Praca oryginalna *Original paper*

Influence of different dietary vitamin E supplementation on some plasma components and egg production of laying Japanese quails during heat stress

SULE KAYA

Deparment of Physiology, Faculty of Veterinary Medicine, University of Mustafa Kemal, Antakya-Hatay, Turkey

Kaya S.

Influence of different dietary vitamin E supplementation on some plasma components and egg production of laying Japanese quails during heat stress

Summary

The experiment was conducted to evaluate the effects of vitamin E (dl-α-tocopherol acetate) supplementation at various concentrations (0-control-, 250, 500, 750 mg/kg of diet) on the feed intake, egg production, plasma enzymes, electrolytes and metabolites of laying quails (Coturnix coturnix japonica) exposed to chronic heat stress at a mean temperature of 31°C from 13 to 17 weeks of age. The quails were randomly assigned to 4 treatment groups, 3 replicates of 15 birds each. Body weight, feed intake and egg production were not affected by vitamin E supplementation. Plasma total cholesterol, total protein, alkaline phosphatase (ALP), blood urea nitrogen (BUN), inorganic P and Mg concentrations did not differ among the groups. Plasma creatinine was higher in the 750 mg/kg vitamin E supplemented group than in other vitamin E supplemented groups. Plasma glucose concentrations decreased as dietary vitamin E increased up to 500 mg/kg of the diet. A higher glucose concentration and activity of glutamic-oxaloacetic transaminase (GOT) were stated in the control group. Triglyceride concentration was the highest whereas albumin and Ca concentrations were higher in the control group than for the 250 and 750 mg/kg vitamin E supplemented groups. Plasma glutamic pyruvic transaminase (GPT) activity was the lowest in control group.

These results indicate that the supplementation of 500 mg vitamin E/kg to the diet may offer a potential protective management practice for preventing heat stress-related damages in laying quails.

Keywords: heat stress, vitamin E, egg production, blood parameters, laying quails

High environmental temperature is one of the most serious factors affecting the production performance of laying hens in both tropical and temperate countries. The adverse effects of chronic heat stress on laying hens depress feed intake (20), egg production (30), egg weight (31) and shell quality (14, 31). There have been many reports of attempts to alleviate the consequences of heat stress by nutritional manipulation (10, 25-27). Laying hens represent a more complex situation, as the egg cycle consists of a period of massive protein secretion followed by periods of massive calcium and bicarbonate secretions. The effect of ambient temperature on eggshell quality is most likely to be mediated through changes in ionised calcium and bicarbonate (11, 14, 23). The diminished ability of duodenal cells to transport calcium may be a critical factor in the detrimental effects of heat stress on egg production and eggshell characteristics in laying hens (14). Furthermore the effects of chronic heat stress on intestinal morphology are decreased villus heights and wet and dry weights per unit length of jejunum (20).

Vitamin E, one of the most powerful intercellular and intracellular antioxidants, has been included into the animal feed to improve performance and alleviate the effects of chronic heat stress in laying hens (3, 17, 27, 30). Evidence was obtained that the depression of egg production during heat stress is a reduction in the circulatory supply of yolk precursors, particularly vitellogenin (3, 30) and very low density lipoprotein (triglyceride) (3). The beneficial effect of vitamin E was associated with increased plasma concentrations of these precursors and it has also been speculated that vitamin E supplementation may have raised the synthesis of these precursors in the liver by protecting the liver from lipid peroxidation and damage to cell membranes by acting as a membrane-bound antioxidant and maintaining the necessary properties for transport mechanisms across membranes (3, 30). On the other hand, it has been reported that high temperature (mean 26.7 and 29.4°C) did not affect egg production (5) except in older hens (16 to 18-months-of-age) exposed to 35°C and 60% relative humidity (31). It has been

indicated that acute heat stress had no adverse effects on dietary amino acid digestibility (13); moreover, heat stress did not significantly affect egg weight or feed conversion (22).

The evaluation of blood composition can be a useful method for detecting changes due to heat stress. Blood composition changes as a result of metabolism, nutrition and cellular damage. Thus blood levels of metabolites such as glucose, blood urea nitrogen, minerals, enzymes, and proteins will be greatly affected by metabolism, nutrition and organ functioning. These changes can be measured and related to the altered metabolism (6).

The aim of this study was to evaluate the effect of vitamin E differing in the diet on the feed intake, egg production, plasma enzymes, electrolytes and metabolites of laying quails during chronic heat stress.

Material and methods

One hundred eighty, 13-week-old Japanese quails (*Coturnix coturnix japonica*) were used in this study. The birds were randomly assigned to 4 treatment groups, 3 replicates of 15 birds each. The quails were fed *ad libitum* either a basal diet (2700 kcal ME/kg, 17.90% CP, 3.2% Ca, 0.7% P, 8000 IU vitamin A/kg, 1500 IU vitamin D_3/kg, 15 IU vitamin E/kg,10 IU vitamin B_{12}/kg, 4 IU vitamin B_2/kg, 2 IU vitamin K_3/kg) which is based on soybean meal and corn, or a basal diet supplemented with 250, 500, 750 mg of α-tocopherol-acetate/kg of diet for 4 weeks. The basal diet met all nutrient requirements for laying quails (1).Vitamin E was specifically produced by a commercial company (Roche, Istanbul, Turkey) as a source of vitamin E for feed. The mean value of daily temperature in the quail house was 31°C, average relative humidity was 60%, and light period was 17 h/d.

The quails were weighed at the beginning and at the end of the study. Egg production was recorded daily, feed intake was measured weekly. At the end of the experiment, 13 birds were randomly chosen from each treatment group and slaughtered; blood samples were taken to heparinized tubes. Plasma glucose, BUN, creatinine, total cholesterol and protein, triglyceride, albumin, ALP, GOT, GPT, Ca, Mg, inorganic phosphorus were measured using a biochemical analyzer kit (Olympus AU 5200). Plasma vitamin E concentration was measured by high pressure liquid chromatography (19).

All data were subjected to analysis of variance and Duncan's multiple range test by using statistical analysis system (SPSS, Release 9.0; SPSS, Inc., Chicago, IL, USA). Significance was set at $p < 0.05$.

Results and discussion

Reduction in feed intake (10, 16) and body weight (5, 16) in response to heat stress were observed in birds. Increased supplemental vitamin E to the diet of layers increased feed intake (10, 26) and live weight gain (26). The changes in feed intake were not observed due to vitamin E supplementation in quails reared under the temperature of 31°C (tab. 1) confirms results of earlier studies in laying hens (3, 21, 25) as well as body weight (25). Plasma vitamin E concentrations were significantly increased by increasing dietary supplementation of vitamin E (tab. 2).

Egg production and feed intake were higher in laying quails reared under a thermo neutral zone (22°C) in comparison to heat stressed (34°C) quails and were 32.8 g – 73.5% and 29.2 g – 64.3%, respectively (24). The decrease of egg production in layers related to high temperature was presented in some reports (10, 16, 22, 30). However, significant differences were not determined in a percent of hen-day egg production at a mean temperature of 29.4°C in cycling chambers (5) and the changes in temperature (35°C) in laying hens except for older hens exposed to 60% relative humidity (31).

A decrease in vitellogenin (Vg), egg yolk precursor, synthesis in the liver, likewise, more importantly, an inhibition in the release of Vg from liver (30) or decrease in feed consumption (16) are the reasons for the depression of egg production during heat stress. Plasma protein concentration decreased in broilers during exposure to 30°C (32), but did not change in laying hens exposed to 38°C (12).

Vitamin E appears to act during heat stress by facilitating the release of Vg from liver and thus increasing the circulatory supply of precursor protein for yolk formation. Dietary supplementation of vitamin E minimised the depression in egg production brought about by heat stress (4, 10, 30). Vitamin E supplementation at different levels (125, 250, and 500 mg/kg of diet) had no significant effects on the measured values under thermo neutral conditions (22°C); however, it improved the performance (namely live weight, feed intake, egg production and feed efficiency as well as egg quality) in laying Japanese quails reared under heat stress (34°C) (28). On the other hand, Mori et al. (21) stated that high vitamin E supplementation declined egg production in laying hens.

Egg production results of laying quails in the control group aged 13 and 17 weeks were higher (86.81--87.83%, respectively) in thermo

Tab. 1. Effects of dietary vitamin E supplementation on body weight, feed intake and egg production of Japanese quails reared under a temperature of 31°C for 4 weeks ($\bar{x}$ ± SEM)

Performance parameters	Vitamin E supplementation, mg/kg of diet			
	0 (Control)	250	500	750
Body weight, g				
13 weeks	198.50 ± 5.02	192.43 ± 5.80	199.32 ± 3.86	204.91 ± 4.27
17 weeks	222.23 ± 5.04	211.16 ± 5.37	215.42 ± 3.01	214.23 ± 4.01
Feed intake, g d^{-1}	24.15 ± 1.16	23.38 ± 1.11	23.25 ± 0.90	23.50 ± 1.92
Egg production, eggs produced hen d^{-1}	0.65 ± 0.02	0.60 ± 0.02	0.64 ± 0.02	0.64 ± 0.02

Tab. 2. Effects of dietary vitamin E supplementation on some blood plasma parameters of Japanese quails reared under a temperature of 31°C for 4 weeks ($\bar{x} \pm SEM$)

Blood parameters	Vitamin E supplementation, mg/kg of diet			
	0 (Control)	250	500	750
Vitamin E, μmol L^{-1}	132609.42 ± 185.44^{a}	136210.30 ± 190.50^{b}	139679.01 ± 263.17^{c}	146412.65 ± 467.66^{d}
Blood urea nitrogen, mmol L^{-1}	0.50 ± 0.05^{a}	0.52 ± 0.05^{a}	0.43 ± 0.04^{a}	0.50 ± 0.06^{a}
Creatinine, μmol L^{-1}	70.04 ± 3.67^{ab}	65.28 ± 3.09^{a}	62.56 ± 2.54^{a}	74.80 ± 2.76^{b}
Total cholesterol, mmol L^{-1}	5.82 ± 0.47^{a}	5.67 ± 0.61^{a}	5.12 ± 0.49^{a}	5.72 ± 0.29^{a}
Triglyceride, mmol L^{-1}	6.50 ± 0.97^{a}	2.05 ± 0.41^{b}	2.61 ± 0.49^{b}	2.33 ± 0.43^{b}
Alkaline phosphatase, U L^{-1}	1237.80 ± 121.07^{a}	1201.50 ± 103.31^{a}	1230.75 ± 159.8^{a}	1345.20 ± 91.52^{a}
Glutamic-oxaloacetic transaminase, U L^{-1}	246.76 ± 11.81^{b}	217.69 ± 9.87^{ab}	205.69 ± 10.52^{a}	230.61 ± 10.17^{ab}
Glutamic pyruvic transaminase, U L^{-1}	10.46 ± 0.96^{a}	21.76 ± 2.44^{b}	20.69 ± 0.94^{b}	27.30 ± 2.65^{c}
Albumin, g L^{-1}	15.92 ± 1.24^{b}	11.30 ± 0.67^{a}	13.61 ± 0.95^{ab}	12.15 ± 0.73^{a}
Total Protein, g L^{-1}	37.38 ± 1.41^{a}	36.46 ± 2.80^{a}	36.46 ± 1.24^{a}	35.53 ± 1.32^{a}
Glucose, mmol L^{-1}	16.97 ± 0.46^{b}	15.78 ± 0.65^{ab}	14.76 ± 0.30^{a}	16.21 ± 0.26^{b}
Ca, mmol L^{-1}	4.13 ± 0.38^{b}	2.84 ± 0.17^{a}	3.43 ± 0.31^{ab}	2.93 ± 0.18^{a}
Inorganic P, mmol L^{-1}	1.38 ± 0.16^{a}	1.39 ± 0.05^{a}	1.32 ± 0.14^{a}	1.24 ± 0.09^{a}
Mg, mmol L^{-1}	1.85 ± 0.14^{a}	1.81 ± 0.12^{a}	1.66 ± 0.13^{a}	1.54 ± 0.08^{a}

Explanations: means in a row with a different letter differ significantly at $p < 0.05$

neutral conditions (9) when compared to presented data of the quails aged 17 weeks reared in high environmental temperature (tab. 1). Moreover, egg production was not changed significantly by supplementing vitamin E to the diet in this investigation (tab. 1) which is in agreement with other reports (8, 25). According to above explanations unchanged feed intake and plasma total protein levels by heat stress and adding vitamin E caused no significant difference in egg production (tab. 1, 2).

An acute heat stress period in laying hens (38°C) did not affect blood plasma glucose, uric acid and creatinine (12). Glucose concentration of blood was decreased insignificantly as dietary vitamin E concentration increased (250 and 750 mg/kg) in this study. There was a significant increase in the glucose of the control group, especially compared with the 500 mg/kg vitamin E supplemented group (tab. 2). This may be explained by the possible disability of the cells to metabolize carbohydrates and use proteolysis as an alternative process for energy production in heat stress (15). Plasma triglyceride levels of the unsupplemented group was the highest compared with vitamin E supplemented groups (tab. 2). Heat stress tended to elevate blood corticosterone and ACTH levels and it resulted in increased serum glucose and triglyceride concentrations (26, 27), as determined in the quails of the control group in this study. These results may probably be due to the greater catabolic effect (or concentration) of ACTH, yielding more glucose and triglycerides in the serum and may have an influence on the lowered effects of heat stress with greater supplementation of vitamin E (26).

In normally hydrated fowls, heat stress did not significantly affect blood constituents. Water deprivation increased SGPT, and hyperthermic dehydration increased BUN, uric acid in the fowl (*Gallus domesticus*) (2).

The significant increase in plasma GOT activity, creatinine and BUN strongly suggest an increased permeability of cell membranes and damage to muscle cells under heat stress (42-44°C) (15). In this study, creatinine was significantly higher in the 750 mg vitamin E supplemented group than other vitamin E supplemented groups. GOT activity was significantly higher but BUN presented a tendency for higher values in the control group compared with the group feeding 500 mg vitamin E/kg of diet (tab. 2). These results may be explained in that the damage of the muscle cells of laying quails in the temperature of 31°C was not too severe. On the other hand, Sahin et al. (26) reported that activities of SGOT and SGPT remained unchanged but blood cholesterol level decreased, activity of serum ALP increased with increasing vitamin E supplementation in broilers reared under heat stress (32°C). Although plasma ALP activity and total cholesterol level were not significantly different among the groups in the present study (tab. 2), it may be suggested that liver tissue was not damaged by heat stress as reported by Marder et al. (15). Increasing vitamin E concentration caused an increase in GPT activity in this study.

Exposure of laying hens to an acute heat stress period (38°C) produced a decrease in blood plasma magnesium (12). Inorganic P and Mg levels normally increase during renal failure (6). In the present study,

plasma Mg and inorganic P were not affected by the supplementation of vitamin E to the diet in quails reared under the temperature of 31°C (tab. 2). Ca uptake by duedonal epithelial cells was decreased by exposure to high environmental temperature (35°C) and this may be a critical factor in detrimental effects of heat stress on egg production (14). Contrarily, Samara et al (29) reported that neither blood concentration of ionized Ca nor total plasma Ca was affected by temperature and the supply of Ca available in blood for shell deposition is not diminished in hens acclimatized to high environmental temperature (21 to 39°C). The disagreement could be due to differences in heat stress treatments or the type or age of birds used. It has been observed that plasma Ca and P concentrations increased in heat stressed Japanese quails (34°C) and broilers (32°C) fed a diet supplemented with vitamin E (26, 27). Horowitz and Adler (7), explained that plasma volume was maintained via the maintenance of adequate albumin mass, while heat acclimation (34°C) resulted in a 48% reduction in albumin synthesis. The Ca bound to albumin 80% and globulin 20% is an important reservoir of Ca (18). It was confirmed in our results that significantly increased albumin concentration of plasma is responded to by an increase in Ca concentration of the unsupplemented group compared with the 250 and 750 mg vitamin E supplemented groups (tab. 2). This may indicate that increasing dietary vitamin E levels (250 and 750 mg/kg) decreased plasma albumin and total plasma Ca levels in quails reared under a mean temperature of 31°C. However, in terms of albumin and Ca concentration, the group with 500 mg vitamin E/kg of diet supplementation responded similarly as the unsupplemented group. According to explanations above (7, 29) Ca and albumin concentrations may differ due to the maintained plasma volume since egg production was not affected by giving vitamin E supplementation to quails reared under 31°C temperature.

In conclusion, although egg production and feed intake were not different, some parameters in the blood changed indicating cell damage which is a result of heat stress. Supplementing 500 mg vitamin E/kg of diet may offer a potential protective management practice for preventing heat stress-related damages in laying quails.

References

1. *Anon.*: National Research Council (NRC): Nutrient requirements of Poultry. National Academy Press, Washington DC 1994.
2. *Arad Z., Marder J., Elylath U.*: Serum electrolyte and enzyme responses to heat stress and dehydration in the fowl (Gallus Domesticus). Comp. Biochem. Physiol. A 1983, 74, 449-453.
3. *Bollengier-Lee S., Mitchell M. A., Utom D. B., Williams P. E. V., Whitehead C. C.*: Influence of a high dietary vitamin E supplementation on egg laying response of hens subjected to heat stress. Brit. Poult. Sci. 1998, 39, 106-112.
4. *Bollengier-Lee S., Williams P. E. V., Whitehead C. C.*: Optimal dietary concentration of vitamin E for alleviating the effect of heat stress on egg production in laying hens. Brit. Poult. Sci. 1999, 40, 102-107.
5. *Emery D. A., Vohra P., Ernst R. A., Morrison S. R.*: The effect of cyclic and constant ambient temperatures on feed consumption, egg production, egg weight and shell tickness of hens. Poultry Sci. 1984, 63, 2027-2035.
6. *Finco D. R.*: Kidney function, [in:] Kaneko J. J., Harvey J. W., Bruss M. L.: Clinical Biochemistry of Domestic Animals. Academic Press, London 1997.
7. *Horowitz M., Adler J. H.*: Plasma volume regulation during heat stress: Albumin synthesis vs capillary permeability. A comparison between desert and non-desert species. Comp. Biochem. Physiol. A 1983, 75, 105-110.
8. *Jiang Y. H., Mcgeachin R. B., Bailey C. A.*: Alpha-tocopherol, beta-carotene and retinol enrichment of chicken eggs. Poultry Sci. 1994, 73, 1137-1143.
9. *Kaya S., Erdogan Z., Erdogan S.*: Effect of different dietary levels of Yucca schidigera powder on the performance, blood parameters and egg yolk cholesterol of laying quails. J. Vet. Med. A 2003, 50, 14-17.
10. *Kirunda D. F., Scheideler S. E., Mckee S. R.*: The efficacy of vitamin E (DL--alpha-tocopheryl acetate) supplementation in hen diets to alleviate egg quality deterioration associated with high temperature exposure. Poultry Sci. 2001, 80, 1378-1383.
11. *Koelkebeck K. W., Odom T. W.*: Laying hen responses to acute heat stress and carbondioxide supplementation: I. Blood gas changes and plasma lactate accumulation. Comp. Biochem. Physiol. A 1994, 107, 603-606.
12. *Koelkebeck K. W., Odom T. W.*: Laying hen responses to acute heat stress and carbon dioxide supplementation. II – Changes in plasma enzymes, metabolites and electrolytes. Comp. Biochem. Physiol. A 1995, 112, 119-122.
13. *Koelkebeck K. W., Parsons C. M., Wang X.*: Effect of acute heat stress on amino acid digestibility in laying hens. Poultry Sci. 1998, 77, 1393-1396.
14. *Mahmoud K. Z., Beck M. M., Scheideler S. E., Forman M. F., Anderson K. P., Kachman S. D.*: Acute high environmental temperature and calcium-Estrogen relationships in the hen. Poultry Sci. 1996, 75, 1555-1562.
15. *Marder J., Eylath U., Moskovitz E., Sharir R.*: The effect of heat exposure on blood chemistry of the hyperthermic rabbit. Comp. Biochem. Physiol. A 1990, 97, 245-247.
16. *Mashaly M. M., Hendricks III G. L., Kalama M. A., Gehad A. E., Abbas A. O., Patterson P. H.*: Effect of heat stress on production parameters and immune responses of commercial laying hens. Poultry Sci. 2004, 83, 889-894.
17. *McDowell L. R.*: Minerals in Animal Human Nutrition. Academic Press, London 1992.
18. *McDowell L. R.*: Vitamins in Animal Nutrition-Comperative Aspects to Human Nutrition. Academic Press, London 1989.
19. *Miller K. W., Yang C. S.*: An isocratic high-performance liquid chromatography method for the simultaneous analysis of plasma retinol, alpha-tocopherol, and various carotenoids. Anal. Biochem. 1985, 145, 21-26.
20. *Mitchell M. A., Carlisle A. J.*: The effects of chronic exposure to elevated environmental temperature on intestinal morphology and nutrient absorption in the domestic fowl (Gallus domesticus). Comp. Biochem. Physiol. A 1992, 101, 137-142.
21. *Mori A. V., Mendonça Jr. C. X., Almedia C. R. M., Pita M. C. G.*: Supplementing hen diets with vitamin A and E affects egg yolk retinal and α-tocopherol levels. J. Appl. Poult. Res. 2003, 12, 106-114.
22. *Muiruri H. K., Harrison P. C.*: Effect of roost temperature on performance of chickens in hot ambient environments. Poultry. Sci. 1991, 70, 2253-2258.
23. *Odom T. W., Harrison P. C., Bottje W. G.*: Effects of thermal induced respiratory alkalosis on blood ionized calcium levels in the domestic hen. Poultry Sci. 1986, 65, 570-573.
24. *Onderci M., Sahin N., Sahin K., Balci T. A., Gursu M. F., Juturu V., Kucuk O.*: Dietary arginine silicate inositol complex during the late laying period of quail at different environmental temperatures. Brit. Poult. Sci. 2006, 47, 209-215.
25. *Puthpongsiriporn U., Scheideler S. E., Sell J. L., Beck M. M.*: Effects of vitamin E and C supplementation on performance, in vitro lymphocyte proliferation, and antioxidant status of laying hens during heat stress. Poultry Sci. 2001, 80, 1190-1200.
26. *Sahin K., Kucuk O., Sahin N., Gursu M. F.*: Optimal dietary concentration of vitamin E for alleviating the effect of heat stress on performance, thyroid status, ACTH and some serum metabolite and mineral concentrations in broilers. Vet. Med. Czech. 2002a, 47, 110-116.
27. *Sahin K., Kucuk O., Sahin N., Sari M.*: 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. Vit. Nutr. Res. 2002b, 71, 91-100.
28. *Sahin K., Sahin N., Onderci M.*: Vitamin E supplementation can alleviate negative effects of heat stress on egg production, egg quality, digestibility of nutrients and egg yolk mineral concentrations of Japanese quails. Res. Vet. Sci. 2002c, 73, 307-312.
29. *Samara M. H., Robbins K. R., Smith M. O.*: Environmental heat stress does not reduce blood ionised calcium concentration in hens acclimated to elevated temperatures. Poultry Sci. 1996, 75, 197-200.
30. *Whitehead C. C., Bollengier-Lee S., Mitchell M. A., Williams P. E. V.*: Vitamin E can alleviate the depressed egg production of heat stressed laying hens. Brit. Poult. Sci. 1998, 39, S44-46.
31. *Yahav S., Shinder D., Razpakovski V., Rusal M., Bar A.*: Lack of response of laying hens to relative humidity at high ambient temperature. Brit. Poult. Sci. 2000, 41, 660-663.
32. *Zhou W. T., Fujita M., Yamamoto S., Iwasaki K., Ikawa R., Oyama H., Orikawa H.*: Effects of glucose in drinking water on the changes in whole blood viscosity and plasma osmolality of broiler chickens during high temperature exposure. Poultry Sci. 1998, 77, 644-647.

Author's address: Assoc. Prof. Dr. Sule Kaya, Deparment of Physiology, Faculty of Veterinary Medicine, University of Mustafa Kemal, Antakya--Hatay, Turkey; e-mail: sule_kaya@hotmail.com, sule_ky@yahoo.com