanese quails reared at high ambient temperature , (n=30)

Item	T r e a t m e n t s *				SEM
	C	Vit C	Vit E	Vit C + E	
Egg weight, g	10.3 <sup>a</sup>	11.6 <sup>b</sup>	11.4 <sup>b</sup>	12.3 <sup>c</sup>	0.4
Specific gravity, 1.07 <sup>x</sup>	5 <sup>a</sup>	7 <sup>b</sup>	7 <sup>b</sup>	9 <sup>c</sup>	0.01
Egg shell thickness, μm	21.3 <sup>a</sup>	22.1 <sup>b</sup>	22.0 <sup>b</sup>	23.2 <sup>c</sup>	0.02
Egg shell weight, g	1.03 <sup>a</sup>	1.15 <sup>b</sup>	1.13 <sup>b</sup>	1.25 <sup>c</sup>	0.03
Haugh unit	87.2 <sup>a</sup>	90.5 <sup>b</sup>	90.2 <sup>b</sup>	93.4 <sup>c</sup>	1.5

a,b,c,d:Mean values within a row with no common superscript differ significantly ( $P < 0.05$ ).

\*C: control (basal) diet, Vit C: control diet + 250 mg of L-ascorbic acid/kg of diet, Vit E: control diet +250 mg of β-tocopherol acetate /kg of diet, Vit C+E: control diet + 250 mg of L-ascorbic acid/kg + 250 mg of β-tocopherol acetate /kg of diet

**Table 4.** Effects of supplemental vitamin C and E on apparent digestibility in laying Japanese quails reared at high ambient temperature , % (n=10).

Nutrients	T r e a t m e n t s *				SEM
	C	Vit C	Vit E	Vit C+E	
Dry matter	62.0 <sup>a</sup>	63.1 <sup>b</sup>	62.9 <sup>b</sup>	63.8 <sup>c</sup>	0.05
Organic matter	65.5 <sup>a</sup>	66.8 <sup>b</sup>	66.6 <sup>b</sup>	67.5 <sup>c</sup>	0.08
Crude protein	66.9 <sup>a</sup>	67.8 <sup>b</sup>	67.9 <sup>b</sup>	70.8 <sup>c</sup>	0.52
Ether extract	67.5 <sup>a</sup>	68.6 <sup>b</sup>	68.5 <sup>b</sup>	70.3 <sup>c</sup>	0.50

a,b,c,d:Mean values within a row with no common superscript differ significantly ( $P < 0.05$ ).

\*C: control (basal) diet, Vit C: control diet + 250 mg of L-ascorbic acid/kg of diet, Vit E: control diet +250 mg of β-tocopherol acetate /kg of diet, Vit C+E: control diet + 250 mg of L-ascorbic acid/kg + 250 mg of β-tocopherol acetate /kg of diet

**Table 5.** The effects of supplemental vitamin C and E on some serum metabolites of laying Japanese quails reared at high ambient temperature (n=10).

Item	T r e a t m e n t s*				SEM
	C	Vit C	Vit E	Vit C+E	
Corticosterone, mol/L	2.2 <sup>c</sup>	1.8 <sup>b</sup>	1.7 <sup>b</sup>	1.2 <sup>a</sup>	0.06
Glucose, mg/dl	251 <sup>c</sup>	226 <sup>b</sup>	231 <sup>b</sup>	209 <sup>a</sup>	12
Cholesterol, mg/dl	192 <sup>c</sup>	166 <sup>b</sup>	164 <sup>b</sup>	146 <sup>a</sup>	9
Total protein, g/dl	4.2 <sup>a</sup>	4.5 <sup>b</sup>	4.5 <sup>b</sup>	4.8 <sup>c</sup>	0.02

<sup>a,b,c,d</sup>: Mean values within a row with no common superscript differ significantly ( $P < 0.05$ ).

\*C: control (basal) diet, Vit C: control diet + 250 mg of L-ascorbic acid/kg of diet, Vit E: control diet +250 mg of  $\beta$ -tocopherol acetate /kg of diet, Vit C+E: control diet + 250 mg of L-ascorbic acid/kg + 250 mg of 250 mg of  $\beta$ -tocopherol acetate /kg of diet

**Table 6.** Effects of supplemental vitamin C and E on serum MDA, ascorbic acid and vitamin E levels of laying Japanese quails reared at high ambient temperature, (n=10).

Item	T r e a t m e n t s*				SEM
	C	Vit C	Vit E	Vit C+E	
MDA, nmol/mL	2.30 <sup>c</sup>	1.90 <sup>b</sup>	1.89 <sup>b</sup>	1.20 <sup>a</sup>	0.07
Ascorbic acid,mg/mL	7.35 <sup>a</sup>	11.53 <sup>c</sup>	8.64 <sup>b</sup>	12.6 <sup>d</sup>	0.09
Vitamin E, mg/mL	0.81 <sup>a</sup>	0.96 <sup>b</sup>	10.23 <sup>c</sup>	11.09 <sup>d</sup>	0.10

<sup>a,b,c,d</sup>: Mean values within a row with no common superscript differ significantly ( $P < 0.05$ ).

\*C: control (basal) diet, Vit C: control diet + 250 mg of L-ascorbic acid/kg of diet, Vit E: control diet +250 mg of  $\beta$ -tocopherol acetate /kg of diet, Vit C+E: control diet + 250 mg of L-ascorbic acid/kg + 250 mg of 250 mg of  $\beta$ -tocopherol acetate /kg of diet

production and egg quality might be explained by the concentration of yolk precursor proteins during heat stress<sup>43</sup>. Egg yolk contains three main macromolecular protein components: lipovitellin, phosvitin and livetin<sup>43,44</sup>. Egg yolk also contains very low density lipoproteins<sup>6</sup>. Bollengier-Lee et al.<sup>3</sup> have shown that dietary supplementation with vitamin E ( $\alpha$ -tocopherol acetate) ameliorated the negative effects of chronic heat stress in laying hens. A supplement of 500 mg vitamin E/kg increased egg production by 7% in heat-stressed hens for 4-week periods at two different ages and stage of lay compared to heat-stressed birds fed 10 mg vitamin E/kg diet<sup>3</sup>. In addition, Bollengier-Lee et al.<sup>4</sup> reported dietary supplement of 250 mg/kg as an optimum level provided before, during, and after heat stress in laying hens for partially alleviating the adverse effects of chronic heat stress. Sahin and Kucuk<sup>47</sup> reported that 250 mg/kg or 500 mg/kg vitamin E supplementation in a broiler diet increased feed intake and improved live weight gain of Japanese quails reared under heat stress (34°C). Results of the apparent digestibility of nutrients of the present study also support the supplementation of dietary vitamin C and E, in terms of alleviating the negative effects of the heat stress in laying Japanese quails. Wallis and Balnava<sup>54</sup> found that the digestibility of amino acids was decreased at a high environmental temperature in broilers. Similarly, Zuprizal et. al<sup>59</sup> have shown that true digestibility of protein and amino acids of two different protein sources (rapeseed and soybean meals) decreased as the temperature increased from 21 to 32°C. Hai et al.<sup>18</sup> reported that the activities of trypsin, chymotrypsin, and amylase decreased significantly by a high temperature (32°C). The reason for the decrease in digestive enzymes is uncertain. However, Osman and Tanios<sup>39</sup> speculated that it is because of adjustment of the pancreas in birds accustomed to a hot environment. Sahin and Kucuk<sup>47</sup> reported that digestibility of nutrients (DM, OM, CP, and EE) were higher when vitamin C and vitamin E was included into diet in broiler Japanese quails reared chronic heat stress. Serum concentration of corticosterone was lower with supplemental dietary vitamin C and E, probably indicating a lowered response to heat stress with supplementation of these two vita-

mins. Similarly, Kutlu and Forbes<sup>27</sup> reported that heat stress elevated plasma corticosterone concentration which was significantly reduced with vitamin C supplementation in a broiler diet. Pardue et al.<sup>40</sup> and McKee and Harrison<sup>33</sup> reported that supplemental vitamin C enhanced weight gain in heat-stressed chickens that corresponded to reductions in plasma corticosterone. Results of the present study showed similar trends for effects of vitamin E to vitamin C for serum concentrations of corticosterone. In the present study, similar effects of vitamin C and vitamin E existed as evidence that serum glucose, and cholesterol concentrations decreased, while protein concentrations increased by supplemental dietary vitamin C and E. Serum concentration of corticosterone was also lower with supplemental dietary vitamin C and vitamin E, indicating a lowered response to heat stress with supplementation of these two vitamins. Increasing concentrations of corticosterone was parallel to increases in serum glucose and cholesterol concentrations. This result was probably due to the greater catabolic effect (or concentration) of corticosterone, yielding more of glucose in the serum with supplemental dietary vitamin C and vitamin E. Sahin et al.<sup>49</sup> reported that vitamin C and E supplementation increased plasma protein concentration while markedly decreased blood ACTH, glucose and cholesterol concentrations in heat-stressed (34°C) Japanese quails. Similarly, Kutlu and Forbes<sup>27</sup> reported that vitamin C supplementation increased plasma protein concentration while markedly decreased blood glucose and cholesterol concentrations in heat-stressed (36°C) broilers. A likely mechanism by which vitamin C causes a reduction in corticosterone concentration is through inhibitory effect of vitamin C on glucocorticoid synthesis, and it has been postulated that the improved performance of poultry results from a decrease in protein-derived gluconeogenesis<sup>40</sup>. Increases in concentrations of glucose may be attributed to increased glucocorticoid secretion which increases gluconeogenesis<sup>55</sup>. Dietary vitamin C may reverse these changes, presumably by reducing the secretion and/or synthesis of glucocorticoids<sup>27</sup>. At the present study, dietary vitamin C and E caused an increase in serum concentrations of vitamin C and E but a decrease in serum MDA concentrations.

This is consistent with previous studies <sup>4,46,48,49</sup>. It is well-stated that vitamin concentrations are influenced by environmental temperature <sup>7,15,26,27</sup>. Sahin et al <sup>46</sup> reported that supplemental vitamin E linearly increased serum vitamin E and A, but decreased MDA concentrations. Kutlu and Forbes <sup>27</sup> reported that adrenal size and vitamin C secretion rate decreased at high temperatures. Some researchers also reported that plasma ascorbic acid levels were increased by dietary ascorbic acid supplementation in broilers under heat stress. It is known that heat stress leads to generation of free radicals. This free radicals can damage cell membranes by inducing lipid peroxidation of polyunsaturated fatty acids in the cell membrane <sup>29</sup>, resulting in abnormal membrane integrity during heat stress. The results are similar to the research of Sahin et al. <sup>49</sup>, who reported that supplemental vitamin C and E reduced MDA concentrations in serum and liver in Japanese quails reared under heat stress (34°C). Vitamin E is well accepted as the first line of defense against lipid peroxidation. 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 <sup>32</sup>. Similar to results of the present study, Morrissey et al. <sup>35</sup> reported that dietary supplementation of chicken diets with β-tocopherol increased tissues β-tocopherol concentrations, while markedly decreasing MDA concentration. An improve in performance, egg quality, digestibility of nutrients, and serum metabolites in Japanese quails in the present study could have been due to positive effects of vitamin C and/or vitamin E, alleviating the negative effects of heat stress. More specifically, the combination of vitamin C and E provided the greatest performance, egg quality, and nutrient digestibility for most parameters measured. It is apparent that a combination of dietary ascorbic acid and β-tocopherol acetate supplementation offers a feasible way to reduce the losses in performance due to heat stress. Overall antioxidant potential has been reported to possibly be more efficient and crucial than single antioxidant nutrients <sup>22</sup>. In this respect, vitamin C and vitamin E work together or act synergistically such that vitamin E is the major chain-breaking antioxidant in lipid phases such as cellular membrane or low density lipoproteins, and the oxidizing free radical chain reactions are terminated in aqueous compartments, with vitamin C as terminal reductant <sup>23,52</sup>. In accordance with this finding, Jacob <sup>21</sup> reported that vitamin C enhances antioxidant activity of vitamin E by reducing the tocopheroxyl radicals back to their active form vitamin E. Similarly, Yin et al. <sup>57</sup> reported that a mixture of β-tocopherol and ascorbate delayed myoglobin oxidation. In addition, vitamin C itself plays an important role not only in reacting with all aggressive oxygen species under formation of a practically inert radical but also in transferring radical equivalents from lipid phases to aqueous compartments <sup>19,23</sup>. Results of the present study showed that a combination of 250 mg of vitamin C and 250 mg of vitamin E provides the greatest performance in Japanese quails reared under heat stress. Such a combination can be considered as a protective management practice in a poultry diet, ameliorating the detrimental effects of heat stress.

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#### References

- <sup>1</sup>Donkoh, A. 1989. Ambient temperature: a factor affecting performance and physiological response of broiler chickens. *Int. J. Biometeorol.* **33**: 259-265.
- <sup>2</sup>Siegel, H. S. 1995 Stress, Strains & Resistance. *Br. Poult. Sci.* **36**: 3-22.
- <sup>3</sup>Edens, F. W., and Siegel, H.S. 1975. Adrenal responses in high and low acth response lines of chickens during acute heat stress. *Gen. Comp. Endocrinol.* **25**: 64-73.
- <sup>4</sup>Freeman, B. A. and Crapo, J. D. 1982. Biology of disease: Free radicals and tissue injury. *Lab. Invest.* **47**: 412-426.
- <sup>5</sup>Sahin, K. Sahin, N., Onderci, M. Yaralioglu, S. and Kucuk, O. 2001. Protective role of supplemental vitamin E on lipid peroxidation, vitamins E, A and some mineral concentrations of broilers reared under heat stress. *Vet. Med. -Czech.* **46** (5): 140-144.
- <sup>6</sup>Sahin, K. Sahin, and N. Yaralioglu, S. 2002. Effects of vitamin C and vitamin E on lipid peroxidation, blood serum metabolites and mineral concentrations of laying hens reared at high ambient temperature. *Biol. Trace Elem. Res.* **85**: 35-45.
- <sup>7</sup>Freeman, B.M. 1967 Effect of stress on the ascorbic acid content of the adrenal gland of gallus domesticus. *Comp. Biochem. Physiol.* **23**: 303-305.
- <sup>8</sup>Sykes, A. H. 1978 Vitamin C for Poultry; some recent research. *Roche Symposium*, pp:5-15
- <sup>9</sup>Bollengier-Lee, S., Mitchell, M. A. Utomo, D. B. Williams, P. E. V. and Whitehead, C. C. 1998. Influence of High Dietary vitamin E supplementation on egg production and plasma characteristics in hens subjected to heat stress. *Br. Poult. Sci.* **39**: 106-112.
- <sup>10</sup>Bollengier-Lee, S., Williams, P. E. V. And Whitehead, C. C. 1999. Optimal dietary concentration of vitamin e for alleviating the effect of heat stress on egg production in laying hens. *Br. Poult. Sci.* **40**: 102-107.
- <sup>11</sup>Feenster, R. 1985. High temperatures decrease vitamin utilization. *Misset Poultry.* **38**: 38-50
- <sup>12</sup>Puthongsiriporn, U. Scheideler, S.E. Sell, J.L. and Beck, M.M. 2001. Effects of vitamin E and C supplementation on performance, in vitro lymphocyte proliferation, and antioxidant status of laying hens during heat stress. *Poult. Sci.* **80**: 1190-1200.
- <sup>13</sup>Whitehead, C. C., Bollengier-Lee, S., Mitchell, M. A. and Williams, P. E. V. 1998 Vitamin E can alleviate the depression in egg production in heat stressed laying hens. *Proc. of Spring meeting, WPSA-UK Branch*, pp-55-56. Scarborough.
- <sup>14</sup>Kafri, I. and Cherry, J.A. 1984 Supplemental ascorbic acid and heat stress in broiler chicks. *Poult. Sci.* **63** (suppl):125.
- <sup>15</sup>Kutlu, H.R. and Forbes, J.M. 1993. Changes in growth and blood parameters in heat-stressed broiler chicks in response to dietary ascorbic acid. *Livestock Prod. Sci.* **36**: 335-350
- <sup>16</sup>Njoku, P.C. 1986. Effect of dietary ascorbic acid (vitamin C) supplementation on the performance of broiler chickens in a tropical environment. *Anim. Feed Sci. Technol.* **16**: 17-24.
- <sup>17</sup>Pardue, S. L. Thaxton, J. P. and Brake, J. 1985. Influence of supplemental ascorbic acid on broiler performance following exposure to high environmental temperature. *Poult. Sci.* **64**: 1334-1338.
- <sup>18</sup>Sahin, K. and Küçük, O. 2001. Effects of vitamin C and Vitamin E on performance, digestion of nutrients and carcass characteristics of Japanese quails reared under heat stress (34°C). *J. Anim. Physiol. a. Anim. Nutr.* **85**: 335-342.
- <sup>19</sup>Jacob, R. A. 1995. The integrated antioxidant system. *Nutr. Res.* **15**: 755-766.
- <sup>20</sup>Gallo-Torres, D.C. 1980 Absorption, blood transport and metabolism of vitamin E. In *A Comprehensive Treatise*, Maclin, L.J. (ed.), Marcel Dekker, New York, pp:170-267.
- <sup>21</sup>Yin, M.C., Faustman, C., Reisen, J.W., and Williams, S.N. 1993. Alfa-tocopherol and ascorbate delay oxymyoglobin and phospholipid oxidation in vitro. *J. Food Sci.* **58**: 1273-1276.
- <sup>22</sup>Gey, K.F. 1998 Vitamins e plus c and interacting conutrients required for optimal health. *BioFactors* **7**: 113-174.
- <sup>23</sup>Tappel, A.L. 1968. Will antioxidant Nutrients Slow aging Processes? *Geri-*

- atrics. **23**: 1371-1376.
- <sup>24</sup>Bendich, A., Apolito, P. D., Gabriel, E. And Machlin, L.J. 1984. Interaction of dietary vitamin C and E on guinea pig immune responses to mitogens. *J. Nutr.* **114**: 1588-1593.
- <sup>25</sup>Gonzalez-Vega-Aguirre, D., Contreras, B.P.A., Klein, R. And Bohmwald, H. 1995. Effect of vitamin C and E supplementation in the diet of broiler chicks on performance and immune response. *Veterinaria.* **26**: 333-340.
- <sup>26</sup>Macpherson, A. 1994. Selenium, vitamin E and biological oxidation. In: Cole D. J. and Garnsworthy P. J. (eds.): *In recent advances in Animal Nutrition.* Butterworth and Heinemann's, Oxford. pp:3-30.
- <sup>27</sup>McDowell, L.R. 1989. Vitamins in Animal Nutrition. Comparative Aspects to Human Nutrition. Vitamin A and E. Mc Dowell L.R. (ed.) Academic Press London, pp-10-52, 93-131.
- <sup>28</sup>Yu, B.P. 1994. Cellular defenses against damage from reactive oxygen species. *Physiol. Rev.* **74**: 139-162.
- <sup>29</sup>National Research Council. 1994. Nutrient requirements of poultry. National Academy Press. Washington, DC. Ninth Revised Edition.
- <sup>30</sup>Hempe J. M, Lauxen, R.C, and Savage, J. E. 1998 Rapid determination of egg weight and specific gravity using a computerized data collection system. *Poult. Sci.* **67**: 902-907.
- <sup>31</sup>Eisen, E.J., Bohren, B.B. and McKean H.E, 1962. The haugh unit as a measure of egg albumen quality. *Poult. Sci.* **41**: 1461-1468.
- <sup>32</sup>Petry, H. and Rapp, W. 1971 Zur Problematik der Chromoxidebestimmung in Verdauungsversuchen. *Z. Tierphysiol. Tierernaehr.* **27**: 181-189.
- <sup>33</sup>A.O.A.C. 1990 Official Methods of Analysis Association of Agricultural Chemists. Virginia, D. C., U.S.A.
- <sup>34</sup>Terpstra, K. and De Hart, N. 1974. The estimation of urinary nitrogen and fecal nitrogen in poultry excreta. *Z. Tierphysiol. Tierernaehr. Futtermittlkd.* **32**: 306-312
- <sup>35</sup>Placer, Z. A., Cushmann, L. L. and Johnson, B. C. 1966. Estimation of products of lipid peroxidation in biochemical systems. *Anal. Biochem.* **16**: 359-364.
- <sup>36</sup>Matkovýcs, B., Szabo, I. Varga, I. S. 1989. Determination of enzyme activities in lipid peroxidation and glutathione pathways (in Hungarian). *Laboratoriumi Diagnosztika* **15**: 248-249.
- <sup>37</sup>McMurry, C. H., Blanchflower, W. j. and Rice, D. A.1980. Influence of extraction techniques on the determination of  $\alpha$ -tocopherol in animal feedstuffs. *J. Assoc. Off. Anal. Chem.* **63**: 1258-1261.
- <sup>38</sup>Kyaw, A. 1978. A Simple calorimetric methods for ascorbic acid determination in blood plasma. *Clin. Chem. Acta.* **86**:153-160.
- <sup>39</sup>SAS Institute, 1996 SAS User's Guide: Version 6.12. SAS Institute Inc., Cary, NC.
- <sup>40</sup>Orban, J.I, Roland, D.A. Jr., Cummins, K., and Lovell, R.T. 1993 Influence of large doses of ascorbic acid on performance, plasma calcium, bone characteristics and eggshell quality in broiler and leghorn hens. *Poult. Sci.* **72**: 691-700.
- <sup>41</sup>Ensminger, M. E., J. E. Oldfield, and Heinemann W. W., 1990. Feeds and Nutrition. pp. 08-110. The Ensminger Publishing Company, USA.
- <sup>42</sup>Brown, K. I. and Nestor, K. E., .1973. Some physiological responses of turkeys selected for high and low adrenal response to cold stress. *Poult. Sci.* **52**: 1948-1950.
- <sup>43</sup>El-Boushy A.R, Simons PCM, and Wiertz G, 1968. Structure and ultra structure of the hen's egg shell as influenced by environmental temperature, humidity and vitamin C additions. *Poult. Sci.* **47**: 456-467.
- <sup>44</sup>Demir E, Öztürkcan, O, Görgülü M. Kutlu H.R and Okan F. 1995. Sıcak kōbullarda yumurta tavuđu rasyonlarına eklenen vitamin A ve C' nin yumurta özelliklerine etkileri. *J.Agric. Fac. Ç.Ü.* **10**, (4): 123-132.
- <sup>45</sup>Dorr P. and Balloun S. L, 1976 Effect of dietary vitamin A, ascorbic acid and their interaction on turkey bone mineralization. *Br. Poult. Sci.* **17**: 581-599.
- <sup>46</sup>Richards, M.P. 1997. Trace mineral metabolism in the avian embryo. *Poult. Sci.* **76**: 152-164
- <sup>47</sup>Burley, R.W., Ewans, A.J. and Pearson, J. A. 1993. Molecular aspects of the synthesis and deposition of hens' egg yolk with special reference to low density lipoprotein. *Poult. Sci.* **72**: 850-855.
- <sup>48</sup>Wallis, I.R. and Balnave, D. 1984.The influence of environmental temperature, age and sex on the digestibility of amino acids in growing broiler chickens. *Br. Poult. Sci.* **25**: 401-407.
- <sup>49</sup>Zuprizal, M., Larbier, A.M., Channeau, and Geraert, P.A., 1993 Influence of ambient temperature on true digestibility of protein and amino acids of rapeseed and soybean meals in broilers. *Poult. Sci.* **72**: 289-295.
- <sup>50</sup>Hai, By. L. Rong, D. Zhang, Z. Y. 2000 The effect of thermal environment on the digestion of broilers. *J. Anim. Physiol Anim. Nutr.* **83**: 57-64.
- <sup>51</sup>Osman, A.M. and Tanios, N.I. 1983 The effect of heat on the intestinal and pancreatic levels of amylase and maltase of laying hens and broilers. *Comp. Biochem. Physiol.* **75A**:563-567.
- <sup>52</sup>McKee, J. S., and Harrison, P.C. 1995. Effects of supplemental ascorbic acid on the performance of broiler chickens exposed to multiple concurrent stressors. *Poult. Sci.* **74**:1772-1785.
- <sup>53</sup>Sahin, K. Kucuk, O. Sahin, N. and Sari, M 2002. Effects of Vitamin C and Vitamin E on Lipid Peroxidation Status, Some Serum Hormone, Metabolite, and Mineral Concentrations of Japanese Quails Reared Under Heat Stress (34 °C). *Int. J. Vitamin and Nutrition Res.* **72**: 91-100.
- <sup>54</sup>Webster, A.J.F. 1983. Nutrition and the thermal environment in nutritional physiology of farm animals, J.A.F. Rook and P.C. Thomas (Eds.), New York, Longman, pp:639-669.
- <sup>55</sup>Cheng, T.K., Coon, C.N. and Hamre, M. L. 1990. Effect of environmental stress on the ascorbic acid requirement of laying hens. *Poult. Sci.* **69**:774-780.
- <sup>56</sup>Klasing, K.C. 1998. Comparative Avian Nutrition. University Press, Cambridge. 277-299.
- <sup>57</sup>Luadicina, D.C. and Marnett, L.J. 1990. Enhancement of hydroperoxide-dependent lipid peroxidation in rat liver microsomes by ascorbic acid. *Arch. Biochem. Biophys.* **278**: 73-80
- <sup>58</sup>Morrissey, P.A., Brandon, S., Buckley, D.J., Sheehy, P.J.A. and Frigg, M.1997. Tissue content of  $\beta$ -tocopherol and oxidative stability of broilers receiving dietary  $\beta$ -tocoperol acetate supplement for various periods post-slaughter. *Br. Poult. Sci.* **38**: 84-88.
- <sup>59</sup>Halliwell, B. and Gutteridge, J.M.C. 1989. Lipid peroxidation: A radical chain reaction. Pages 188-218. In: *Free Radicals in Biology and Medicine.* 2<sup>nd</sup> ed. Oxford University Press, New York, NY.