Effects of dietary resveratrol supplementation on egg production and antioxidant status

K. Sahin,*[1] F. Akdemir,† C. Orhan,* M. Tuzcu,‡ A. Hayirli,§ and N. Sahin*

*Department of Animal Nutrition & Nutritional Disorders, Faculty of Veterinary Medicine, Firat University, Elazig 23119, Turkey; †Department of Animal Nutrition & Nutritional Disorders, Faculty of Veterinary Medicine, Dicle University, Diyarbakir 21100, Turkey; ‡Department of Biology, Faculty of Science, Firat University, Elazig 23119, Turkey; and §Department of Animal Nutrition & Nutritional Disorders, Faculty of Veterinary Medicine, Ataturk University, Erzurum 25240, Turkey

ABSTRACT Resveratrol, a polyphenol derived from red grapes, berries, and peanuts, exerts antiinflammatory, antioxidant, and immunomodulatory effects. The objective of this study was to investigate the effects of dietary resveratrol supplementation on performance and serum and egg yolk antioxidant status in quail (*Coturnix coturnix japonica*). A total of 150 five-week-old quails were allocated randomly to 1 of 3 dietary treatments: basal diet and basal diet supplemented with 200 or 400 mg of resveratrol/kg of diet. Each diet was offered to 10 cages of 5 birds in each from 4 to 16 wk of age. Serum and egg samples were collected at the beginning and end the experimental period to be evaluated for malondialdehyde (MDA), vitamin A, and vitamin E. Data were subjected to analysis of covariance using the MIXED procedure. There was no treatment effect on feed intake, egg production, or egg quality parameters related to shell, yolk, and albumen. There were no effects of resveratrol supplementation on serum and egg yolk vitamin A concentrations. The quails supplemented with resveratrol had a lower serum MDA concentration (0.56 vs. 0.88 mg/L, $P < 0.03$) and a higher serum vitamin E concentration (5.72 vs. 3.56 mg/L, $P < 0.008$) than those not supplemented with resveratrol. Moreover, there was a linear decrease in serum MDA concentration ($P < 0.02$) and a linear increase in serum vitamin E concentration ($P < 0.01$) as supplemental resveratrol level increased. The treatment groups had less egg yolk MDA concentration than the control group (0.21 vs. 0.15 μg/g, $P < 0.002$). Egg yolk MDA concentration decreased linearly in response to increasing dietary resveratrol level ($P < 0.0003$). In conclusion, inclusion of resveratrol up to 400 mg/kg into quail diets enhanced antioxidant status of birds and eggs. Further studies should investigate the carryover effects of dietary resveratrol supplementation on product quality with respect to shelf life, antioxidant stability, and its nutritive value for human consumption.

Key words: resveratrol, egg yolk, malondialdehyde, antioxidant status, Japanese quail

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INTRODUCTION

Resveratrol (trans-3,5,4'-trihydroxystilbene) is a stilbene-type aromatic phytoalexin predominantly found in grapes, peanuts, berries, and turmeric and it exerts numerous biological effects (Frémont, 2000; Pirola and Fröjdö, 2008), including cardioprotective, antioxidant, antiaging, anticancer and antiinflammatory, immunomodulatory, and metabolic modifier activities that are considered in the treatment and management of a vast array of human diseases. Moreover, deacetylase silent information regulation 2/silent information regulation 2 homolog 1, an enzyme promoting stress resistance and aging, is the target of resveratrol (Chen and Guarente, 2007). Antiaging, anticarcinogenic, and immunostimulant effects of resveratrol are also linked to inhibitions of phosphoinositide 3-kinase that cause downregulation of insulin-like pathways (Fröjdö et al., 2007) and of nuclear factor κB activation and cyclooxygenase-2 and matrix metalloprotease-9 activities (Banerjee et al., 2002; Shakibaei et al., 2009).

Lipid oxidation is a process that has significant effect on the food industry because it can alter food quality (rancidity, flavor, odor, and color) and may lead to toxic end product accumulation (Lin and Liang, 2002). Antioxidant agents and some natural substances (e.g., resveratrol) that possess antioxidant potentials are com-

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[1]Corresponding author: nsahinkm@yahoo.com

Table 1. Ingredients and nutrient composition of the basal diet[1]

Item	Amount
Ingredient, %	
Corn	53.76
Soybean meal, 44% CP	29.27
Soy oil	4.85
Salt	0.31
DL-Methionine	0.20
Limestone	9.50
Dicalcium phosphate	1.76
Vitamin and mineral premix[2]	0.35
Nutrient composition	
ME,[3] kcal/kg	2,830
CP, %	17.95
Ca, %	3.96
P, %	0.63
Methionine, %	0.42
Lysine, %	1.05

[1]Resveratrol [200 and 400 mg/kg of resveratrol (50%, Resveratrol, Solgar, Istanbul, Turkey)] was added to basal diet at the expense of corn.

[2]Per kilogram contained the following: vitamin A, 8,000 IU; vitamin D_3, 3,000 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B_{12}, 0.02 mg; biotin, 0.1 mg; folacin, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; thiamin, 3 mg; copper (copper sulfate), 10.00 mg; iodine (ethylenediamine dihydriodide), 1.00 mg; iron (ferrous sulfate monohydrate), 50.00 mg; manganese (manganese sulfate monohydrate), 60.00 mg; zinc (zinc sulfate monohydrate), 60.00 mg; and selenium (sodium selenite), 0.42 mg.

[3]Calculated value according to tabular values listed for the feed ingredients (Jurgens, 1996).

monly used to control oxidation of polyunsaturated fats in foods. Due to rich nutrient contents (triacylglycerols, phospholipids, and proteins), egg yolk is widely used as an ingredient in various food products and is thus exposed to lipid peroxidation. Dietary supplementation of antioxidant nutrients (e.g., vitamin E, vitamin A, and lycopene) is one of the effective ways to minimize lipid peroxidation in eggs at farm level because these compounds are transferred into egg yolk and meat (Guo et al., 2001; Sahin and Kucuk, 2003; Sahin et al., 2008). It is also well-established that high dietary antioxidant content improves food quality parameters such as color, tenderness, and storage properties (Flachowsky et al., 2002).

Resveratrol exerts antioxidant effect via prevention of peroxidation of the apolipoprotein B protein associated with low-density lipoprotein (Shakibaei et al., 2009) and restoration of tissue glutathione and plasma total antioxidant capacity and tissue malondialdehyde (**MDA**) and myeloperoxidase activity (Zhong et al., 1999; Sehirli et al., 2008) through acting as an electron donor (Kohnen et al., 2007). Antiinflammatory effects of resveratrol are associated with increased proinflammatory mediators such as tumor necrosis factor α; interleukins 2, 6, and 12; interferon-γ; and prostaglandin E_2 (Zhong et al., 1999; Sehirli et al., 2008). To our knowledge, this is the first experiment dealing with antioxidant and antiinflammatory effects of resveratrol in poultry. The objective of this experiment was therefore to examine the effects of resveratrol supplementation on egg production, egg quality, and antioxidant and antiinflammatory statuses as reflected by serum and egg yolk vitamins (A and E), MDA, and liver heat shock protein 70 (**Hsp70**) levels.

MATERIALS AND METHODS

Birds, Diets, and Management

One hundred fifty 5-wk-old Japanese quails (*Coturnix coturnix japonica*), provided by a commercial company (Insanay AY Kanatli Hayvan Uretim Paz. Tic. Inc., Elazig, Turkey) were used in accordance with animal welfare regulations at the Veterinary Control and Research Institute of Elazig, Turkey. All quails were hatched from a large group of the parent stock that were of identical age.

Birds were fed a diet containing no resveratrol during the pretest period (0 to 4 wk). After grouping homogenously by BW, the quails were assigned randomly to 1 of 3 diets (Table 1) for 12 wk: basal diet not supplemented with resveratrol and basal diet supplemented with 200 or 400 mg/kg of resveratrol (50%, Resveratrol, Solgar, Istanbul, Turkey). Resveratrol contained naturally derived resveratrol from *Polygonum cuspidatum*. The diets were prepared in batches and stored in black plastic containers at 4°C to avoid photooxidation.

Each diet was replicated in 10 cages (300 cm^2/bird), each containing 5 quails. During the experimental period (12 wk), the quails were exposed to a 17-h daily photoperiod and had free choice of feed and water.

Performance Variables and Egg Quality

Feed consumption was measured weekly and egg production was recorded daily during the experimental period. Egg samples collected randomly (1 egg from each cage; 10 per treatment) at the beginning (wk 4) and end (wk 16) of the experimental periods were analyzed to assess egg quality, using the following formulas as summarized by Ergün et al. (1987): shape index (%) = (egg width, cm/egg length, cm) × 100; albumen index (%) = (albumen height, mm/average of albumen length, mm and albumen width, mm) × 100; yolk index (%) = (yolk height, mm/yolk diameter, mm) × 100; and Haugh unit = $100 \times \log (H + 7.57 - 1.7 \times W^{0.37})$, where H = albumen height, mm (TLM-N1010, Saginomiya, Tokyo, Japan) and W = egg weight, g. Yolk color was determined by using a commercially available yolk color fan according to the CIE standard colorimetric system (F. Hoffman-La Roche Ltd., Basel, Switzerland).

Sample Collections and Laboratory Analyses

At the end of the pretest (wk 4) and experimental (wk 16) periods, blood samples were taken from the axillary vein of 1 bird from each cage (10 samples per treatment in both periods) and put into additive-free

vacutainers. After letting samples stand for 1 h, they were centrifuged at 3,000 × *g* for 10 min and aliquots were transferred to microfuge tubes. The sera were kept on ice and protected from light to avoid any artifactual oxidation during sampling and were then stored at −80°C until analyses. One egg from each cage was also collected at the end of the pretest and experimental periods and stored at −80°C until analyses. Blood sera and egg yolk were analyzed for vitamins (A and E) and MDA.

The diet protein content was analyzed using the Kjeldahl method (AOAC, 1990) and energy, mineral (calcium and phosphorus), and amino acid (methionine and lysine) contents were calculated from tabular values listed for the feedstuffs (Jurgens, 1996). Contents of vitamins (Franchini et al., 2002) and MDA (Karatepe, 2004) in serum and egg yolk were determined using the fully automatic HPLC (Shimadzu, Kyoto, Japan), consisting of a pump (LC-20AD), a UV-visible detector (SPD-20A), an inertsil ODS-3 C_{18} column (250 × 4.6 mm, 5 m), a column oven (CTO-10ASVP*)*, an autosampler (SIL-20A), a degasser unit (DGU-20A5), and a computer system with LC solution software (Shimadzu). Intra- and interassay CV were 4.5 and 5.8% for vitamin E, 3.5 and 4.9% for vitamin A, and 2.9 and 4.2% for MDA.

After blood sampling, the quails were killed to assess Hsp70 in the liver using the Western blot technique. Briefly, after homogenization in PBS with protease inhibitor cocktail (Calbiochem, San Diego, CA), the supernatants (20 mg of protein per lane) were mixed with sample buffer, boiled for 5 min, separated by SDS-PAGE (12%) under denaturing conditions, and then electroblotted onto nitrocellulose membranes. Nitrocellulose blots were washed in PBS and blocked with 1% BSA in PBS for 1 h before application of the primary antibody. Chicken antibodies against Hsp70 antibody (ab6535) were commercially available (Abcam, Cambridge, UK). Primary antibody was diluted (1:1,000) in the same buffer containing 0.05% Tween-20. The nitrocellulose membrane was incubated overnight at 4°C with proteins' antibody. The blots were washed and incubated with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (Abcam). Specific binding was detected using diaminobenzidine and H_2O_2 as substrates. Protein loading was controlled using a monoclonal mouse antibody against β-actin antibody (A5316, Sigma, St. Louis, MO). Protein levels were analyzed densitometrically using an image analysis system (Image J, National Institutes of Health, Bethesda, MD).

Statistical Analyses

In sample size calculation, a 10% improvement in egg MDA concentration was considered to be significant at type I error of 0.05 with the power of 0.85. Data were subjected to 1-way analysis of covariance using the PROC MIXED procedure (SAS, 2002). The linear model to test the effects of dietary resveratrol supplementation on performance and egg quality as well as serum and egg yolk vitamins and MDA concentrations was $y_{ij} = \mu + b_0 + R_i + e_j$, where y = response variable; μ = population mean; b_0 = covariate, measurements obtained at the end of the pretest period; R = resveratrol supplementation; and e = residual error being N (σ, μ; 0, 1). The model also included orthogonal and polynomial contrast to determine resveratrol supplementation effect and changes in response variable with increasing dietary resveratrol supplementa-

Table 2. Effect of resveratrol supplementation on performance and egg quality in quails

	Resveratrol,[2] mg/kg				Contrasts,[3] $P <$		
Response variable[1]	0	200	400	SEM	C vs. R	LR	QR
Performance							
Feed intake, g/d	31.9	32.8	32.3	0.97	0.61	0.76	0.81
Egg production, %	92.0	93.0	93.0	1.54	0.78	0.81	0.87
Egg quality							
Egg weight, g	12.41	12.12	11.68	0.31	0.19	0.11	0.84
Eggshell weight, g	1.097	1.133	1.086	0.029	0.73	0.79	0.26
Shell thickness, mm·10^{-2}	3.027	3.123	3.016	0.094	0.71	0.94	0.38
Shape index, %	77.76	77.17	77.19	1.08	0.66	0.71	0.82
Albumen height, mm	0.460	0.500	0.480	0.019	0.21	0.47	0.21
Albumen width, mm	4.210	4.110	4.100	0.113	0.45	0.50	0.75
Albumen index, %	21.93	24.54	23.63	1.15	0.14	0.30	0.22
Haugh unit, %	57.00	57.88	58.31	0.47	0.07	0.06	0.70
Yolk color	6.60	6.10	6.50	0.30	0.42	0.81	0.23
Yolk height, cm	1.180	1.200	1.140	0.023	0.72	0.22	0.16
Yolk diameter, cm	2.460	2.400	2.300	0.052	0.10	0.04	0.76
Yolk width, cm	2.610	2.560	2.520	0.026	0.04	0.02	0.88
Yolk length, cm	3.360	3.320	3.270	0.042	0.22	0.14	0.92
Yolk index, %	48.12	50.16	49.66	1.16	0.22	0.35	0.38

[1]Haugh unit = 100 × log(H + 7.57 − 1.7 × $W^{0.37}$), where H = albumen height, mm, and W = egg weight, g.

[2]Values are least squares means for 10 cages, each containing 5 quails.

[3]C vs. R = resveratrol effect, contrasting control vs. resveratrol; LR = linear effect of resveratrol supplementation; QR = quadratic effect of resveratrol supplementation.

tion, respectively. Correlations among independent and dependent variables were determined using the CORR procedure (SAS, 2002). Statistical significance was declared at probability value less than 0.05.

RESULTS

Performance and Egg Quality

Dietary resveratrol supplementation affected neither feed intake nor egg production (Table 2). The mean daily feed intake and egg production were 32.3 g and 92.7%, respectively. Overall, resveratrol supplementation did not affect egg quality parameters, except for yolk width. Comparing with the quails fed the basal diet, the quails supplemented with resveratrol produced eggs with narrower egg yolk (2.61 vs. 2.54 cm, $P < 0.04$; Table 2). Moreover, egg yolk width decreased linearly as the amount of supplemental resveratrol increased ($P < 0.02$; Table 2). Haugh unit tended to be higher ($P < 0.07$), whereas egg yolk diameter tended to be lower ($P < 0.10$) for the quails supplemented with resveratrol than for those not supplemented with resveratrol (Table 2). As the amount of supplemental resveratrol increased (Table 2), there was a tendency of linear increase in Haugh unit ($P < 0.06$) and a linear decrease in egg yolk diameter ($P < 0.04$).

Serum and Egg Yolk Antioxidant Status

There were no differences in the pretest serum MDA (0.87 ± 0.25, mg/L; $P < 0.95$), vitamin A (1.56 ± 0.20, mg/L; $P < 0.89$), and vitamin E (3.55 ± 0.93, mg/L; $P < 0.91$) concentrations (mean $\pm$ SE). Dietary resveratrol supplementation did not affect serum vitamin A concentration (Figure 1). The quails supplemented with

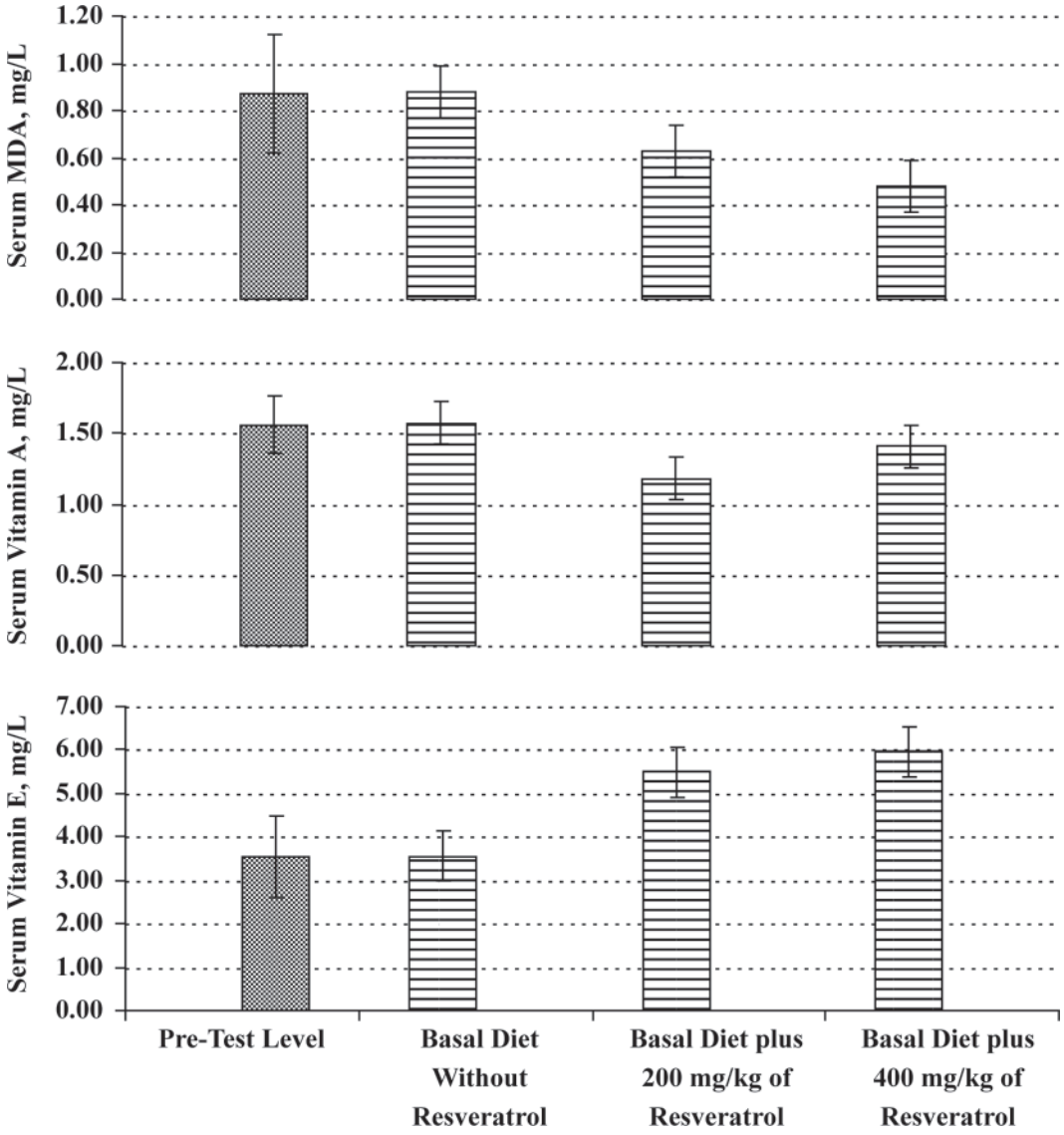

Figure 1. Effects of dietary resveratrol supplementation on serum malondialdehyde (MDA), vitamin A, and vitamin E levels in quails. Probabilities of significance for basal diet versus diet supplemented with resveratrol and linear effect of resveratrol supplementation were less than 0.03 and 0.02 for serum MDA, 0.15 and 0.47 for serum vitamin A, and 0.008 and 0.01 for serum vitamin E, respectively. There was no quadratic effect of resveratrol supplementation on serum measurements.

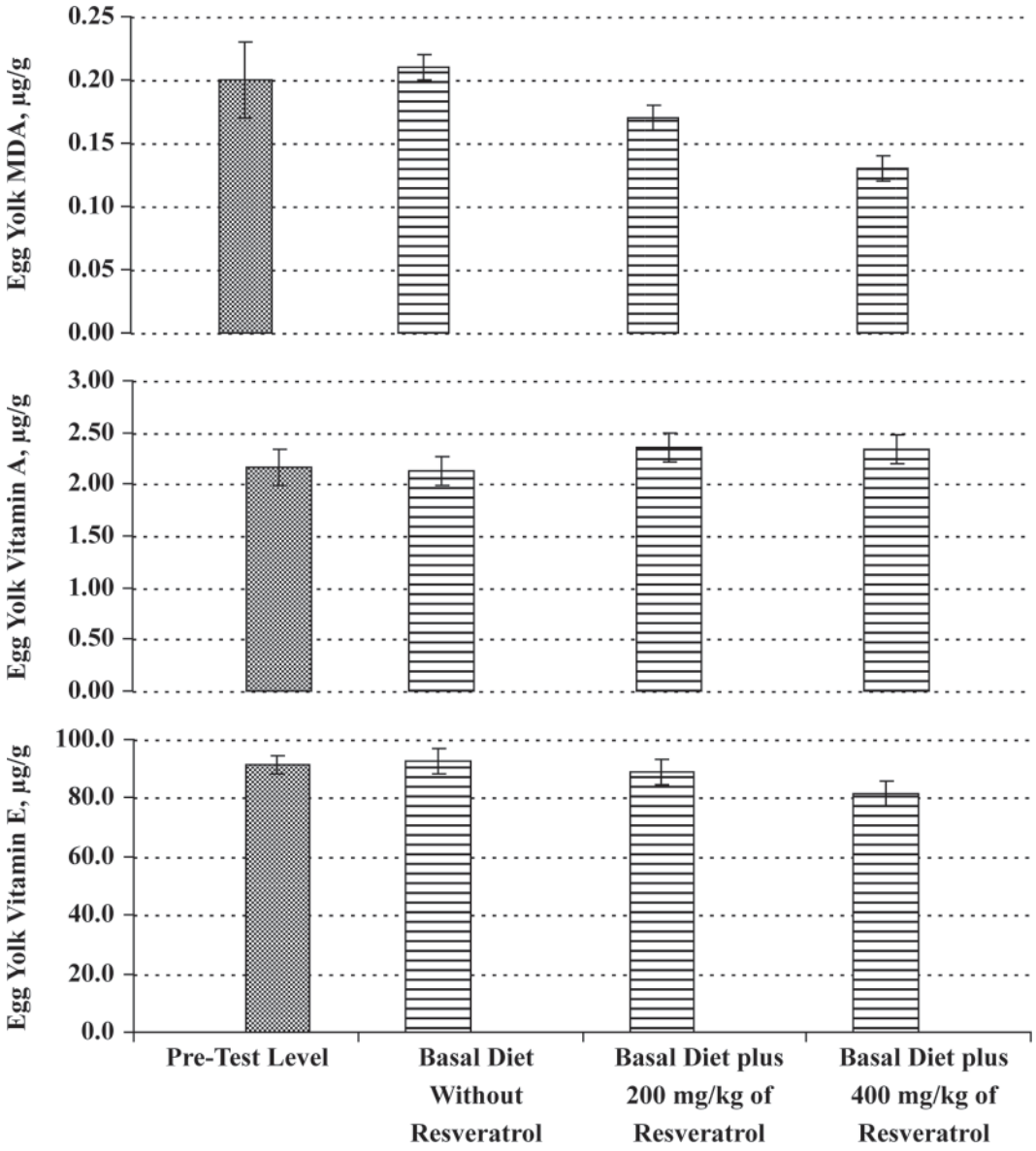

Figure 2. Effects of dietary resveratrol supplementation on egg yolk malondialdehyde (MDA), vitamin A, and vitamin E levels in quails. Probabilities of significance for basal diet versus diet supplemented with resveratrol and linear effect of resveratrol supplementation were less than 0.002 and 0.0003 for egg yolk MDA, 0.22 and 0.30 for egg yolk vitamin A, and 0.18 and 0.08 for egg yolk vitamin E, respectively. There was no quadratic effect of resveratrol supplementation on egg yolk measurements.

resveratrol had lower serum MDA (0.88 vs. 0.56 mg/L, $P < 0.03$) concentration and had higher serum vitamin E concentration (3.56 vs. 5.72 mg/L, $P < 0.008$) than those fed the basal diet. With increasing the amount of supplemental resveratrol in the diet (Figure 1), serum MDA ($P < 0.02$) concentration decreased linearly and serum vitamin E concentration increased linearly ($P < 0.01$).

The pretest egg yolk MDA (0.20 ± 0.03, µg/g; $P < 0.82$), vitamin A (2.16 ± 0.18, µg/g; $P < 0.83$), and vitamin E (91.42 ± 3.09, µg/g; $P < 0.93$) concentrations were also similar across the experimental groups (mean ± SE). Egg yolk vitamin A concentration was not affected by dietary resveratrol supplementation (Figure 2). The egg yolk MDA concentration for quails supplemented with resveratrol was lower than for those not supplemented with resveratrol (0.21 vs. 0.15 µg/g, $P < 0.002$). Moreover, egg yolk MDA concentration decreased linearly with increasing dietary resveratrol level ($P < 0.0003$; Figure 2). Overall, there was no difference in egg yolk vitamin E concentrations between quails fed the basal diet and quails supplemented with resveratrol (92.57 vs. 85.27 µg/g, $P < 0.18$), but it tended to decrease with increasing the amount of dietary resveratrol supplementation ($P < 0.08$; Figure 2).

Liver Hsp70 protein (Figure 3) content was 25% lower for the control quails than for those supplemented with resveratrol ($P < 0.0001$) and it decreased linearly to 60% ($P < 0.0001$).

Resveratrol intake was correlated positively with serum ($r = 0.59$; $P < 0.01$) and egg yolk ($r = -0.42$; $P < 0.01$) vitamin E concentrations and negatively corre-

lated with serum (r = −0.53; $P < 0.05$) and egg yolk (r = −0.72; $P < 0.01$) MDA concentrations. Serum MDA concentration was correlated positively with serum vitamin A (r = 0.59; $P < 0.01$) and egg yolk MDA (r = 0.44; $P < 0.10$) concentrations and negatively correlated with serum vitamin E concentration (r = −0.46; $P < 0.10$) (Table 3). Serum vitamin A concentration was not correlated with any variables, but there was a negative correlation between serum vitamin E and egg yolk MDA (r = 0.44; $P < 0.10$) concentrations. There was also a negative correlation between egg yolk MDA and vitamin E (r = −0.42; $P < 0.10$) concentrations. Serum and egg yolk concentrations of MDA and vitamins (A and E) were not correlated with yolk color and yolk index.

DISCUSSION

Resveratrol was first isolated in 1940 as a constituent of the roots of white hellebore (*Veratrum grandiflorum* O. Loes) but has since been found in various plants, including grapes, berries, and peanuts. It exerts a wide range of biological effects, of which antioxidant (Kasdallah-Grissa et al., 2007; Bujanda et al., 2008; Shakibaei et al., 2009) and antiinflammatory effects (Donnelly et al., 2004) may be pertinent to improving quality of poultry products.

Biological effects of resveratrol have been enormously tested in in vivo experiments involving laboratory animals and in vitro experiments as well as retrospective clinical trials involving humans. To our knowledge, however, there is no production report coping with dietary resveratrol supplementation to poultry and its metabolic fate in poultry species in the literature. The oral bioavailability of resveratrol is independent from dose and aqueous solubility (Das et al., 2008). Juan et al. (2002) tested a very high dose of oral supplementation of resveratrol to rats (20 mg/kg for 20 d), 1,000 times the amount consumed by a 70-kg person taking 1.4 g of *trans*-resveratrol/d, and reported no adverse effects on growth, water and feed intakes, and hematological and biochemical variables as well as histopathological alteration. Pharmacokinetic studies revealed that the target organs of resveratrol are liver and kidney, where it is concentrated after absorption and is mainly converted to a sulfated form and a glucuronide conjugate (Vitrac et al., 2003; Wenzel et al., 2005) and excreted via feces and urine (Wenzel and Somoza, 2005). No phenolic degradation products were detected in urine or tissues indicating that unlike flavonoids, resveratrol does not serve as a substrate for colonic microflora (Abd El-Mohsen et al., 2006). In this experiment (Table 2), feed intake, egg production, and outer and inner egg quality indicators were not affected by dietary resveratrol supplementation. Egg production was expected to increase because resveratrol was shown to have estrogenic activity through binding to estrogen receptor α and stimulating Michigan Cancer Foundation-7, a breast cancer cell line proliferation (Schmitt et al., 2002). Resveratrol may also augment calcium metabolism. Using ovariectomized rats, Liu et al. (2005) showed that resveratrol (0.7 mg/kg) supplementation increased bone mineral density and inhibited femur calcium loss associated with estrogen deficiency. In this experiment, however, eggshell thickness was not affected by resveratrol supplementation (Table 2).

Studies to improve oxidative stability of eggs and meat via dietary antioxidant enrichments (Cherian et al., 1996; Guo et al., 2001; Sahin et al., 2008) reveal an inverse relationship between levels of dietary antioxidant and MDA contents in egg yolk, meat, and blood serum, a lipid peroxidation indicator in the poultry products. Despite its well-known antioxidant and hypolipidemic effects, antioxidant parameters in poultry species supplemented with resveratrol are lacking. In this experiment, serum and egg yolk resveratrol content were not assayed. However, decreases in both serum (Figure 1) and egg yolk (Figure 2) MDA level may indicate validity of antioxidant effects of resveratrol in poultry species. A decrease in serum and egg yolk MDA level could also be related to reduction of serum and egg yolk cholesterol level (Zhu et al., 2008), which were not measured. Resveratrol is an effective scavenger of hydroxyl, superoxide, and metal-induced radicals (Frémont et al., 1999; López-Vélez et al., 2003) and increases activities of antioxidant enzymes (Young et al., 2000), such catalase, superoxide dismutase, glutathione peroxidase, NADPH quinone oxidoreductase, and glutathione S-transferase, as well as activates erythroid-derived

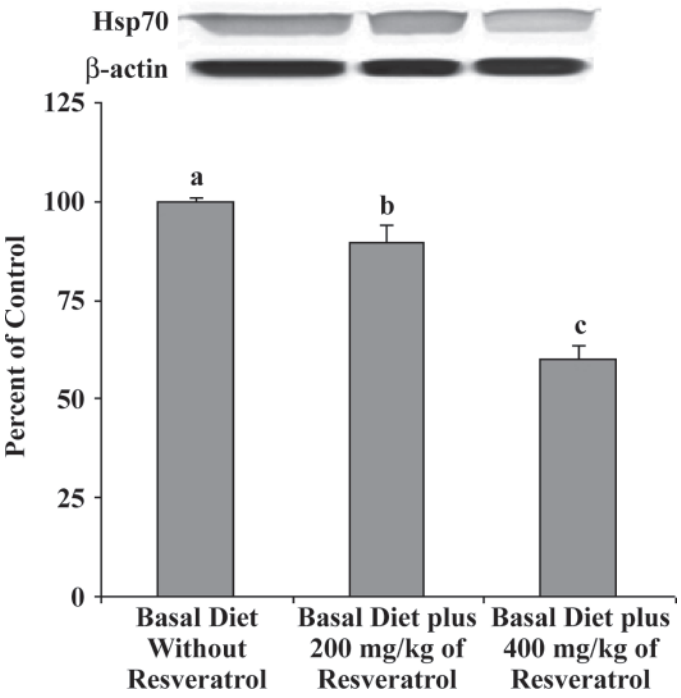

Figure 3. Effects of dietary resveratrol supplementation on liver heat shock protein (Hsp70) content. Probability of significance was less than 0.0001 for resveratrol supplementation effect and linear resveratrol supplementation effect. Different letters above bars indicate significant difference ($P < 0.05$).

Table 3. Correlations among serum and egg yolk concentrations of malondialdehyde (MDA) and vitamins and some egg characteristics

Item	Resveratrol intake	Serum MDA	Serum vitamin A	Serum vitamin E	Egg yolk MDA	Egg yolk vitamin A	Egg yolk vitamin E	Albumen index	Egg weight	Shell weight	Shell thickness	Yolk color	Haugh unit
Feed intake	0.24	−0.29	−0.51	0.33	−0.20	−0.13	−0.36	0.18	−0.07	−0.06	−0.04	−0.17	0.14
Resveratrol intake	1.00	−0.53**	−0.20	0.59*	−0.72*	0.22	0.42***	0.20	−0.31***	−0.05	−0.02	−0.05	0.36***
Serum MDA		1.00	0.59*	−0.46***	0.44***	−0.25	0.02	−0.20	0.19	0.01	−0.31	−0.33	−0.25
Serum vitamin A			1.00	−0.21	0.26	0.02	0.07	−0.39	0.18	0.16	−0.48	−0.06	−0.30
Serum vitamin E				1.00	0.44***	0.38	0.11	0.09	0.10	−0.02	−0.13	0.29	−0.10
Egg yolk MDA					1.00	−0.09	−0.42***	−0.41***	0.27	0.16	−0.05	−0.11	−0.44**
Egg yolk vitamin A						1.00	0.36***	0.31	0.01	−0.20	0.01	−0.12	0.10
Egg yolk vitamin E							1.00	−0.10	0.42***	0.05	0.02	−0.14	−0.46**
Albumen index								1.00	−0.10	−0.01	−0.09	−0.31	0.48***
Egg weight									1.00	0.41**	−0.16	−0.13	−0.89*
Shell weight										1.00	0.17	−0.03	−0.33***
Shell thickness											1.00	0.03	0.13
Yolk color												1.00	0.03
Haugh unit													1.00

*$P < 0.01$; **$P < 0.05$; ***$P < 0.10$.

nuclear factor, a major transcription factor regulating antioxidant response (Rubiolo et al., 2008). Resveratrol is highly hydrophilic and lipophilic; hence, it is likely to be more effective than some other antioxidants, such as vitamin E and C (Chanvitayapongs et al., 1997; Murcia and Martinez-Tome, 2001). In other experiments involving rats, reversed oxidative stress by resveratrol was shown upon exposure to H_2O_2 (De Almeida et al., 2008) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Lu et al., 2008) in brain tissue and CCl_4 in liver (Vitaglione et al., 2009). Hypolipidemic effect of resveratrol through suppressing hepatic lipogenesis (Zern et al., 2003; Tian, 2006; Cho et al., 2008) and inducing apoptosis in adipocytes (Park et al., 2008; Rayalam et al., 2008a,b) was shown to be accompanied by decreased plasma and hepatic TBA reactive substances, another lipid peroxidation indicator, and increased serum superoxide dismutase, glutathione peroxidase, and catalase activities (Zhu et al., 2008).

At the cellular level, different stress factors including chemical and physiological stress factors (i.e., heat, radiation, toxins, viral infections, ethanol, arsenite, and gene transfer) increase heat shock protein synthesis. Increased heat shock protein protects cells against the additional stress via making the cells resistant to harmful insults and apoptosis (Lindquist and Craig, 1988; Morimoto et al., 1997; Wang and Edens, 1998). Constitutive expression of Hsp70, a major heat shock protein, mediates the protection against cell lysis induced by the toxic effect of NO, a reactive oxygen intermediate created through oxygen-derived free-radical action (Bellmann et al., 1996). Resveratrol activates the vitagene system, which encodes for cytoprotective Hsp70 and heme oxygenase 1, as well as thioredoxin reductase and sirtuins (Calabrese et al., 2008). Through these mechanisms, resveratrol also activates the silent information regulation 2 homolog 1 and 5′ adenosine monophosphate-activated protein kinase that are related to extending lifespan and improving metabolic disease (Ajmo et al., 2008). The quails in this experiment were not subjected to any stress-causing practices. However, Hsp70 content decreased by 40% in response to increasing dietary resveratrol supplementation (Figure 3).

In conclusion, the inclusion of resveratrol into diets could enhance antioxidant status in quails, as reflected by dose-dependent decreases in serum MDA and liver Hsp70 levels and an increase in serum vitamin E level. Moreover, supplemental resveratrol or perhaps its degradation product(s) in the body reduced egg yolk MDA content, which may prolong shelf life benefit consumers.

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