11-1-2015

Influence of soy oil source and dietary supplementation of vitamins E and C on the oxidation status of serum and egg yolk, and the lipid profile of egg yolk

H. Irandoust
Agriculture Jihad

Dong U. Ahn
Iowa State University, duahn@iastate.edu

Influence of soy oil source and dietary supplementation of vitamins E and C on the oxidation status of serum and egg yolk, and the lipid profile of egg yolk

Abstract

An experiment was conducted to determine the effects of adding vitamins E and C to diets containing 3.5% refined soy oil (SO), recycled soy oil (RSO), or acidulated soy oil soapstocks (ASS) on 1) fatty acid (FA) profile, and cholesterol, triglyceride (TG) and α-tocopherol (α-T) concentrations of yolk, and 2) the oxidation status of serum and yolk. Twelve dietary treatments, using 3 oil sources, 2 levels of vitamin E (0 vs. 250 mg/kg), and 2 levels of vitamin C (0 vs. 250 mg/kg), were prepared. A total of 300 W36 Hy-line laying hens, from 44 to 56 weeks of age, were placed in 60 cages (5 birds/cage) and 5 cages were randomly assigned to one of the 12 diets. Blood samples and eggs were collected after 84 d on trial. No interactions among main effects were found for any of the traits studied. Oil sources had little effects on the FA profile of the yolk, except for C18:3 that was higher (*P*-value of < 0.01) in the hens fed SO than those fed RSO or ASS. Vitamin E supplementation significantly (*P*-value of < 0.05) increased the concentration of C16:0, C18:0, and C16:1 but decreased that of C18:2 and C22:6n3 in the yolk. Vitamin C supplementation significantly (*P*-value of < 0.05) increased C18:0 and C18:3 concentrations in the yolk but decreased the n6 to n3 FA ratio. The concentrations of cholesterol and triglyceride in serum and yolk were not affected by dietary treatment but α-tocopherol concentration increased (*P*-value of < 0.01) by the dietary vitamin E. Compared with the hens fed the SO diets, malondialdehyde (MDA) concentration in serum was higher with RSO diet but lower with ASS diet. Vitamin E and vitamin C supplementation decreased (*P*-value of < 0.05) serum MDA. Yolk FA profile was affected not only by the FA profile of the oil source used in diet, but also by the supplementation of vitamin E and C. The results showed that triglyceride profile, but not cholesterol content, of egg was affected by fatty acid profile of the supplemental oil and the vitamin C and E supplementations.

Keywords

soy oil, vitamin C, vitamin E, yolk fatty acid profile, yolk stability

Disciplines

Agriculture | Animal Sciences | Nutrition | Poultry or Avian Science

Comments

This article is published as Irandoust, H., and D. U. Ahn. "Influence of soy oil source and dietary supplementation of vitamins e and c on the oxidation status of serum and egg yolk, and the lipid profile of egg yolk." *Poultry science* 94, no. 11 (2015): 2763-2771. doi:10.3382/ps/pev290.

Influence of soy oil source and dietary supplementation of vitamins E and C on the oxidation status of serum and egg yolk, and the lipid profile of egg yolk

H. Irandoust*,[1] and D. U. Ahn†

*Department of Animal Sciences, Institute of Technical and Vocational Higher Education, Agriculture Jihad, 8168116676, Isfahan, Iran; and †Department of Animal Science, Iowa State University Ames, Iowa 50011–3150, USA

ABSTRACT An experiment was conducted to determine the effects of adding vitamins E and C to diets containing 3.5% refined soy oil (SO), recycled soy oil (RSO), or acidulated soy oil soapstocks (ASS) on 1) fatty acid (FA) profile, and cholesterol, triglyceride (TG) and α-tocopherol (α-T) concentrations of yolk, and 2) the oxidation status of serum and yolk. Twelve dietary treatments, using 3 oil sources, 2 levels of vitamin E (0 vs. 250 mg/kg), and 2 levels of vitamin C (0 vs. 250 mg/kg), were prepared. A total of 300 W36 Hy-line laying hens, from 44 to 56 weeks of age, were placed in 60 cages (5 birds/cage) and 5 cages were randomly assigned to one of the 12 diets. Blood samples and eggs were collected after 84 d on trial. No interactions among main effects were found for any of the traits studied. Oil sources had little effects on the FA profile of the yolk, except for C18:3 that was higher (P-value of < 0.01) in the hens fed SO than those fed RSO or ASS. Vitamin E supplementation significantly (P-value of < 0.05) increased the concentration of C16:0, C18:0, and C16:1 but decreased that of C18:2 and C22:6n3 in the yolk. Vitamin C supplementation significantly (P-value of < 0.05) increased C18:0 and C18:3 concentrations in the yolk but decreased the n6 to n3 FA ratio. The concentrations of cholesterol and triglyceride in serum and yolk were not affected by dietary treatment but α-tocopherol concentration increased (P-value of < 0.01) by the dietary vitamin E. Compared with the hens fed the SO diets, malondialdehyde (MDA) concentration in serum was higher with RSO diet but lower with ASS diet. Vitamin E and vitamin C supplementation decreased (P-value of < 0.05) serum MDA. Yolk FA profile was affected not only by the FA profile of the oil source used in diet, but also by the supplementation of vitamin E and C. The results showed that triglyceride profile, but not cholesterol content, of egg was affected by fatty acid profile of the supplemental oil and the vitamin C and E supplementations.

Key words: soy oil, vitamin C, vitamin E, yolk fatty acid profile, yolk stability

2015 Poultry Science 94:2763–2771
http://dx.doi.org/10.3382/ps/pev290

INTRODUCTION

Egg is a high-quality source of proteins, vitamins, phospholipids and unsaturated fatty acids (**FA**) for humans. Dietary composition of hen feed influences the nutritive value of the eggs (Grobas et al., 2002), especially fatty acid peofile, and the contents of some minerals and fat-soluble vitamins (Naber, 1979). Moreover, supplementation of diet with recycled or wasted vegetable oils may affect sensorial quality of meat and egg due to the development of oxidation-derived off-flavors (Bou et al., 2006), which could limit their use in poultry diets. Vitamin E protects cells and tissues from oxidative damage induced by free radicals (Yu, 1994). Vitamin C acts as an antioxidant due to its properties as a radical scavenger, and can donate an electron to reactive free radicals (Niki, 1991). Vitamin C can also act in synergy with tocopherol by regenerating tocopheroxyl radicals (Mäkinen et al., 2001). Therefore, vitamins E (**VE**) and C (**VC**) supplementation may be good alternatives to minimize lipid oxidation of the diet and may help maintain the quality of eggs (Grobas et al., 2001).

The effects of dietary VE and oil sources on yolk FA profile, sensory quality of the eggs, and yolk oxidation stability have been studied extensively (Meluzzi et al., 2000; Pál et al., 2002). However, little information on the combined effects of VE and VC supplementation in soy oil based diets on the lipids profile of egg yolk. The aim of this research was to investigate the effect of soy oil source and VE and VC supplementation, alone and in combinations, on fatty acid profile, cholesterol, triglyceride (**TG**), and α-tocopherol concentrations of yolk, and oxidative stability of blood serum and yolk.

Received April 27, 2015.
Accepted July 30, 2015.
[1]Corresponding author: h.irandoust@ag.iut.ac.ir or hirandoust@yahoo.com

MATERIALS AND METHODS

The procedures used in this research were approved by the Animal Ethics Committee of the Isfahan University of Technology, and were in compliance with the guidelines of the Iranian Council of Animal Care (1995).

Dietary Treatments and Sampling

Briefly, a batch of refined soy oil (**SO**) and a batch of acidulated soy oil soapstocks (**ASS**) were collected from a soybean crushing and soy oil refinery plant (Naz, Isfahan, Iran). In addition, a batch of recycled soy oil (**RSO**) was collected from an industrial plant (Golab, Isfahan, Iran), which produces foods based on wheat flour. Details on soy oil characteristics and hen husbandry have been reported elsewhere (Irandoust et al., 2012). A total of 300 W36 Hy-line laying hens from 44 to 56 weeks of age were randomly allocated to 12 groups of 5 cages, and each cage contained 5 birds. The diets were based on corn and soybean meal, and included 3.5% of SO, RSO, or ASS, and 0 or 250 mg VE/kg diet, and 0 or 250 mg VC/kg diet in a factorial arrangement.

Hens were received their respective experimental diets for 12 weeks (Tables 1 and 2). On the last day of the feeding trial, 5 eggs were collected at random from each cage to determine FA profile, the content of cholesterol, and vitamin E in the yolk. Five additional eggs per replicate were used for assessment of susceptibility to oxidation. Blood samples (3 mL) were taken from the branchial vein of 2 hens from each replicate. Serum samples were prepared by centrifugation of blood at 1200 × *g* for 15 min, frozen immediately and stored at −20°C until further analysis. Fatty acid profiles of oil sources and the diet were also determined.

Table 1. Ingredient composition and calculated and determined nutrient content (% as-fed basis, unless otherwise indicated) of the experimental diets.

	Soybean Oil	Recycled Soy Oil	Acidulated Soy Oil Soapstocks
Ingredient			
Yellow corn	56.45	56.71	58.22
Soybean meal (45.7% CP)	21.41	21.49	21.90
Wheat bran	4.93	4.58	2.67
Soy oil	3.50	–	–
Recycled soy oil	–	3.5	–
Acidulated soy oil soapstocks	–	–	3.50
Calcium carbonate	10.63	10.63	10.61
Dicalcium phosphate	1.93	1.94	1.96
Sodium chloride	0.34	0.34	0.34
DL-Methionine, (98%)	0.18	0.18	0.18
L-Lysine-HCL, (78%)	0.13	0.13	0.12
Vitamin and mineral premix[1]	0.50	0.50	0.50
Calculated analysis[2]			
AMEn (kcal/kg)	2,800	2,800	2,800
Lys	0.88	0.88	0.88
Met	0.43	0.43	0.43
Met + Cys	0.69	0.69	0.69
Thr	0.57	0.57	0.58
Trp	0.18	0.18	0.18
Ca	4.00	4.00	4.00
Available P	0.42	0.42	0.42
Sodium	0.17	0.17	0.17
Determined analyses[3]			
Gross energy (kcal/kg)	3,770	3,815	3,810
Dry matter	93.4	93.5	92.8
CP	16.4	16.8	16.8
Ether extract	6.7	6.4	6.4
Linoleic acid	3.56	3.36	3.28
Crude fiber	3.7	3.7	3.5
Total ash	13.6	13.6	13.5
Ca	4.08	4.03	3.99
Total P	0.65	0.65	0.62

[1]Provided the following (per kg of diet): vitamin A (trans-retinyl acetate), 7,700 IU; vitamin D_3 (cholecalciferol), 3,300 IU; vitamin E (all-rac-tocopherol acetate), 10 IU; vitamin K (bisulfate menadione complex), 0.55 mg; thiamine (thiamine mononitrate), 1 mg; riboflavin, 4.4 mg; pyridoxine (pyridoxine HCl), 1 mg; pantothenic acid (D-calcium pantothenate), 5.5 mg; nicotinic acid, 22 mg; choline (choline chloride), 275 mg; vitamin B_{12} (cyanocobalamin), 20 μg; Mn, ($MnSO_4$, H_2O), 66 mg; Zn (ZnO), 66 mg; Fe ($FeSO_4$, H_2O), 33 mg; Cu ($CuSO_4$, $5H_2O$), 8.8 mg; I (KI), 0.9 mg; Co, 0.2 mg; and Se (Na_2SeO_3), 0.3 mg.

[2]Based on NRC (1994) energy values for all ingredients except for the oil sources for which values determined by difference in experiment 1 were used (9,127, 8,947, and 7,967 kcal AME/kg SBO, RSO, and ASO, respectively).

[3]Analyzed in triplicate.

Table 2. Fatty acid (FA) profile of the experimental soy oils and diets (% of total fatty acids).[1]

	Soy Oils			Diets		
Fatty Acid	SO[2]	RSO[3]	ASS[4]	SO	RSO	ASS
Myristic (C14:0)	0.1	0.1	0.1	0.1	0.1	0.1
Palmitic (C16:0)	14.2	15.8	18.0	14.4	14.3	17.0
Margaric (C17:0)	0.1	0.1	0.1	0.1	0.1	0.1
Stearic (C18:0)	3.9	3.7	4.4	3.5	3.5	3.4
Arachidic (C20:0)	0.2	0.2	0.3	0.1	0.1	0.0
Behenic (C22:0)	0.5	0.4	0.5	Nd[5]	Nd	Nd
Lignoceric (C24:0)	0.1	0.1	0.1	Nd	Nd	Nd
Saturated FA (SFA[6])	19.1	20.4	23.5	18.2	18.1	20.6
Palmitoleic (C16:1)	0.1	0.1	Nd	0.1	0.1	0.1
Oleic (C18:1)	19.5	22.6	22.0	24.6	26.0	25.0
Monounsaturated FA(MUFA[7])	19.6	22.7	22.0	24.7	26.1	25.1
Linoleic (C18:2)	55.3	51.7	48.6	53.4	52.6	51.0
$\sum$n6	55.3	51.7	48.6	53.4	52.6	51.0
Linolenic (C18:3n3)	6.2	5.2	5.9	3.7	3.3	3.3
$\sum$n3	6.2	5.2	5.9	3.7	3.3	3.3
Polyunsaturated FA (PUFA[8])	61.5	56.9	54.5	57.1	55.9	54.3
n6:n3 ratio	8.9	9.9	8.2	14.4	15.9	15.5
PUFA:SFA ratio	3.2	2.8	2.3	3.1	3.1	2.6
Vitamin E (mg/kg)	348.0	115.0	111.0	37.0	25.0	26.0
Vitamin C (mg/kg)	14.0	1.0	1.0	No[9]	No	No

[1]Analyzed in triplicate samples
[2]Soy oil
[3]Recycled soy oil
[4]Acidulated soy oil soapstocks
[5]Not detected
[6]Saturated FA (Σ of C14:0, C16:0, C17:0, C18:0, C20:0, C22:0, and C24:0)
[7]Monounsaturated FA (Σ of C16:1 and C18:1)
[8]Polyunsaturated (Σ of C18:2n6 and C18:3n3)
[9]Not determined.

Fatty Acid Profile

For fatty acid profile, total lipids were extracted from feeds and yolk using chloroform: methanol (2:1 vol/vol) as indicated by Folch et al. (1957). The lipid fraction was methylated (Metcalfe et al., 1961) and the FA methyl esters were separated and identified using an automated GC/MS Agilent gas chromatograph equipped with autosampler, MS detector and a Supelco SP-2560 (100 m × 0.25 mm inside diameter, 0.2 μm film thickness) chromatography column. The GC was programmed with an initial temperature of 180°C for 10 min, allowing increases of 1°C/min until 200°C remaining for 20 min and then increased by 5°C/min until a temperature of 240°C was reached. The temperatures of injector and detector were 230 and 240°C, respectively. Helium, at 1 mL/min, was used as the carrier gas.

Cholesterol and Triglyceride Concentration in Serum and Yolk

Serum and egg yolk total cholesterol and TG concentrations were measured using a standard enzymatic method (TECO diagnostic kits, California, CA).

α-Tocopherol Concentration

α-Tocopherol content of plasma samples was determined as described by Gonzalez-Corbella et al. (1994) with modifications. A 100 μL aliquot of ethanol containing an internal standard (211.54 μ mol d-α-tocopheryl acetate/L) was added to 100 μL of sample in an amber-coloured polypropylene micro centrifuge tube. After vortex mixing, the mixture was extracted with 100 μL of n-hexane. The tubes were centrifuged at 1,200 × g for 5 min. The supernatant was evaporated at room temperature under a stream of nitrogen and the residue was reconstituted in 100 μL of methanol. A 20 μL aliquot of the solution was injected. A Zorbax Eclipsed XDB-C18 HPLC column (250 mm × 4.6 mm) was used for the assay with a flow rate of 2 mL/min. For feed samples, 6 g feed were defatted in a Soxhlet apparatus for 3 h using acetone as a solvent. Evaporated lipid extract was redissolved in 10 mL hexane, stored overnight in darkness and analyzed by HPLC (Hewlett Packard 1050, Waldbronn 76337, Germany).

The α-tocopherol content was determined by HPLC as indicated by Ahn et al. (1995) with modifications. Briefly, 0.5 g yolk, 6 mL acetone, 0.5 mL of internal standard (IS; d-α-tocopheryl acetate, 1mg/mL), and 5 μL butylatedhydroxytoluene (1 mg/mL) were placed into a 50 mL tube and homogenized for 30 s. The

mixture was centrifuged at 1,200 × *g* for 10 min, and the supernatant was collected and evaporated under nitrogen at 60°C. The dried sample was redissolved in 2 mL methanol and analyzed using an Agilent ChemStation HPLC (UV detector fixed at 292 nm, 1200 Series), fitted with a XDB-C18 column[1] and run isocratically at a flow of 1.5 mL/min.

Thiobarbituric Acid Reactive Substances

The thiobarbituric acid reactive substances (**TBARS**) were measured in serum as described by Esterbauer and Cheeseman (1990). Briefly, an aliquot serum sample was mixed with 2 volumes of cold 10% trichloroacetic acid for protein precipitation. Following centrifugation, the supernatant was mixed with an equal volume of 0.67% TBA in a boiling water bath for 10 min. After cooling, the absorbance was measured at 532 nm. Measurements were performed in duplicate and expressed as μM of malonaldehyde per litre of serum.

The TBARS content of the yolks was determined using the method described by Cherian et al. (1996). Briefly, yolk samples (2 g) were weighed and placed into a test tube with 18 mL of 3.86% perchloric acid. The mixture was homogenized with a Brinkman Polytron (Type PT 10/35, Westbury, NY) for 15 s at high speed. The homogenate was filtered through a Whatman #1 filter paper. The filtrate (2 mL) was mixed with 2 mL of 20 mM TBA in distilled water and incubated in a boiling water bath for 30 min. After cooling, the absorbance of filterate was read at 531 nm against blank containing 1 mL distilled water and 1 mL of 20 mM TBA solution.

Sensory Evaluation

To evaluate the organoleptic qualities of hard-boiled eggs from hens on the various oil source diets, the triangle difference test (Roessler et al., 1948) was employed. Eggs were collected from the experimental cages on last week 3 days and were kept at 5°C for 14 days to facilitate peeling. Eggs were boiled for 15 min and kept in water at around 35°C to keep them warm until they were served for sensory evaluation (Caston and Leeson, 1990). Each egg was randomly placed and coded using three digit randomized numbers. In the test, each panelist was given 3 coded samples and was only asked to distinguish which egg was different from the others. Thirty untrained panelists were asked to evaluate eggs after cutting them in half. Unsalted crackers and water were offered as carriers.

[1]ZORBAX Eclipse XDB-C18 column, 4.6 × 250 mm, 5 μm (Agilent Technologies, Inc. Wilmington, NC).

Statistical Analysis

This experiment was conducted as a completely randomized 3 × 2 × 2 factorial design with 3 oil sources, 2 levels of vitamin E (0 and 250 mg/kg diet), and 2 levels of vitamin C (0 and 250 mg/kg diet). The data were analyzed using the GLM procedure of SAS (2001). Significant differences at 5% among treatment means were evaluated using Tukey's new multiple range test.

RESULTS AND DISCUSSION

Diet did not affect the productive performance of the hens (Irandoust et al., 2012). No interactions among main effects were found for any of the traits studied, and therefore, only main effects are discussed.

Effect of Diets on the FA Profile of Yolk

Soy oil source affected the FA profile of the yolk (Tables 3 and 4). The Saturated fatty acid (**SFA**) contents of yolk were not affected by dietary oil source except for C20:0, which was lower in the ASS diet than in the SO and RSO diets (P-value of < 0.001). The content of Polyunsaturated fatty acid (**PFA**) and the ratios of n6:n3, PUFA to SFA, and arachidonic acid to linoleic acid (**ARA:LA**) were not affected by oil source (P-value of > 0.05). Previous studies (Meluzzi et al., 2000; Mazalli et al., 2004) indicated that the concentration of SFA in eggs usually did not respond to dietary manipulation. A tendency to lower values (P-value = 0.059) of MUFA were observed in eggs from hens fed SO than those from hens fed RSO and ASS, which reflected the low content of MUFA in SO. Baucells et al. (2000) and Mazalli et al. (2004) found similar results with 4% and 3% canola oil in the diets, respectively. This finding showed that the changes of fatty acids induced by dietary treatment were not uniformly distributed among all FA types of egg yolk (Jiang et al., 1991). Measurable amounts of odd-chain FAs, C15:0 and C17:0, were observed in all eggs, irrespective to their presence in the dietary treatments, with the greatest quantities found for the VC supplemented diets. Garton et al. (1972) and López-Bote et al. (1997) reported that the production of volatile FA, mainly propionate by fermentation in the hind gut, could have ended up in the production of certain odd-numbered FA residues in non-ruminants. The concentration of yolk LNA and DHA (C22:6n-3) was not affected much by dietary oil sources. Grobas et al. (2001) reported that the conversion of LNA to DHA is a very inefficient process in the laying hen.

Vitamin E supplementation significantly increased the percentage of SFA (38.3% vs. 39.5) and C16:1 (1.41 vs. 1.60%), whereas n-6 and n-3 fatty acids and subsequently PUFA concentrations decreased (P-value of < 0.05). Vitamin E supplementation also lowered the concentration of docosahexanoic acid (**DHA**; C22:6n-3) in the egg yolk (0.64 vs. 0.60; P-value of < 0.05). However, the changes were very small. Most of the

Table 3. Effect of diet on saturated and monounsaturated fatty acid profile of the egg yolk (% of total fatty acids).

Item	C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	ΣSFA[1]	C14:1	C16:1	C18:1	C20:1	ΣMUFA[2]
Oil source												
SO[3]	0.19	0.03	30.32	0.13	8.33	0.09[a]	39.09	0.02	1.47	36.63	0.11	39.7
RSO[4]	0.22	0.03	30.59	0.13	8.05	0.08[a]	39.09	0.02	1.46	37.16	0.12	40.2
ASS[5]	0.19	0.03	30.23	0.13	7.88	0.06[b]	38.52	0.01	1.58	37.53	0.12	40.8
Vitamin E, mg/kg												
0	0.18[b]	0.03	30.07[b]	0.13	7.82[b]	0.08	38.31[b]	0.02	1.41[b]	37.19	0.12	40.1
250	0.21[a]	0.03	30.69[a]	0.13	8.36[a]	0.08	39.49[a]	0.02	1.60[a]	37.02	0.12	40.4
Vitamin C, mg/kg												
0	0.19	0.03[a]	30.48	0.14[a]	7.76[b]	0.08[a]	38.68	0.01[b]	1.48	37.09	0.11	40.19
250	0.20	0.02[b]	30.28	0.12[b]	8.42[a]	0.07[b]	39.12	0.02[a]	1.52	37.12	0.12	40.30
SEM[6] (n = 5)	0.001	0.001	0.169	0.003	0.119	0.003	0.181	0.001	0.030	0.199	0.001	0.210
Effects[7]						Probabilities						
Oil source (OS)	0.11	0.91	0.62	0.92	0.16	0.001	0.16	0.69	0.14	0.091	0.77	0.059
Vitamin E (VE)	0.014	0.76	0.053	0.74	0.006	0.10	0.001	0.65	0.001	0.61	0.67	0.57
Vitamin C (VC)	0.37	0.001	0.52	0.007	0.001	0.002	0.12	0.027	0.45	0.95	0.49	0.78
OS × VE	0.59	0.91	0.061	0.97	0.36	0.79	0.059	0.27	0.67	0.061	0.66	0.059
OS × VC	0.071	0.32	0.67	0.31	0.15	0.48	0.074	0.37	0.27	0.086	0.31	0.16
VE × VC	0.072	0.37	0.11	0.51	0.32	0.063	0.057	0.65	0.72	0.056	0.33	0.083
OS × VE × VC	0.95	0.081	0.17	0.97	0.075	0.31	0.63	0.15	0.41	0.072	0.77	0.12

[a,b]Means with different superscripts within a column of the same item are significantly different ($P < 0.05$).

[1]Saturated fatty acid (Σ of C14:0, C15:0, C16:0, C17:0, C18:0, and C20:0).

[2]Monounsaturated fatty acid (Σ of C14:1, C16:1, C18:1, and C20:1).

[3]Soy oil.

[4]Recycled soy oil.

[5]Acidulated soy oil soapstocks.

[6]Standard error of the mean (5 replicates of 5 hens per replicate). Data are the average of 5 eggs per replicate.

[7]The interactions between the main effects were not significant ($P > 0.05$).

decrease in n-6 FA was due to a decrease in LA, whereas the decrease of n-3 FA was due primarily to a decrease in DHA. The ratio of PUFA to SFA decreased (*P*-value of < 0.001), but that of ARA: LA increased (*P*-value of < 0.05). Supplementation of both vitamins E and C reduced or tended to reduce C18:2 content in yolk and increased ARA: LA ratio. Supplementation of vitamin E alone did not affect the content of odd-number FA concentration.

Vitamin E supplementation reduced C18:2n-6, C20:4n-6, C22:6n-3, PUFA content and PUFA: SFA ratio in yolk. These results are consistent with the findings of some other studies (Meluzzi et al., 1999, 2000; Galobart et al., 2001; Pál et al., 2002). Meluzzi et al. (1999), which suggested α-tocopherol at high doses can interfere with the intestinal absorption of some long-chain FA. The present study indicated that supplemental VE tendency to reduce the synthesis of long-chain PUFA in the liver or ovary and its deposition in the yolk. The concentration of C15:0, C17:0 and C20:0 decreased (*P*-value of < 0.05), but that of C18:0 increased with VC supplementation. Concentration of MUFA was not affected by VC supplementation except for C14:1, which was increased (*P*-value of < 0.05). The n-6:n-3 FA ratio decreased from 16.8 to 14.9 (*P*-value of < 0.001), while arachidonic acid to LA ratio increased (*P*-value of < 0.05). Hens fed with VC supplemented diets laid eggs with lower concentrations of odd-number FA (C15:0 and C17:0) than those fed diets without the vitamin.

Vitamin C supplementation caused some changes in FA profile of yolk. C18:0, C18:3n-3, n-3 FA, and ARA:LNA ratio in the yolk were increased but the ratio of n-6 FA: n-3 FA was decreased by vitamin C supplementation. However, Bou et al. (2006) did not observe any effect of vitamin C (110 mg VC/kg diets) on the FA profile of the meat.

Cholesterol and Triglyceride Concentration in Serum and Yolk

The concentrations of total cholesterol and TG in serum were not affected by dietary treatment (Table 5). The concentrations of total cholesterol in serum were 157, 151, and 158 mg/dL and those of TG were 191, 183, and 199 mg/dL in hens fed SO, RSO, and ASS, respectively (Table 5), which were similar to those of the other reports (Mazalli et al., 2004; Anderson, 2013). Total cholesterol concentration of the yolk from hens fed SO, RSO, and ASS were 13, 12.6, and 12.5 mg/g, respectively. Yolk TG was lower (*P*-value of < 0.05) in yolks from hens fed SO than those fed RSO or ASS diet (28.2 vs. 30.2, and 31.4 mg TG/g yolk, respectively). Supplementation of the diet with VC decreased yolk TG (30.8 vs. 29.1 mg/g). Murata et al. (2003) compared different oil sources (3% of SO, fish oil, canola oil, and poultry by-product) in laying hens diet and did not find any differences in concentrations of total cholesterol and TG in plasma and egg

Table 4. Effect of diet on polyunsaturated fatty acids of egg yolk (% of total fatty acids).

Item	C18:2	C20:2	C20:3	C20:4	Σn6	C18:3n3	C22:6n3	Σn3	Σn6: Σn3	ΣPUFA[1]	ΣPUFA: ΣSFA[2]	ARA[3]: LNA[4]
Oil source												
SO[5]	18.08	0.15[a]	0.14	1.54	19.92	0.67[a]	0.62	1.29	15.54	21.21	0.54	0.09
RSO[6]	17.62	0.15[a]	0.14	1.56	19.48	0.60[b]	0.63	1.23	15.99	20.70	0.53	0.09
ASS[7]	17.64	0.13[b]	0.13	1.54	19.44	0.60[b]	0.62	1.21	16.07	20.66	0.54	0.089
Vitamin E, mg/kg												
0	18.42[a]	0.15	0.14	1.57	20.28[a]	0.64	0.64[a]	1.28[a]	15.94	21.55[a]	0.56[a]	0.08[b]
250	17.14[b]	0.14	0.13	1.53	18.95[b]	0.61	0.60[b]	1.21[b]	15.79	20.16[b]	0.51[b]	0.09[a]
Vitamin C, mg/kg												
0	18.12	0.15	0.14	1.53	19.94	0.57[b]	0.62	1.19[b]	16.84[a]	21.13	0.55	0.08[b]
250	17.43	0.14	0.13	1.57	19.29	0.68[a]	0.62	1.30[a]	14.89[b]	20.58	0.53	0.09[a]
SEM[8] (n = 5)	0.206	0.003	0.002	0.015	0.208	0.012	0.010	0.017	0.226	0.216	0.007	0.001
Effects[9]							Probabilities					
Oil source (OS)	0.54	0.006	0.51	0.72	0.54	0.003	0.89	0.066	0.41	0.47	0.67	0.26
Vitamin E (VE)	0.002	0.068	0.67	0.15	0.002	0.11	0.034	0.017	0.62	0.001	0.001	0.049
Vitamin C (VC)	0.082	0.17	0.20	0.078	0.10	0.001	1.00	0.001	0.001	0.18	0.15	0.049
OS × VE	0.86	0.13	0.29	0.60	0.88	0.79	0.93	0.81	0.92	0.87	0.90	0.92
OS × VC	0.99	0.36	0.85	0.12	0.98	0.46	0.51	0.25	0.28	0.96	0.84	0.55
VE × VC	0.66	0.53	0.056	0.062	0.84	0.72	0.31	0.70	0.47	0.86	0.37	0.058
OS × VE × VC	0.46	0.74	0.63	0.34	0.43	0.41	0.057	0.053	0.063	0.40	0.71	0.82

[a,b]Means with different superscripts within a column of the same item are significantly different ($P < 0.05$).

[1]Polyunsaturated fatty acid (Σ of C18:2n6, C20:2n6, C20:3n6, C20:4n6, C18:3n3, and C22:6n3).

[2]Saturated fatty acid.

[3]Arachidonic acid.

[4]Linoleic acid ratio.

[5]Soy oil.

[6]Recycled soy oil.

[7]Acidulated soy oil soapstocks.

[8]Standard error of the mean (5 replicates of 5 hens per replicate). Data are the average of 5 eggs per replicate.

[9]The interactions between the main effects were not significant ($P > 0.05$).

Table 5. Effect of oil source and vitamin E and vitamin C supplementation on total cholesterol (TC), triglyceride (TG), α-tocopherol, and malondialdehyde (MDA) content in serum and yolk.

	Serum				Yolk				
Item	TC (mg/dl)	TG (mg/dl)	α-T (μM/L)	MDA (μM/L)	TC (mg/g)	TG (mg/g)	α-T (μg/g)	MDA0d[1] (μg/g)	MDA90d[1] (μg/g)
Oil source									
SO[2]	157	191	32.8	0.49[a,b]	13.0	28.2[b]	149	0.50	0.55
RSO[3]	151	183	35.3	0.53[a]	12.6	30.2[a]	125	0.54	0.59
ASS[4]	158	199	29.6	0.42[b]	12.5	31.4[a]	139	0.52	0.55
Vitamin E, mg/kg									
0	157	189	12.2[b]	0.53[b]	13.0	29.5	43[b]	0.51	0.52[b]
250	153	192	52.8[a]	0.44[a]	12.4	30.4	232[a]	0.53	0.60[a]
Vitamin C, mg/kg									
0	154	186	33.5	0.52[b]	12.9	30.8[a]	139	0.51	0.55
250	157	196	31.5	0.44[a]	14.3	29.1[b]	136	0.53	0.57
SEM[5] (n = 5)	4.856	3.794	2.352	0.022	0.202	0.426	7.0	0.026	0.012
Effects[6]						Probabilities			
Oil source (OS)	0.61	0.18	0.37	0.039	0.62	0.001	0.17	0.70	0.28
Vitamin E (VE)	0.60	0.72	0.001	0.005	0.14	0.20	0.001	0.66	0.001
Vitamin C (VC)	0.63	0.14	0.55	0.015	0.28	0.014	0.76	0.47	0.39
OS × VE	0.49	0.52	0.22	0.073	0.66	0.68	0.15	0.14	0.94
OS × VC	0.93	0.55	0.58	0.19	0.65	0.16	0.063	0.49	0.52
VE × VC	0.46	0.29	0.54	0.38	0.23	0.065	0.56	0.22	0.49
OS × VE × VC	0.20	0.066	0.75	0.50	0.89	0056	0.057	0.80	0.16

[a,b]Means with different superscripts within a column of the same item are significantly different ($P < 0.05$).

[1]Malondialdehyde was determined in fresh yolk (0 day) and 90 days storaged yolk at 5°C.

[2]Soy oil.

[3]Recycled soy oil.

[4]Acidulated soy oil soapstocks.

[5]Standard error of the mean (5 replicates of 5 hens per replicate). Data are the average of 5 eggs per replicate.

[6]The interactions between the main effects were not significant ($P > 0.05$).

yolk. Similar findings on the effect of dietary fat on concentration of cholesterol or TG in serum or egg yolk were reported by others (Hirata et al., 1986; Shafey et al., 2003).

Supplemental VE and VC did not affect serum cholesterol or TG concentrations: Narimani-Rad et al. (2011) used semi-refined sunflower oil (2, 4, and 6% of the diet) supplemented with 75 and 150 mg VE/kg of diet and reported no differences in serum total cholesterol and TG of laying hens. Mazalli et al. (2004) used 12 and 100 IU VE/kg of diets containing 3% of different oil sources for laying hens and reported that the VE levels did not affect the cholesterol concentration in eggs. Mohiti Asli et al. (2007) used 0, 100, 200, and 400 mg VE or VC/kg of diet and did not find any differences in serum cholesterol and TG concentration of laying hens. Zaghari et al. (2013) used 0, 200, and 400 mg VE/kg of diet and did not find any changes in blood and yolk cholesterol concentration of the broiler breeder hens. In the current trial, the serum total cholesterol and TG were similar for all the dietary treatments, and the egg yolk cholesterol and TG were also unchanged.

α-Tocopherol Concentration

Soy oil source or VC supplementation did not affect α-Tocopherol concentration in the serum (on average 32.6 μM/L) and yolk (on average 138 μg/g). Supplementing VE to the diet, however, increased α-tocopherol concentration in serum (P-value of < 0.001; 52.8 vs. 12.2 μM/L) as well as in yolk (232 vs 43 μg/g yolk; P-value of < 0.001) (Table 5). The concentration of α-tocopherol in serum and yolk was not affected by type of soy oil, which is in agreement with others (Meluzzi et al., 2000; Galobart et al., 2001; Pál et al., 2002). Cherian et al. (1996) also did not detect differences in α-tocopherol content in yolk of hens fed diets supplemented with 3.5% menhaden, flax, or sunflower oil without added VE. However, studies reported by others generally agree on the negative impact of PUFA on tocopherol absorption from the intestine (Hollander, 1981; Meluzzi et al., 1999).

Dietary VE supplementation increased the α-tocopherol concentration in serum and yolk, which is in agreement with Frigg et al. (1992), Meluzzi et al. (2000), and Galobart et al. (2001). Jiang et al. (1994) showed significantly higher levels of α-tocopherol in yolk when a basal diet was supplemented with 100, 200, or 400 mg of dl-α-tocopheryl acetate/kg of feed. Grobas et al. (2002) reported that there was a direct relationship between the dietary level of α-tocopheryl acetate and α-tocopherol in the yolk. Like Jiang et al. (1994), Qi and Sim (1998) achieved a linear increase of tocopherols in egg yolk when a natural tocopherol was added to laying hen diets.

The deposition efficiency of dietary α-tocopherol in egg yolk was between 14 and 24% when 10 or 260 mg VE/kg was used, respectively, which was similar to other authors. Naber (1993) calculated the transfer efficiency of VE by using data of other authors and found that this parameter oscillated between 16 and 39% with dietary levels of VE ranging from 12.6 to 30 mg/kg. Galobart et al. (2001) obtained an efficiency of 26% for 20 to 40 mg VE/kg feed. A possible explanation of the different efficiencies found in the above-mentioned experiments could be the analytical procedures and the stability of dietary VE used in these experiments. Dju et al. (1950) found a higher efficiency of vitamin E incorporation into egg yolk at lower dietary inclusion levels. A possible explanation for the lower efficiency at higher dietary supplementation might be the higher competition for absorption (Galobart et al., 2001).

Thiobarbituric Acid Reactive Substances

The data for serum and yolk malondialdehyde (**MDA**) concentration is shown in Table 5. Serum MDA concentration of the RSO treatment was higher than the ASS treatment (0.53 vs. 0.42 μM/L), whereas the serum MDA content of the SO treatment was in between the 2 treatments (0.49 μM/L) (P-value of $<$ 0.039). Supplementation of a diet with either VE or VC alone and in combination decreased serum MDA concentration respective to control (0.53 vs. 0.44, 0.52 vs. 0.44, and 0.58 vs. 0.41 μM/L, P-value of < 0.015). Fresh yolk MDA concentration was not affected by dietary treatments, but the MDA concentration of 90 d stored yolks was increased by VE supplementation (0.522 vs. 0.604 μg MDA/g yolk).

Serum MDA, unlike yolk MDA, was influenced by dietary treatments: serum MDA level was more influenced by the dietary manipulation than yolk MDA. RSO increased serum MDA compared with ASS as expected. Higher peroxide value in RSO and lower PUFA in ASS can explain this effect. Lipid peroxidation in serum generated by dietary oils containing high levels of PUFA can be ameliorated by the supplementation of 250 mg VE/kg. This finding leads to the assumption that the decreased lipid peroxidation may be able to enhance stability of blood cellular membranes and lead to longer shell-life of eggs. This assumption needs further study.

Oxidation of fresh egg yolk was low and was not affected by dietary oils or vitamins E or C. Pike and Peng (1988) and Marshall et al. (1994) also reported a low susceptibility of fresh egg yolk to oxidation. Cherian et al. (1996) and Galobart et al. (2001) detected a clear antioxidant effect of dietary α-tocopheryl acetate in n-3 PUFA-enriched eggs. The former authors did not find any change in TBARS values of eggs not enriched with n-3 PUFA, which confirms our results. At 90 d storage time, supplementation of a diet with VE increased MDA concentration of the yolk. In the literature there is some agreement on this matter. Gebert et al. (1998) reported that an elevated concentration of vitamin E (100 to 200 ppm) increased TBARS number, and Chen

et al. (1998) postulated that vitamin E acted is as a prooxidant at 75 ppm or above that corresponded to 120 ppm in the diet.

Values of MDA showed that supplementation of a diet with vitamin E at 250 mg/kg leads to increased deposition of α-tocopherol and thus inhibited the chain reaction of peroxidation in the serum. Hens supplemented with 250 mg VE/kg maintained lower levels of MDA values in serum, whereas MDA values in hens fed diets without supplemental VE were prone to increase. This result is due to the antioxidant property of VE as discussed earlier. These results are similar to the study of Cherian et al. (1996), who reported that supplemental VE reduced TBA values in eggs.

Vitamin C is found in very small amounts in the yolk compared to other yolk vitamins (Gutierrez et al., 1997). It is probable that supplementation of VC did not express an additive effect to VE in yolk because of the very low deposition levels in the yolk. A high level of VC in the current study significantly (P-value of < 0.05) decreased MDA values in serum. Levels of MDA in serum and yolk from hens fed a combination of VE and VC, however, were not significantly different from those supplemented with VE alone. Most of the fat-soluble vitamins in hen eggs are contained in the yolk.

Serum MDA levels decreased when vitamin E and C were supplemented, indicating antioxidant effects of the 2 vitamins. Antioxidant effects of the vitamins were not increased when they were supplemented as a combination. Vitamin E, a nutritional antioxidant in biological systems, functions as free radical scavenger and inhibits lipid peroxidation within membranes (Halliwell and Gutteridge, 1989; McDowell, 1989). However, vitamin C can exhibit either antioxidative or prooxidative effects. At high concentrations of vitamin C, in the presence of relatively low concentrations of free or activated metal ions, the antioxidative properties usually predominate and vitamin C acts as a free-radical chain terminator (McDowell, 1989). There was no synergism between VE and VC in this study, which is inconsistent with other reports that have indicated that the effects of VE and VC are additive (Gallo-Torres, 1980; Gey, 1998).

Sensory evaluation

The data demonstrated that the majority of the panellists were not able to detect significant differences among the coded samples. Panelists detected no significant differences among the eggs from layers fed 3.5% of SO, RSO, and ASO. Approximately 80% and 63% of the panellists reported that all eggs from RSO and ASO were similar in flavor as compared to SO.

CONCLUSION

This study revealed that feeding hens with diets including 3.5% RSO or ASS had no adverse effect on fatty acid profile, cholesterol and triglyceride concentrations, and the oxidative stability of yolk. Because RSO and ASS could be successfully incorporated into the diet of laying hens at 3.5%, use of RSO and ASS in laying hen diets can alleviate the problem of food processesing industry. It was concluded that dietary supplementation of high levels of vitamin E showed antioxidant effects, but vitamin C acted as a prooxidant in long-time stored eggs from hens fed high-PUFA diets.

ACKNOWLEDGMENTS

The authors express their appreciation to Professor T. Trziszka and the staff of the Department of Animal Products Technology and Quality Management (Wrocław University of Environmental and Life Sciences, Wrocław, Poland) for their help and technical support. Authors thank for technical assistance of the Cardiovascular Research Centre and Isfahan Endocrine and Metabolism Research Centre (Isfahan University of Medical Sciences, Isfahan, Iran) especially M. Boshtam and Z. Pournaghshband.

REFERENCES

Ahn, D. U., H. H. Sunwoo, F. H. Wolfe, and J. S. Sim. 1995. Effect of dietary α-linolenic acid and strain of hen on the fatty acid composition, storage stability, and flavor characteristics of chicken eggs. Poult. Sci. 74:1540–1547.

Anderson, K. E.. 2013. Comparison of fatty acid, cholesterol, vitamin A and E composition, and trans fats in eggs from brown and white egg strains that were molted or nonmolted. Poult. Sci. 92:3259–3265.

Baucells, M. D., N. Crespo, A. C. Barroeta, S. López-Ferrer, and M. A. Grashorn. 2000. Incorporation of different polyunsaturated fatty acids into eggs. Poult. Sci. 79:51–59.

Bou, R., S. Grimpa, F. Guardiola, A. C. Barroeta, and R. Codony. 2006. Effects of various oil sources, α-tocopheryl acetate, and ascorbic acid supplements on fatty acid composition and α-tocopherol content in raw and vacuum-packed, cooked dark chicken meat. Poult. Sci. 85:1472–1481.

Caston, L., and S. Leeson. 1990. Research note: Dietary flax and egg composition. Poult. Sci. 69:1617–1620.

Chen, J. Y., I. D. Latshaw, H. O. Lee, and D. B. Min. 1998. α-Tocopherol content and oxidative stability of egg yolk as related to dietary α-tocopherol. J. Food Sci. 63:919–922.

Cherian, G., F. W. Wolfe, and J. S. Sim. 1996. Dietary oils with added tocopherols: effects on egg or tissue tocopherols, fatty acids, and oxidative stability. Poult. Sci. 75:423–431.

Dju, M., M. L. Quaife, and P. L. Harris. 1950. Utilization of pure α, β, δ-tocopherols by laying hens. Am. J. Physiol. 160:259–263.

Esterbauer, H., and K. H. Cheeseman. 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Meth Enzymol 186:407–421.

Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–507.

Frigg, M., C. C. Whitehead, and S. Weber, 1992. Absence of effects of dietary α-tocopherol on egg yolk pigmentation. Br. Poult. Sci. 33:247–353.

Gallo-Torres, D. C. 1980. Absorption, bloodtransport and metabolism of vitamin E. Pages 170–267 in A Comprehensive-Treatise, L.J. Maclin, ed. Marcel Dekker, New York.

Galobart, J., A. C. Barroeta, M. D. Baucells, and F. Guardiola. 2001. Lipid oxidation in fresh and spray-dried eggs enriched with ω3 and ω6 polyunsaturated fatty acids during storage as affected

by dietary vitamin E and canthaxanthin supplementation. Poult. Sci. 80:327–337.

Garton, G. A., F. D. Howell, and W. R. H. Duncan. 1972. Influence of dietary volatile fatty acids on the fatty-acid composition of lamb trygliceride with special reference to the effect of propionate on the presence of branched-chain components. Br. J. Nutr. 28:409–416.

Gebert, S., R. Messikommer, H. P. Pfirter, G. Bee, and C. Wenk. 1998. Dietary fats and vitamin E in diets for laying hens: effects on laying performance, storage stability and fatty acid composition of eggs. ArchivGeflulk. 62:214–222.

Gey, K. F. 1998. Vitamins E plus C and interactingco-nutrients required for optimal health. Bio-Factors. 7:113–174.

Gonzalez-Corbella, M. J., N. Lioberas-Blanch, A. I. Castellote-Bargallo, M. C. Lopez-Sabater, and M. Rivero-Urgell. 1994. Determination of α-tocopherol in plasma and erythrocytes by high-performance liquid chromatography. J. Chromatography B. 660:395–400.

Grobas, S., J. Mendez, C. Lopez-Bote, C. Blas, and G. G. Mateos. 2002. Effect of vitamin E and A supplementation on egg yolk α-tocopherol concentration. Poult. Sci. 81:376–381.

Grobas, S., J. Mendez, R. Lazaros, C. D. Blas, G. G. Mateos, and B. C. De. 2001. Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens. Poult. Sci. 80:1171–1179.

Gutierrez, M. A., H. Takahashi, and L. R. Juneja. 1997. Nutritive evaluation of hen eggs. Pages 25–35 in Hen Eggs: Their Basic and Applied Science. T. Yamamoto, L. R. Juneja, H. Hatta, and M. Kim, eds. CRC Press, Boca Raton, FL.

Halliwell, B., and J. M. C. Gutteridge. 1989. Lipid peroxidation: A radical chain reaction. Pages 188–218 in Free Radicals in Biology and Medicine. 2nd ed. Oxford University Press, New York, NY.

Hirata, A., G. B. M. Nishino, T. Kimura, and Y. Ohtake. 1986. Effects of dietary fats for laying hens in the fatty acid composition and yolk lipids. J. Jap. Food. Sci. Tech. 33:631–639.

Hollander, D. 1981. Intestinal absorption of vitamins A, E, D and K. J. Lab. Clin. Med. 97:449–462.

Irandoust, H., A. H. Samie, H. R. Rahmani, M. A. Edriss, and G. G. Mateos. 2012. Influence of source of fat and supplementation of the diet with vitamin E and C on performance and egg quality of laying hens from forty four to fifty six weeks of age. Anim. Feed Sci. Technol. 177:75–85.

Iranian Council of Animal Care. 1995. Guide to the Care and Use of Experimental Animals. Isfahan University of Technology, Isfahan, Iran.

Jiang, Z., D. U. Ahn, and J. S. Sim. 1991. Effects of feeding flax and two types of sunflower seeds on fatty acid composition of yolk classes. Poult. Sci. 70:2467–2475.

Jiang, Z., R. B. McGeachin, and C. A. Bailey. 1994. α-Tocopherol, β-carotene, and retinol enrichment of chicken eggs. Poult. Sci. 73:1137–1143.

López-Bote, C., A. Rey, B. Isabel, and R. Sanz. 1997. Dietary fat reduces odd-numbered and branched-chain fatty acids in depot lipids of rabbits. J. Sci. Food Agric. 73:517–524.

Mäkinen, M., M. Kähkönen, and A. Hopia. 2001. Ascorbic acid and ascorbylpalmitate have only minor effect on the formation and decomposition of methyl linoleatehydroperoxides. European J. Lipid Sci. and Technol. 103: 683–687.

Marshall, A. C., A. R. Sams, and M. E. Van Elswyk. 1994. Oxidative stability and sensory quality of stored eggs from hens fed 1.5% menhaden oil. J. Food Sci. 59:561–563.

Mazalli, M. R., D. E. Faria, D. Salvador, and D. T. Ito. 2004. A comparison of the feeding value of different sources of fats for laying hens: 1. Performance characteristics. J. Appl. Poult. Res. 13:274–279.

McDowell, L. R. 1989. Vitamins in Animal Nutrition. ComparativeAspects to Human Nutrition. Pages 93–131 in Vitamin C, A and E, L. R. McDowell, ed. Academic Press, London.

Meluzzi, A., F. Sirri, G. Manfreda, N. Tallarico, and A. Franchini. 2000. Effects of dietary vitamin E on the quality of table eggs enriched with n-3 long-chain fatty acids. Poult. Sci. 79:539–545.

Meluzzi, A., F. Sirri, N. Tallarico, and L. Vandi. 1999. Dietary vitamin E in producing eggs enriched with n-3 fatty acids. Proc. 8th European Symposium on the Quality of Eggs and Egg Products, Bologna, Italy. WPSA Italian branch, September 19–23, 1999, Bologna, Italy, pp. 153–159.

Metcalfe, L. D., A. Smitz, and J. B. Pelka. 1961. The rapid preparation of fatty acid esters for gas chromatography. Ann. Chem. 33:363–364.

Mohiti Asli, M., S. AbdollahHosseini, H. Lotfollahian, and F. Shariatmadari. 2007. Effect of probiotics, yeast, vitamin E and vitamin C supplements on performance and immune response of laying hen during high environmental temperature. Int. J. Poult. Sci. 6:895–900.

Murata, L. S., J. Ariki, C. R. Machado, L. Silva, and M. J. M. Rezende. 2003. Effect of oils sources on blood lipid parameters of commercial laying hens. Braz. J. Poult. Sci. 5:203–206.

Naber, E. C. 1979. The effect of nutrition on the composition of eggs. Poult. Sci. 58:518–528.

Naber, E. C. 1993. Modifying vitamin composition of eggs: A review. J. Appl. Poult. Res. 2:385–393.

Narimani-Rad, M., A. Nobakht, and A. Lotfi. 2011. Influence of dietary supplemented semi-refined sunflower oil with vitamin E on some of serum biochemical and immunological measures in laying hens. Adv. Environ. Biol. 5:1955–1959.

National Research Council (NRC). 1994. Nutrient requirement for poultry. Ninth revision, National Academy Press, Washington, D.C.

Niki, E. 1991. Action of ascorbic acid as a scavenger of active and stable oxygen radicals. Am. J. Clin. Nutr. 54:1119s–1124s.

Pál, L., K. Dublecz, F. Husvéth, L. Wágner, Á. Bartos, and G. Kovács. 2002. Effect of dietary fats and vitamin E on fatty acid composition, vitamin A and E content and oxidative stability of egg yolk. Poult. Sci. 81:1694–1702.

Pike, O. A., and I. C. Peng. 1988. Effect of protein disruption by denaturation and hydrolisis on egg yolk lipid oxidation. J. Food Sci. 53:428–431.

Qi, G. H., and J. S. Sim. 1998. Natural tocopherol enrichment and its effect in n-3 fatty acid modified chicken eggs. J. Agric. Food. Chem. 46:1920–1926.

Roessler, E. B., J. Warren, and J. F. Guymon. 1948. Significance in triangular taste tests. Food Res. 13:503–505.

SAS Institute. 2001. Statistical Analysis Systems User's Guide. SAS Institute Inc., Cary, NC.

Shafey, T. M., J. G. Dingle, M. W. McDonald, and K. Kostner. 2003. Effect of type of grain and oil supplement on the performance, blood lipoproteins, egg cholesterol and fatty acids of laying hens. Int. J. Poult. Sci. 2:200–206.

Yu, B. P., 1994. Cellular defenses against damage from reactive oxygen species. Physiol. Rev. 74:139–162.

Zaghari, M., V. Sedaghat, and M. Shivazad. 2013. Effect of vitamin E on reproductive performance of heavy broiler breeder hens. J. Appl. Poult. Res. 22:808–813.