Effects of Dietary Lycopene and Vitamin E on Egg Production, Antioxidant Status and Cholesterol Levels in Japanese Quail*

N. Sahin, K. Sahin[1], M. Onderci, M. Karatepe[2], M. O. Smith[3] and O. Kucuk[4]**
Veterinary Control and Research Institute, 23100 Elazig, Turkey

ABSTRACT : Japanese Quails were used to evaluate the effects of dietary supplementation with vitamin E (dl-a-tocopheryl-acetate), lycopene, and their combination on egg production, egg quality, concentrations of malondialdehyde (MDA), vitamin E, A and cholesterol in serum and egg yolk. Quails (n = 120; 55 d old) were divided into four groups (n = 30/group) and fed a basal diet or the basal diet supplemented with lycopene (100 mg/kg diet), vitamin E (250 mg dl-α-tocopheryl-acetate/kg diet) or a combination of lycopene and vitamin E (100 mg/kg lycopene plus 250 mg dl-α-tocopheryl-acetate/kg diet). Vitamin E and lycopene did not affect ($p>0.05$) body weight, feed intake or egg weight. Egg production and Haugh unit were greater ($p<0.05$) in each supplemental group compared with the control group ($p<0.05$). Serum and liver MDA levels were decreased in supplemented groups compared with the control group. Separately or as a combination, supplemental lycopene and vitamin E increased serum and egg yolk vitamin E and A but decreased cholesterol concentrations ($p<0.05$). In general, when a significant effect was found for a parameter, the magnitude of the responses to vitamin and lycopene supplements was greatest with the combination of the lycopene and vitamin E, rather than that observed with each supplement separately. Results of the present study indicate that supplementing with a combination of dietary lycopene and vitamin E reduced serum and yolk cholesterol concentrations and improved antioxidant status. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 2 : 224-230)***

Key Words : Lycopene, Vitamin E, Egg, MDA

INTRODUCTION

Several beneficial effects of some micronutrients known as antioxidants have been reported (McDowell, 1989; DiMascio et al., 1989; Diplock, 1991; Angelo, 1992; Rao and Agarwal, 1999). Some measurements of quality for foods of animal origin such as colour, oxidative stability, tenderness, storage properties, etc. have been shown to be improved by antioxidant supplementation (Angelo, 1992; Flachowsky, 2000; Flachowsky et al., 2003). Vitamin E, one of the most powerful antioxidants, have been included into animal feed to improve performance, strengthen immunological status, improve the quality of meat and egg and to increase the vitamin E content of food of animal origin and thus increase the vitamin E intake of man (McDowell, 1989; Sunder et al., 1997; Flachowsky, 2000). Poultry cannot synthesize vitamin E, therefore, vitamin E requirements must be met from dietary sources (Chan and Decker, 1994). Vitamin E has been reported to be an excellent biological chain-breaking antioxidant that protects cells and tissue from lipoperoxidative damage induced by free radicals (McDowell, 1989). This vitamin is also 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 the 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). Sahin et al. (2001, 2002) reported that broilers supplemented with dietary vitamin E had a significant reduction in malondialdehyde (MDA) values, an indicator of lipid peroxidation, in serum and tissue of poultry. However vitamin E concentration above the physiological requirements does not have any effects (Jakobsen, 1997; Sunder et al., 1997; Engelmann, 1999; Sunder and Flachowsky, 2001). Incorporation of vitamin E into poultry diets has been shown to provide oxidative stability and increase the quality of their eggs and reduce the development of off-flavors while increasing egg production (Ajuyah et al., 1993; Buckley et al., 1995; Cherian et al., 1996a). In a previous study, it was observed that supplemental vitamin E and C significantly alleviated the heat stress-related decrease in the performance of growing Japanese quails suggesting that additional vitamin E and C supplementation may be necessary under heat stress conditions (Sahin and Kucuk, 2001a).

Lycopene, a member of the carotenoid family and mostly found in tomato, is a highly potent antioxidant that

* Supported by VETAL Company, 02100 Adıyaman- Turkey.
** Corresponding Author: N. Sahin. Tel: +90-532-7473506, Fax: +90-424-233-8720, E-mail: nsahinkm@yahoo.com
[1] Department of Animal Nutrition, Faculty of Veterinary Science, Turkey.
[2] Department of Biochemistry, Faculty of Science, Firat University, 23119 Elazig, Turkey.
[3] Department of Animal Science, The University of Tennessee, 2640 Morgan Circle, Knoxville, Tennessee 37996-4588, USA.
[4] Karmanos Cancer Institute, Wayne State University, Detroit, MI, USA.
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Table 1. Ingredients and chemical composition of the basal diet fed to laying Japanese quails

Ingredients	%
Ground corn	58.0
Soybean meal	28.3
Vegetable oil	2.0
Limestone	9.0
Dicalcium phosphate	2.0
Vitamin+mineral premix[1]	0.20
DL-methionine	0.10
Sodium Chloride	0.30
Sodium bicarbonate	0.10
Chemical analyses (dry matter (DM) basis)	
ME (kcal/kg[2])	2700
Crude protein[3] (%)	17.1
Calcium[3] (%)	3.8
Phosphorus[3] (%)	0.60

[1] Mix supplied per kg of diet: retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; dl-α-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 ($MnSO_4 \cdot H_2O$), 50 mg; Fe ($FeSO_4 \cdot 7H_2O$), 30 mg; Zn (ZnO), 30 mg; Cu ($CuSO_4 \cdot 5H_2O$), 5 mg; I (KI), 0.5 mg; Se (Na_2SeO_3), 0.15 mg; Co ($CoCl_2$-$6H_2O$), 0.1 mg; choline chloride, 125 mg.

[2] ME: Metabolizable energy, calculated from the tabular values (22).

[3] Analyzed value.

provides protection against cellular damage caused by reactive oxygen species (DiMascio et al., 1989; Rao and Agarwal, 1999; Agarwal and Rao, 1998; Rao and Shen, 2002). Dietary intake of lycopene has been shown to be associated with lower risk for prostate cancer (Kucuk, 2002; Kucuk et al., 2001, 2002) and tumor incidence (Sharoni et al., 1997). This subject is of major interest because lipid oxidation has been shown to adversely affect sensory properties and utilization of food (Riemersma et al., 1991; Gridley et al., 1992; Wenk et al., 2000). Guo et al. (2001) reported that there is a significant inverse relationship between thiobarbituric acid reactive substances (TBARS) value in the thigh meat and egg and the dietary antioxidants.

While several studies on the effect of vitamin E on oxidative stability of eggs have been described, there is a lack of information on the effects of both vitamin E and lycopene on egg quality. The aim of this study was to evaluate the effects of dietary lycopene and vitamin E, supplemented alone and together, on the egg production, egg quality, concentrations of malondialdehyde (MDA), vitamin E, A and cholesterol in serum and egg yolk in Japanese Quail.

MATERIALS AND METHODS

Animals, diets and experimental design

Japanese quail (n = 120; 55-d-old) (Coturnix coturnix japonica) were used in the study. The birds were fed either a basal diet or the basal diet supplemented with either 100 mg of lycopene/kg of diet, 250 mg of α-tocopherol-acetate/kg of diet and 100 mg of lycopene plus 250 mg of α-tocopherol-acetate/kg of diet. Vitamin E was specifically produced as a stabilized source of vitamin E for feed by a commercial company (Farmavet A.Ş., Istanbul). Lyc-O-Mato (Healthy Origin, UK) was used as lycopene source. Small amounts of the basal diet were first mixed with the respective amounts of vitamin E and lycopene as a small batch, then with a larger amount of the basal diet until the total amount of the respective diets were homogeneously mixed. Ingredients and chemical composition of the basal diet are shown in Table 1. The basal diet was a typical layer diet containing 2700 ME kcal/kg and 17.1% crude protein, and was calculated to meet or slightly exceed the nutrient requirements recommended by the National Research Council (1994).

The birds were randomly assigned to four groups, 30 birds each (consisting of three replicates of 10 birds), according to their egg production which were similar among treatments. Average ambient relative humidity inside the hen house was 55±3% and the mean value of daily temperature was 20±2.5°C. The experimental period lasted 70 d with birds on a 17L:9D light: dark photo schedule. Water and diets were offered for *ad libitum* consumption throughout the experiment.

Performance variables and egg quality

Body weights were recorded at the beginning and at the end of the study and feed consumption measured weekly. The number of eggs and egg weights were recorded daily. Egg quality measurements were conducted using all eggs produced in one day from all treatments. Parameters examined for egg quality measurement were egg weight and Haugh unit. Haugh unit values were calculated using the HU formula (Eisen, 1962) based on the height of albumen determined by a micrometer (Saginomiya, TLM-N1010, Japan) and egg weight.

Sample collection and laboratory analyses

Ten eggs randomly selected from each group hard-boiled for 15 minutes then egg yolks were separated from albumen and stored at 4°C for the cholesterol extraction. For this purpose, 0.1 g of yolk was weighed and mixed on a vortex for 3 min. in 4 ml of isopropyl alcohol, then centrifuged at 3,000 rpm for 10 min. The supernatant was used for cholesterol analysis using a cholesterol diagnostic kit (Valtek, Chile) and the cholesterol content of yolk was calculated (Berrio and Hebert, 1990). Ten eggs were randomly collected from each group for vitamin E and vitamin A analysis. At the end of the experiment, serum samples from 36 birds (9 birds from each group; 3 per replicate) randomly chosen from each treatment were collected. Levels of MDA, vitamins C, E, and A in serum

Table 2. Effects of supplemental vitamin E and lycopene on performance of laying Japanese quails (n = 30)

Item	Final BW (g)	Feed intake (g/d)	Hen day egg production (%)	FCR (g feed:g egg)	Egg weight (g)	Haugh unit
Control	184	29.2	77.8	2.57	11.36	85.0
Vitamin E	183	30.2	85.6	2.64	11.42	90.0
Lycopene	182	29.6	84.6	2.59	11.40	90.0
VitE+lycopene	183	30.0	90.8	2.65	11.36	94.4
Pooled SEM	2.2	1.45	0.75	0.10	0.5	0.5
Orthogonal contrast[1]	---------- Probabilities ----------					
C vs. V	0.691	0.611	0.0001	0.698	0.702	0.0001
C vs. L	0.478	0.838	0.0001	0.879	0.798	0.0001
C vs. V+L	0.634	0.683	0.0001	0.674	0.823	0.0001
V vs. L	0.751	0.759	0.320	0.812	0.898	0.578
V vs. V+L	0.936	0.918	0.0001	0.974	0.702	0.0001
L vs. V+L	0.811	0.820	0.0001	0.788	0.703	0.0001

[1] C = birds not supplemented with vitamin E or lycopene or their combination.

Statistical contrast: C vs. V = contrasting quails not supplemented with vitamin E versus quails supplemented with vitamin E; C vs. L = contrasting quails not supplemented with lycopene vs. quails supplemented with lycopene; C vs. V+L = contrasting quails not supplemented with vitamin E plus lycopene vs. quails supplemented with vitamin E plus lycopene; V vs. L = contrasting quails supplemented with vitamin E versus quails supplemented with lycopene; V vs. V+L = contrasting quails supplemented with vitamin E versus quails supplemented with vitamin E plus lycopene; L vs. V+L = contrasting quails supplemented with lycopene versus quails supplemented with vitamin E plus lycopene.

were determined as described previously (Ohkawa et al., 1979; Sahin et al., 2002). Content of vitamin E and A in egg yolk were determined by High Performance Liquid Chromatograph (HPLC) using method previously described with minor modifications. Vitamin E concentration was determined by methods of Surai et al. (1996) and vitamin A determined by method of Irie and Seki (2002). Chromatographic determinations were performed on a Cecil 1100 series HPLC equipped with an 1100 series pump and UV absorbance detector. An HP 3395 integrator was employed to record retention times, chromatograms, and evaluate peak heights. A Wakosil II 5C18 RS 5 μm (150×4.6 mm SS) column was used to monitor ambient temperature.

Statistical analyses

The data were initially analyzed by analysis of variance (ANOVA) using General Linear Models procedure of SAS (1999) for the effects of vitamin E and lycopene and their combination. Moreover, we constructed orthogonal contrasts to compare the mean response variables for birds fed the control diet vs. birds fed vitamin E or vs. birds fed lycopene as well as birds fed vitamin E vs. birds fed lycopene and their combination. LSD option was employed to determine contrast. The effects of the experimental diets on response variables were considered to be significant at $p<0.05$.

RESULTS

The effects of vitamin E and lycopene supplementation on performance and egg quality are shown in Table 2. Vitamin E and lycopene did not affect ($p>0.05$) body weight, feed intake and egg weight. Egg production and Haugh unit were greater ($p<0.05$) in each supplemental group compared with the control group ($p<0.05$). Serum and liver MDA levels were decreased in supplemented groups compared with the control group. Separately, or as a combination, supplemental lycopene and vitamin E increased serum and egg yolk vitamin E and A but decreased cholesterol concentrations ($p<0.05$) (Table 3). In general, when a significant effect was found for a parameter, the magnitude of the responses to vitamin and lycopene supplements was greatest with the combination of the lycopene and vitamin E, rather than that observed with each supplement separately.

DISCUSSION

Antioxidants compounds are used in feed mills as well as the food industry. They are added to feeds or directly to the foods for a better stabilization. As the most potent antioxidant, α-tocopherol is used in animal feeds. It exhibits an antioxidant activity at low concentrations and a prooxidant activity at high concentrations (Chen et al., 1998). The addition of *α*-tocopherol to hen diets increases the content of vitamin E in the egg yolk in a dose-dependent manner (Jiang et al., 1994; Surai et al., 1997; Meluzzi et al., 2000). Lycopene and tocopherols may also provide health benefits mainly in preventing cancer and coronary diseases (Diplock, 1991; Knekt et al., 1991), so that the incorporation of vitamin E into the egg may both increase the oxidative stability and provide a source of tocopherols that is useful for human nutrition and health. The methods by which dietary lycopene supplementation affects egg stability and content of egg yolk is not known. In the present study, the effects of dietary vitamin E and lycopene supplementation on egg production, egg quality,

Table 3. Effects of supplemental vitamin E and lycopene on serum MDA, vitamins C, E, and A and cholesterol levels of laying Japanese quails[1]

Item	Serum MDA (nmol/L)	Vitamin C (mol/L)	Vitamin E (mol/L)	Vitamin A (mol/L)	Cholesterol (mmol/L)
Control	1.23	40.80	1.54	1.12	3.82
Vitamin E	0.96	44.00	1.81	1.33	3.55
Lycopene	0.83	44.20	1.64	1.36	3.48
VitE+lycopene	0.57	46.80	1.89	1.57	2.96
Pooled SEM	0.05	1.3	0.09	0.05	0.01
Orthogonal contrast[2]	----------	----------	Probabilities	----------	----------
C vs. V	0.0001	0.0001	0.0001	0.0001	0.001
C vs. L	0.0001	0.0001	0.0001	0.0001	0.001
C vs. V+L	0.0001	0.0001	0.0001	0.0001	0.001
V vs. L	0.0206	0.7001	0.0001	0.1984	0.235
V vs. V+L	0.0001	0.0001	0.0001	0.0001	0.002
L vs. V+L	0.0001	0.0001	0.0001	0.0001	0.001

[1] Values are means, n = 10.

[2] C = birds not supplemented with vitamin E or lycopene or their combination.

Statistical contrast: C vs. V = contrasting quails not supplemented with vitamin E versus quails supplemented with vitamin E; C vs. L = contrasting quails not supplemented with lycopene vs. quails supplemented with lycopene; C vs. V+L = contrasting quails not supplemented with vitamin E plus lycopene vs. quails supplemented with vitamin E plus lycopene; V vs. L = contrasting quails supplemented with vitamin E versus quails supplemented with lycopene; V vs. V+L = contrasting quails supplemented with vitamin E versus quails supplemented with vitamin E plus lycopene; L vs. V+L = contrasting quails supplemented with lycopene versus quails supplemented with vitamin E plus lycopene.

concentrations of malondialdehyde (MDA), vitamin E, A and cholesterol in serum and egg yolk in japanese quails was investigated. Inclusion of vitamin E and lycopene in the diet caused improvements in egg production, Haugh unit, serum and egg yolk vitamin E and A but decreased serum and liver MDA and cholesterol concentrations. Vitamin E is the first line of defence 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). According to antioxidant theory, when the concentrations of antioxidants decreases, lipid peroxidation increases in the plasma and tissues leading to damage of cell membranes (Gallo-Torres 1980; McDowell, 1989). Causing oxidative damage on membrane of hepatic cells, stress has been shown to decrease plasma egg yolk precursor proteins, vitellogenin and tryglyceride (Bollengier-Lee et al., 1998). It was reported that these negative effects can be diminished via dietary vitamin E supplementation by the elevation of concentration of these precursor proteins (Puthpongsiriporn et al., 2001). The results of the study reported here is consistent with the findings Bollengier-Lee et al. (1998) who demonstrated that dietary supplementation of vitamin E (α-tocopherol acetate) could alleviate heat stress-related regression in performance of laying hens. Cherian et al. (1996) observed no effects of dietary tocopherol supplement on the Haugh units of fresh and stored eggs. In the current study, egg weight was unaffected by vitamin E supplementation, similar to reports by Gebert et al. (1998) and Meluzzi et al. (2000). As observed in the present study, Engelmann (1999) reported that egg production were slightly improved by vitamin E supplementation in laying hens.

In the present study, serum MDA levels were decreased in supplemented groups compared with the control group. Separately, or in combination, supplemental lycopene and vitamin E increased serum and egg yolk vitamin E and A while decreasing cholesterol concentration. It is known that vitamin E and lycopene are part of the first line of defense against lipid peroxidation (McDowell, 1989; Rao and Agarwal, 1999). Similar to results obtained in the present study, Morrissey et al. (1996, 1997) reported that dietary supplementation of chicken diets with α-tocopherol increased tissue α-tocopherol concentrations, while markedly decreasing MDA concentration. It has also been reported that egg yolk content of vitamin E is increased when this vitamin is included in the diet (Naber, 1993; Grobas et al., 1997). There is some evidence indicating a direct relationship between dietary α-tocopheryl acetate level and egg yolk concentration (Dju et al., 1950; Frigg et al., 1992; Jiang et al., 1994; Grobas et al., 1997; Surai et al., 1997), but the magnitude of response and the potential interaction with other dietary constituents have not been clearly established. The beneficial effects of vitamin E on lipid peroxidation observed in the present work, is consistent with that reported by Guo et al. (2001) and Bartov and Frigg (1992). Lycopene, synthesized by plants and microorganisms but not by animals, is the singlet most potent oxygen quencher amongst the natural carotenoids (Dimascio et al., 1989; Rao and Agarwal, 1999; Rao and Agarwal, 1999**).** Lycopene has also been reported to inactivate hydrogen peroxide and nitrogen dioxide (Rao and Agarwal, 1999). However the effects of such a strong

Table 4. The effects of supplemental vitamin E and lycopene on cholesterol, vitamins E and A concentrations of egg yolk[1]

Item	Cholesterol (mg/g)	Vitamin E (g/g)	Vitamin A (g/g)
Control	13.51	55.00	5.19
Vitamin E	12.38	162.00	5.66
Lycopene	12.29	72.20	5.54
VitE+Lycopene	11.60	186.60	6.16
Pooled SEM	0.6	8.6	0.8
Orthogonal contrast[2]	---------- Probabilities ----------		
C vs. V	0.001	0.0001	0.0001
C vs. L	0.001	0.0009	0.0001
C vs. V+L	0.0001	0.0001	0.0001
V vs. L	0.083	0.0001	0.0002
V vs. V+L	0.002	0.0001	0.0001
L vs. V+L	0.001	0.0001	0.0001

[1] Values are means, n = 10.

[2] Control = birds not supplemented with vitamin E or lycopene.

Statistical contrast: C vs. V = contrasting quails not supplemented with vitamin E versus quails supplemented with vitamin E; C vs. L = contrasting quails not supplemented with lycopene vs. quails supplemented with lycopene; C vs. V+L = contrasting quails not supplemented with vitamin E plus lycopene vs. quails supplemented with vitamin E plus lycopene; V vs. L = contrasting quails supplemented with vitamin E versus quails supplemented with lycopene; V vs. V+L = contrasting quails supplemented with vitamin E versus quails supplemented with vitamin E plus lycopene; L vs. V+L = contrasting quails supplemented with lycopene versus quails supplemented with vitamin E plus lycopene.

antioxidant compound on the quality markers of eggs and egg production in laying hens was not investigated. In the present study, lycopene supplementation decreased serum MDA concentrations and increased vitamins E and A concentration in serum and eggs (Table 4). The protective action of lycopene on MDA confirms previously reported findings of other investigators (Rao and Agarwal, 1999; Jain et al., 1997; Rao and Shen, 2002). Similar to the results of the current study, Jain et al. (1999) reported that dietary lycopene decreased serum TBARS concentration in rats by 14%. Leal et al. (1999) also reported that the broilers exposed to lycopene showed a reduction in MDA production. Paran et al. (2001) reported that lycopene supplementation reduced oxidative stress markers such as homocysteine in hypertansive patients. We could not find any study on lycopene-vitamin C, E, A interrelation to compare our results. However, an opposite correlation between MDA, vitamin E, and lycopene is stated (Dimascio et al., 1989; Rao and Agarwal, 1999; Halliwell and Gutteridge, 1999).

Results of the present study showed similar trends for the effects of vitamin E and lycopene. Overall antioxidant potential has been reported to possibly be more efficient and crucial than single antioxidant nutrients (Gallo-Torres, 1980). Based on these findings it is suggested that vitamin E and lycopene may act synergistically. Therefore, supplement of a combination of vitamin E and lycopene should offer a better results than when supplemented separately. Vitamin E and lycopene supplementation of poultry feed increased vitamin E and A concentrations and decreased MDA of human food of poultry origin. These supplementaion of poultry diets with these substances has the potential to provide protection against some cancer types as well as cardiovascular human diseases.

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