Natural Tocopherol Enrichment and Its Effect in *n*−3 Fatty Acid Modified Chicken Eggs†

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Two hundred and forty White Leghorn laying hens were fed an *n*−3 fatty acid modified diet supplemented with a natural tocopherol (TOC) mixture at 0, 200, 400, and 800 mg/kg for 7 weeks. Egg yolk TOC content increased linearly ($P < 0.05$) up to day 8 at each level of dietary supplementation. The fatty acid profile of egg yolks became relatively constant at day 8 with the exception of C20:4*n*6, which stabilized by day 16. From day 0 to day 8 of the feeding, the contents of all *n*−3 polyunsaturated fatty acids in egg yolk lipids significantly increased, with a concomitant decrease in C18:1 ($P < 0.05$). Lipid stability of egg yolk was significantly improved with increasing dietary TOC supplementation ($P < 0.05$). In short, *n*−3 fatty acid modified eggs can be also simultaneously enriched with natural tocopherols through dietary modification to make egg yolk lipid more stable.

Keywords: *Tocopherol; n−3 fatty acid; chicken egg; flaxseed*

INTRODUCTION

Attention given to modifying the fatty acid composition of eggs as well as poultry meat has recently focused on the elevation of *n*−3 fatty acids (Sim and Qi, 1995) with the intention of making these products more favorable for human consumption because of their beneficial effects (Fernandes and Verkatraman, 1993). It has been proposed that shell eggs enriched with *n*−3 fatty acids may provide an excellent alternative food source for these fatty acids, both independently and as an ingredient in processed foods (Simopoulos and Salem, 1992). A recent survey in the United States showed that ∼60% of consumers were willing to purchase *n*−3 fatty acid enriched eggs, and of those, 71% were willing to pay more for these eggs (Marshall et al., 1994). This indicates that *n*−3 fatty acid enriched eggs represent a viable means for increasing *n*−3 fatty acids in the diets of health-conscious consumers.

The presence of fats containing polyunsaturated fatty acids (PUFA) involves the risk of the development of oxidative rancidity as the fatty acids become oxidized in the feed or in the gastrointestinal tract. The problem is more serious with oils of high PUFA content and high levels of the more highly unsaturated fatty acids. In addition, oxidized fatty acids and their degeneration products, when absorbed, are harmful to animals as well as humans and may cause adverse biological effects (Kubow, 1993). Thus, this has emphasized the need for minimizing lipid oxidation in PUFA-enriched products.

Reports on the potential of antioxidants in prolonging the life span of some animal species and inhibiting the toxic, mutagenic, and carcinogenic actions of some chemicals have encouraged the public to consume antioxidants as a prophylactic means against aging and disease (Schuler, 1990). However, two commonly used synthetic antioxidants, butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA), appear to be tumor promoters in long-term animal and human studies, although the concentrations of BHA and BHT used in food are probably harmless (Kappus and Kahl, 1993). These adverse effects prevent them from being used at high level in foodstuffs, including eggs.

Moreover, there is a general trend toward replacing the use of synthetic antioxidants in food by the addition of natural oxidation inhibitors or by the preferential use of ingredients that naturally possess antioxidant activity. These natural antioxidants offer the following unique characteristics: they are naturally occurring and presumably safe and have a lower health risk, and thus they are more easily approved by authorities and accepted by consumers in comparison to their synthetic counterparts (Schuler, 1990). Of the natural antioxidants, vitamin E is one of the best alternatives because of its powerful antioxidative activity both in vivo and in vitro. In the biological system, it can function as an intercellular and intracellular antioxidant (Burton, 1994), thus neutralizing free radicals and preventing oxidation of lipids within membranes to benefit humans and animals. Evidence of the role of tocopherols in limiting and protecting biological systems from oxidative damage is increasing (Schuler, 1990). The oral application of vitamin E in the prevention of human diseases associated with free radical mechanisms was reviewed by Bendich and Machlin (1992). They suggest that it is safe to consume 750 IU/day of vitamin E, which is >50 times the U.S. Recommended Dietary Allowance (RDA).

Most of the publications concerning vitamin E enrichment in eggs have focused on the utilization of α-toco-

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Table 1. Composition of the Basal Diet

ingredient/nutrient	basal diet, g/kg
corn	78.68
wheat	562.70
soybean meal (47% CP)	89.76
full-fat flax seed	150.00
limestone	83.19
Biofos (15% Ca, 21% P)	20.00
sodium chloride	3.00
DL-methionine	0.67
menhaden oil	5.00
vitamin and mineral premix[a]	7.00
calculated value	
ME, kcal/kg	2800
crude protein, %	16.00
Ca, %	3.6
available P, %	0.53
lysine, %	0.68
methionine + cystine, %	0.56
determined value	
total tocopherol,[b] mg/kg	67.03
total lipid, %	7.06

[a] Supplied per kilogram of the diet: vitamin A, 12 000 IU; vitamin D_3, 3 000 IU; vitamin K, 2.0 mg; pantothenic acid, 14.0 mg; riboflavin, 6.5 mg; folic acid, 1.0 mg; niacin, 40.0 mg; thiamin, 3.3 mg; pyridine, 12.0 mg; vitamin B_{12}, 0.02 mg; biotin, 0.2 mg; vitamin E (α-tocopherol acetate), 40 IU; iodine, 0.5 mg; manganese, 75.0 mg; copper, 15.0 mg; zinc, 80.0 mg; selenium, 0.1 mg; iron, 100.0 mg. [b] α-, γ-, and δ-tocopherols accounted for 67.23, 30.06, and 2.70%, respectively.

pherol or its derivatives (Jiang et al., 1994). Moreover, there is not enough consideration given to γ- and δ-tocopherols, which often exist in great quantities in natural sources and have higher antioxidative activities in vitro (Duithie et al., 1992) than α-tocopherol. In light of the health benefits of both *n*−3 fatty acids and natural tocopherols, the objective of this study was to enrich *n*−3 fatty acid modified eggs with tocopherols by feeding the laying hens the *n*−3 fatty acid modified diets supplemented with a natural mixture of α-, γ-, and δ-tocopherols.

MATERIALS AND METHODS

Experimental Design. Two hundred and forty Single Comb White Leghorn laying hens, 34 weeks old, were housed in three double-deck cage batteries with two birds in each cage (31 × 36 cm). Each battery had 40 cages and was divided into four units with 10 cages per unit, thus generating a total of 12 experimental units. The birds were assigned at random into one of the four dietary treatments, with each treatment replicated three times. Four experimental diets were prepared by supplementing a basal diet containing 15% full-fat Norlin flaxseed produced in Western Canada and 0.5% menhaden oil (Zapata Protein, Inc., Reedville, VA) with 0, 200, 400, and 800 mg of natural tocopherol mixtures (Henkel Corp., Kankakee, IL) per kilogram of diet (Tables 1−3). Whole flaxseed with hulls were ground before the diets were mixed. Feed samples were taken for fatty acid and tocopherol analysis. Feed and water were provided for ad libitum consumption. Egg production was recorded continually throughout the experiment. Feed consumption was measured for the whole period of the experiment. Both egg production and feed consumption were calculated as a mean of the experiment unit containing 20 birds each.

On day 28 of the feeding, 12 eggs from each treatment of 4 each replicate were randomly taken to install the panel test of scrambled eggs. For scrambling eggs, 12 eggs per treatment were pooled and thoroughly homogenized. All eggs were cooked in a nonstick saute pan on an electric stove using a medium heating to a final internal temperature of 76 °C. Twenty untrained volunteers from the University of Alberta participated. Four samples were coded and presented to panelists on a coded plate each time. Cold tap water was provided to panelist to rinse between samples. Two 9 cm linear hedonic scales (1−5), anchored with the words "no off-flavor" and "strong off-flavor" for flavor of sample and "dislike extremely" and "like extremely" for taste of sample at opposite ends, were used to record the sample on flavor and taste. The responses from the panelists were expressed in numerical values ranging from 1 (no off-flavor) to 5 (strong off-flavor) for flavor and from 1 (dislike extremely) to 5 (like extremely) for taste to the nearest 0.5 cm.

Table 2. Major Fatty Acid Composition of the Basal Diet

fatty acid	% of total fatty acid methyl esters	fatty acid	% of total fatty acid methyl esters
C16:0	7.79	SFA[a]	10.46
C18:0	2.25	MUFA[b]	16.75
C18:1*n*9	15.95	*n*3[c]	35.09
C18:2*n*6	21.58	*n*6[d]	21.68
C20:4*n*6	0.10	*n*6/*n*3	0.61
C18:3*n*3	33.72		
C20:5*n*3	0.71		
C22:5*n*3	0.14		
C22:6*n*3	0.51		

[a] SFA, total saturated fatty acids, including C14:0, C16:0, C17:0, and C18:0. [b] MUFA, total monounsaturated fatty acids, including C16:1, C18:1, and C20:1*n*9. [c] *n*3, total *n*3 polyunsaturated fatty acids, including C18:3*n*3, C20:5*n*3, C22:5*n*3, and C22:6*n*3. [d] *n*6, total *n*6 polyunsaturated fatty acids, including C18:2*n*6 and C20:4*n*6.

Table 3. Tocopherol Contents of the Experimental Diets

supplemental level, mg/kg of diet	dietary tocopherol content, mg/kg of diet			
	α-	γ-	δ-	total
0	45.07	20.15	1.81	67.03
200	64.55	135.85	66.63	267.03
400	84.03	251.55	131.45	467.03
800	122.99	482.95	261.09	867.03

Sampling and Analyses. On days 0, 4, 8, 16, and 28 of feeding, 4 eggs from each of the three experimental units, totaling 12 eggs per treatment, were randomly collected. The eggs were weighed and cracked. After Haugh units were measured according to the method of Haugh (1937), two egg yolks were pooled together (six samples per treatment) for the determination of tocopherol contents and fatty acid composition.

Tocopherol contents were analyzed according to the method of Zaspel and Csallany (1983) with some modifications. Egg yolk was weighed into a test tube with 3 mL of acetone, an appropriate amount of internal standard (5,7-dimethyltocol, Matreya Inc., Pleasant Gap, PA) based on the tocopherol content of the sample, and 5 μL of BHA (1 mg/mL). The mixture was then homogenized with a Polytron (Brinkman Instruments Inc., Westbury, NY) for 30 s at high speed, and the Polytron head was rinsed twice with 3 mL of acetone twice. The homogenate was centrifuged at 2000*g* for 10 min, and the supernatant was collected. The resulting pellet was dissolved with 4 mL of acetone, vortexed, and then centrifuged. The supernatant was pooled and evaporated under nitrogen at 60 °C. The dried sample was dissolved with methanol, vortexed, and centrifuged at 2000*g* for 15 min. The clear upper layer was injected on the column. A Varian 5000 high-performance liquid chromatography system (Varian Associates Inc., Walnut Creek, CA) with a Shimadzu Sil-9A model autosampler (Shimadzu Corp., Kyoto, Japan) was used to determine α-, γ-, and δ-tocopherol concentrations in the samples. Separation was achieved on a Supelcosil 3 μm reversed phase LC-18 column (Supelco Canada Ltd., Mississauga, ON, Canada) (150 mm × 4.6 mm) with a guard column (5 cm, 20−40 μm LC-18 packing) and a mobile phase of methanol/acetonitrile (50:50 v/v) at a 1.5 mL/min flow rate. Tocopherols were detected with a Shimadzu RF-535 fluorescence detector (Shimadzu Corp.) at an excitation wavelength of 295 nm and an emission wavelength of 330 nm. Samples

were identified and quantified on a chromatography data system (Shimadzu Scientific Instruments Inc., Columbia, MD) by measuring the retention times of tocopherol standards (Sigma Chemical Co., St. Louis, MO) and the response factors of the standards against the internal standard. Tocopherol contents of feed samples were analyzed after saponification (Rizzolo and Polesello, 1992).

Total lipids were extracted from egg yolk and feed samples with chloroform/methanol (2:1 v/v) according to the method of Folch et al. (1957). The fatty acid methyl esters were prepared from the lipid extracts by transesterification with BF_3/methanol (Morrison and Smith, 1964). Analysis was done on a Varian 3600 gas chromatograph equipped with an 8200 autosampler, an on-column injector, and a flame ionization detector. A DB-23 30 m × 0.25 mm i.d. × 0.25 μm film thickness fused silica capillary column (J&W Scientific, Folsom, CA) was used to separate fatty acid methyl esters. Column temperature was programmed from 70 °C for 0.5 min to 180 °C at 30 °C/min, held for 10 min, then increased to 230 °C at 5 °C /min, and held at the final temperature for 3 min. Injector temperature was programmed from 80 to 230 °C at 150 °C /min and held at 230 °C for 17 min. Liquid CO_2 was used to cool the injector. The carrier gas, helium, head pressure was set at 20 psi with a flow rate of 30 mL/min as makeup gas to the detector. The peak was identified and integrated by using fatty acid methyl ester standards (Nu-Chek-Prep, Inc., Elysian, MN) and a chromatography data system.

The pooled egg yolk samples of six per treatment day 28 of the feeding were also used to determine the thiobarbituric acid reactive substance (TBA-RS) value with some modifications to the method of Yagi (1984). About 0.2 g of egg yolk was weighed into a test tube and mixed with 8 mL of 0.17 M H_2SO_4 solution. To the mixture was added 1 mL of 10% phosphotungstic acid with mixing. After standing at room temperature for 5 min, the mixture was centrifuged at 3000*g* for 10 min. The supernatant was discarded, and the sediment was mixed with 2 mL of 0.17 M H_2SO_4 and 0.3 mL of 10% phosphotungstic acid solution. The mixture was centrifuged at 3000*g* for 10 min. The sediment was suspended in 4.0 mL of distilled water and 1.0 mL of thiobarbituric acid (TBA) solution (a mixture of equal volume of 0.67% TBA solution and glacial acetic acid). The reaction mixture was heated at 95 °C for 1 h in a water bath, then cooled with tap water, and shaken vigorously with the addition of 5 mL of *n*-butanol. After 15 min of centrifugation at 3000*g*, the *n*-butanol (top) layer was taken for fluorometric measurement by a Nova spectrofluorometer (Baird Atomic Ltd., Springwood Industries Est., Braintree Essex, England) at 535 nm with an excitation wavelength of 515 nm. 1,1,3,3-Tetramethoxypropane (Fluka Chemical AG, Buchs, Switzerland) was used as the external standard.

Statistical Analyses. Two-way General Linear Models (GLM) of the SAS program were used to analyze the effects of dietary TOC supplementation and time (day of the experiment) on egg yolk TOC content, fatty acid composition, and egg quality. Main effects (TOC and time) were separated by the Student−Newman−Keuls (SNK) multiple-range test. Interaction of TOC by time was compared using the PDIFF function of the SAS program (SAS Institute, 1985). One-way GLM procedure was used to analyze the effects of diet on egg production, feed consumption, preference scores of the panel test, and egg yolk TBA-RS level. Upon significant *F* test ($P < 0.05$), differences among treatments were determined using the SNK multiple-range test.

RESULTS AND DISCUSSION

Tocopherol Contents. Changes in α-, γ-, and δ-tocopherols and total tocopherols in egg yolk with dietary treatments and time of feeding are presented in Figure 1. Significant incorporation of tocopherols into egg yolk was obtained by feeding laying hens a basal diet supplemented with the natural TOC mixture at 200, 400, and 800 mg/kg. The tocopherol contents of egg yolks increased linearly with increasing levels of dietary TOC up to day 8. Egg yolk TOC contents of the supplemented treatments increased with the time up to day 8. At 8 days, contents of α-, γ-, and δ-TOC in egg yolks were 83.62, 7.89, and 1.27 μg/g when no TOC was added, 119.02, 57.89, and 10.48 μg/g when 200 mg/kg of TOC was added, 175.38, 108.71, and 18.27 μg/g when 400 mg/kg of TOC was added, and 234.93, 134.33, and 26.67 μg/g when 800 mg/kg of TOC was added, respectively. As shown in Figure 1, there was no apparent increase in the egg yolk tocopherol contents after day 8 of the feeding.

Tocopherol Composition. The three tocopherol isomers, α, γ, and δ, that exist in the diets were also detected in the eggs. This indicates that the different tocopherol isomers were transferred through the hen's diet. Percentages of α-, γ-, and δ-tocopherols, respectively, accounted for about 10, 60, and 30% of total tocopherol in the tocopherol mixture used in this study. Consequently, with supplemental levels of TOC increasing from 0 to 800 mg/kg diet, dietary γ- and δ-TOC increased from 30.06 and 2.70% to 55.70 and 30.11%, respectively, with a concomitant decrease of α-TOC from 67.24 to 14.19%. However, egg yolk α-TOC still accounted for 57.60% of total TOC, though decreased from 90.96%, with the compensational increase of γ- and δ-TOC from 1.00 and 8.05% to 7.09 and 35.31%, respectively (Figure 2). The compositional differences of the tocopherol isomers in the diets and the corresponding eggs reflect the transfer efficiencies of tocopherol isomers from diets to eggs; that is, α-TOC has a higher efficiency of dietary transfer into eggs than the γ- and δ-tocopherols.

The present consumer concerns on the nutritive value of foods have precipitated interest in the vitamin composition of eggs. The interest in modifying the level of vitamins in eggs now extends beyond production consideration to designing a high-quality food for consumption by health-conscious humans (Naber, 1993). It is well-known that the fat-soluble vitamin content of egg yolk, such as tocopherol, can be manipulated by supplementing the diet with the vitamin (Naber, 1993). The vitamin E content in egg yolk is markedly influenced by the hen's dietary level (Jiang et al., 1994). Up to now, studies on tocopherol enrichment in eggs have mainly focused on α-tocopherol or its esters. The present study clearly shows that not only α-tocopherol but also γ- and δ- tocopherols could be enriched by dietary supplementation, though there were lower dietary transfer efficiencies of γ- and δ-tocopherols to eggs. Piironen et al. (1991) found that transfer efficiencies of the α-tocopherol stereoisomers are proportional to the order of their biological activities. In the present experiment, the higher transfer efficiency of α-tocopherol, relative to those of γ- and δ-tocopherols, may also be due to their differences in biological activityy; that is, the higher the biological activity, the more efficient the dietary transfer to eggs. In experiments with chickens, Hartfiel and Jakobsen (1991) found that tocopherol addition to the diet, derived from natural sources, containing mainly γ-tocopherol had at least the same effect as the addition of α-tocopherol. The higher antioxidative activities of γ- and δ-tocopherols in vitro compared with that of α-TOC (Duithie et al., 1992) indicate their enrichment in $n-3$ fatty acid modified eggs more meaningful.

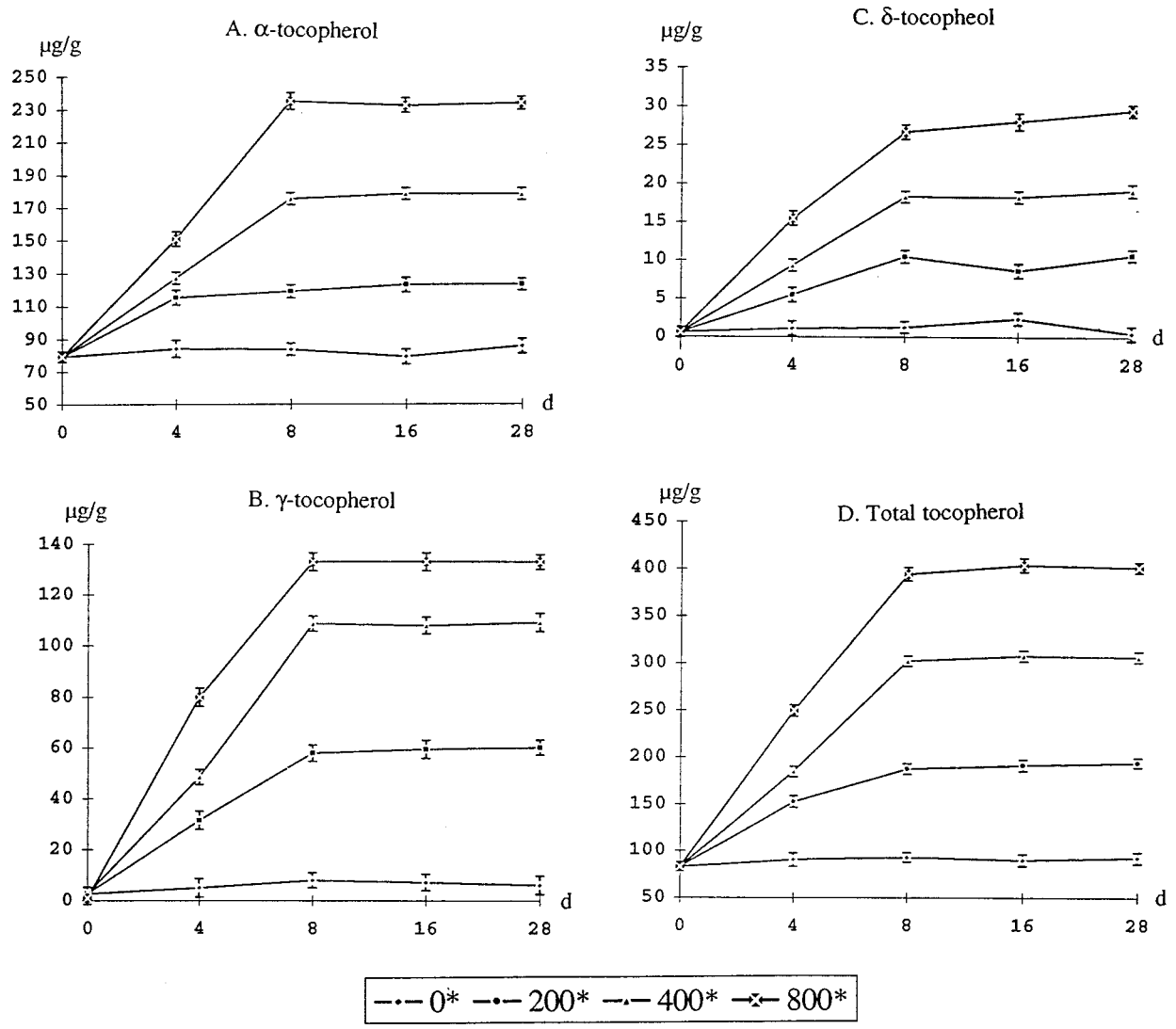

Figure 1. Tocopherol accumulation in egg yolks with time at different levels of tocopherol supplementation.

Fatty Acid Composition. Table 4 shows the partial individual fatty acid profile of yolk lipids after feeding of a basal diet containing 15% full-fat flaxseed and 0.5% menhaden oil supplemented with a natural tocopherol mixture of 0, 200, 400, and 800 mg/kg. C18:3*n*3, C20:5*n*3, C22:5*n*3, and C22:6*n*3 in yolk lipids gradually increased to their maximum level at day 8 of the feeding, whereas C18:1 decreased. However, C20:4*n*6 was not at its maximum value until day 16 of the feeding. As a result, indicated in Table 5, total *n*−3 PUFA in egg yolk increased from 1 to >7%, and monounsaturated fatty acids (MUFA) decreased by ~11.54% from 52 to 46% on average. The ratio of *n*−6 to *n*−3 PUFA decreased from 10:1 on day 0 to 1.6:1 by day 8. Saturated fatty acids in yolk lipids also decreased with time of feeding of the experimental diets, although the trend was not as apparent as those of *n*−3 PUFA and MUFA.

Cruickshank (1934) first reported that dietary unsaturated fatty acids affect the proportion of fatty acids present in the yolk. Since the chicken is monogastric, much of the dietary fat, especially unsaturated fats, is directly assimilated with minimal modification. Varying the type and amount of dietary unsaturated fat dramatically modifies the fatty acid composition of lipids in the hen egg yolk (Sim et al., 1973). Because of the unique merits of flaxseed, it was considered to be one of the most suitable sources for enriching poultry products, including eggs, with *n*−3 fatty acids. The present study agrees with the previous papers (Cherian and Sim, 1991; Scheideler et al., 1994), where *n*−3 fatty acids in egg yolks could be enriched by feeding flaxseed to laying hens. As a consequence, the eggs designed in such a way are more favorable for human consumption.

On the other hand, no significant effects of dietary TOC supplementation on fatty acid composition of egg yolk lipids were noted in the present study. However, significant differences in TBA-RS values of egg yolks among different treatments were observed as shown in Figure 3, which will be discussed later.

This study shows that the fatty acid profile of egg yolk becomes relatively constant by day 8 with the exception of C20:4*n*6, which stabilized by day 16 of the feeding. This is similar to the findings of Sim and Qi (1995), who suggested that a plateau of *n*−3 PUFA levels in the

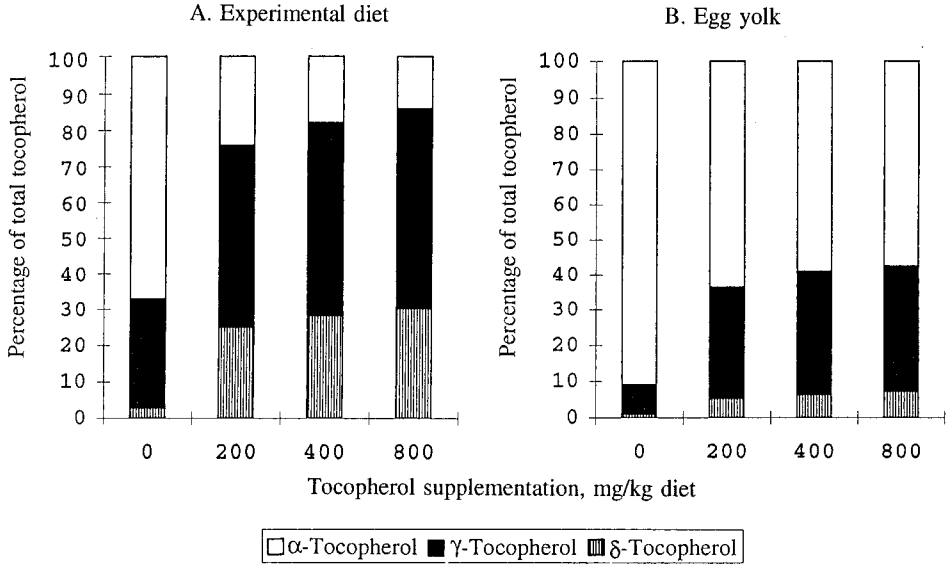

Figure 2. Comparison of tocopherol composition of diets and eggs.

Table 4. Changes in Individual Fatty Acid Composition[a] (Percent) of Egg Yolk Lipid after Feeding of the Experimental Diet

fatty acid	TOC[b]	time of feeding 0 days	4 days	8 days	16 days	28 days	SEM
C18:1	0	47.44[a]	46.35[a]	42.68[b]	42.42[b]	41.98[b]	0.81
	200	49.25[a]	45.47[b]	42.81[c]	43.31[bc]	42.07[c]	0.73
	400	49.11[a]	48.00[a]	42.54[b]	43.14[b]	41.33[b]	0.78
	800	47.77[a]	45.10[ab]	41.20[b]	44.01[b]	42.09[b]	1.05
C18:2*n*6	0	9.83[a]	9.59[a]	10.76[a]	9.56[a]	11.02[a]	0.34
	200	9.63[a]	10.95[a]	10.94[a]	10.11[a]	11.35[a]	0.48
	400	9.52[ab]	8.88[b]	10.61[a]	9.99[ab]	10.90[a]	0.40
	800	9.68[a]	10.43[a]	11.07[a]	10.11[a]	11.43[a]	0.44
C18:3*n*3	0	0.43[c]	2.09[b]	4.46[a]	4.71[a]	4.94[a]	0.25
	200	0.37[c]	2.13[b]	4.79[a]	4.96[a]	5.16[a]	0.31
	400	0.41[c]	1.68[b]	4.39[a]	4.55[a]	4.79[a]	0.19
	800	0.38[c]	1.88[b]	4.13[a]	4.82[a]	4.87[a]	0.21
C20:4*n*6	0	1.54[a]	1.53[a]	1.01[b]	0.79[c]	0.77[c]	0.03
	200	1.63[a]	1.60[a]	1.09[b]	0.87[c]	0.75[c]	0.05
	400	1.54[a]	1.50[a]	1.20[b]	0.78[c]	0.72[c]	0.08
	800	1.56[a]	1.62[a]	1.15[b]	0.89[c]	0.78[c]	0.05
C20:5*n*3	0	ND[c]	ND	0.25[a]	0.25[a]	0.25[a]	0.02
	200	ND	ND	0.23[a]	0.26[a]	0.26[a]	0.01
	400	ND	ND	0.27[a]	0.26[a]	0.25[a]	0.02
	800	ND	ND	0.22[a]	0.23[a]	0.25[a]	0.01
C22:5*n*3	0	ND	0.20[b]	0.34[a]	0.35[a]	0.31[a]	0.02
	200	ND	0.21[b]	0.36[a]	0.34[a]	0.33[a]	0.02
	400	ND	0.16[b]	0.33[a]	0.33[a]	0.33[a]	0.03
	800	ND	0.16[b]	0.33[a]	0.30[a]	0.28[a]	0.02
C22:6*n*3	0	0.67[c]	1.41[b]	2.33[a]	2.41[a]	2.27[a]	0.08
	200	0.74[c]	1.36[b]	2.26[a]	2.29[a]	2.22[a]	0.07
	400	0.66[c]	1.36[b]	2.41[a]	2.23[a]	2.23[a]	0.18
	800	0.71[c]	1.34[b]	2.27[a]	2.29[a]	2.17[a]	0.08

[a] Means in a row with no common superscript differ significantly($P < 0.05$). Fatty acid composition of different levels of tocopherol supplementation at given day are not significantly different ($P > 0.05$). [b] Tocopherol supplemental level (mg/kg of diet). [c] ND, nondetectable.

Table 5. Changes in the Fatty Acid Composition (Percent)[a] of Different Groups in Egg Yolk Lipid after Feeding of the Experimental Diet

fatty acid	TOC[b]	time of feeding 0 days	4 days	8 days	16 days	28 days	SEM
SFA[c]	0	36.10[a]	34.64[b]	33.98[b]	34.78[b]	34.54[b]	0.33
	200	34.56[a]	34.00[a]	33.40[a]	33.43[a]	34.54[a]	0.39
	400	34.73[a]	34.45[a]	34.26[a]	34.14[a]	35.06[a]	0.42
	800	36.05[a]	35.10[a]	34.62[a]	33.61[b]	33.89[b]	0.64
MUFA[d]	0	51.45[a]	50.30[a]	46.38[b]	47.03[b]	46.07[b]	0.39
	200	53.06[a]	49.54[b]	47.30[c]	46.94[c]	45.90[c]	0.63
	400	53.13[a]	51.76[a]	46.55[b]	47.33[b]	45.72[b]	0.77
	800	51.61[a]	49.26[ab]	45.15[b]	48.02[b]	46.33[b]	0.95
*n*3[e]	0	1.10[c]	3.70[b]	7.86[a]	7.46[a]	7.44[a]	0.16
	200	1.12[c]	3.70[b]	7.64[a]	7.85[a]	7.97[a]	0.35
	400	1.07[c]	3.19[b]	7.39[a]	7.37[a]	7.61[a]	0.34
	800	1.09[c]	3.38[b]	7.64[a]	6.94[a]	7.57[a]	0.23
*n*6[f]/*n*3	0	10.32[a]	3.14[b]	1.50[c]	1.42[c]	1.60[c]	0.12
	200	10.10[a]	3.44[b]	1.59[c]	1.52[c]	1.45[c]	0.19
	400	10.34[a]	3.29[b]	1.62[c]	1.54[c]	1.50[c]	0.11
	800	10.32[a]	3.57[b]	1.61[c]	1.63[c]	1.62[c]	0.12

[a] Means in a row with no common superscript differ significantly($P < 0.05$). Fatty acid composition of different levels of tocopherol supplementation at given day are not significantly different ($P > 0.05$). [b] Tocopherol supplemental level (mg/kg of diet). [c] SFA, total saturated fatty acids, including C14:0, C16:0, and C18:0. [d] MUFA, total monounsaturated fatty acids, including C16:1, C18:1, and C20:1*n*9. [e] *n*3, total *n*3 polyunsaturated fatty acids, including C18:3*n*3, C20:5*n*3, C22:5*n*3, and C22:6*n*3. [f] *n*6, total *n*6 polyunsaturated fatty acids, including C18:2*n*6 and C20:4*n*6.

egg yolk could be reached within 9−12 days after feeding of the experimental diet. Cherian and Sim (1991) and Maurice (1994), respectively, implied that ~2 weeks is required to bring egg lipid into equilibrium with dietary lipids. However, Lin et al. (1991) indicated that it takes ≈1 month for the fatty acid composition of eggs to stabilize after hens have been fed the fatfree diet with oils of widely divergent of *n*−6 and *n*−3 fatty acid contents. The reasons for these differences are not clear. It may be due to the differences of diets and birds used in their studies. It is reasonable that tocopherols and fatty acids share the same deposition pattern because they are both fat-soluble substances.

Egg Production and Quality. Dietary TOC supplementation had no significant effect on egg production, feed consumption, and egg quality (Table 6), which generally agrees with the work of Jiang et al. (1994), who reported no significant differences in egg production and egg weight when laying hens were fed a basal diet supplemented with 50, 100, 200, and 400 mg of DL-α-tocopherol acetate/kg. However, they found feed consumption decreased with their highest level of vitamin E supplementation. Yolk weight decreased ($P < 0.05$) and Haugh units increased ($P < 0.05$) after feeding of the *n*−3 fatty acid enriched diets (Table 7). The decrease of yolk weight, after laying hens were fed a

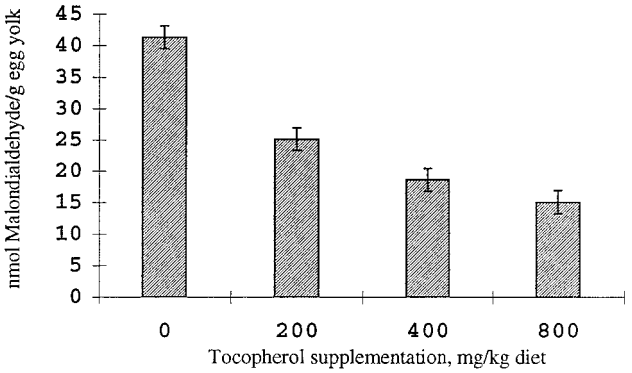

Figure 3. Effect of tocopherol supplementation on lipoperoxide level (mean with SE bar) in egg yolk.

Table 6. Effects of Dietary Tocopherol Supplementation on Egg Production, Feed Consumption, and Egg Quality[a]

tocopherol supplementation, mg/kg of diet	productivity, %	daily feed consumption, g/bird	egg wt, g	yolk wt, g	Haugh unit
0	91.87	116.5	60.07	15.87	89.43
200	90.50	114.9	59.19	15.96	89.41
400	93.63	118.5	57.15	15.66	89.77
800	91.50	114.8	58.74	15.39	91.40
SEM	2.07	2.83	0.55	0.17	0.76

[a] Means in a column have no significant difference ($P > 0.05$).

Table 7. Changes in Egg Quality[a] after Feeding of the Experimental Flaxseed-Based Diets

time of feeding, days	egg wt, g	yolk wt, g	Haugh unit
0	58.49[a]	16.46[a]	79.42[c]
4	58.63[a]	15.89[b]	89.36[b]
8	59.13[a]	15.43[b]	94.85[a]
16	58.58[a]	15.67[b]	91.56[b]
28	58.89[a]	15.50[b]	1.31
SEM	0.64	0.19	1.31

[a] Means in a column with no common superscript differ significantly ($P < 0.05$).

Table 8. Panel Test Result[a] of Scrambled Eggs from Laying Hens Fed Different Levels of Tocopherols

	tocopherol supplementation				
parameter	0 mg/kg of diet	200 mg/kg of diet	400 mg/kg of diet	800 mg/kg of diet	SEM
flavor[b]	2.49	2.73	2.55	3.04	0.27
taste[c]	2.94	3.07	2.93	3.63	0.28

[a] Values expressed as mean of 20 observations and in a row have no significant difference ($P > 0.05$). [b] Flavor score: 1, no off-flavor; 5, strong off-flavor. [c] Taste score: 1, dislike extremely; 5, like extremely.

diet containing flaxseed, was also reported by Scheideler et al. (1994). There was no significant change in egg weight with time of the feeding ($P > 0.05$). No significant differences were observed in taste and flavor measurement of scrambled egg samples among treatments (Table 8). Scheideler et al. (1994) also reported that vitamin E had no significant effects on sensory analysis of *n*−3 fatty acid enriched eggs. With increasing levels of TOC supplementation in laying hen diets from 0 to 800 g/kg of diet, TBA-RS values significantly decreased from 41.32 to 18.57 nmol of malondialdehyde/g of egg yolk ($P < 0.05$). However, there were no significant differences ($P > 0.05$) between the 400 and 800 mg of TOC/kg supplemented diets (Figure 3). This indicates that lipid stability could be improved by increasing tocopherol contents of eggs. It also shows the TBA-RS assay employed in this study is sensitive in determining the lipid stability of egg yolks.

In conclusion, eggs can be feasibly enriched with natural tocopherols as well as with *n*−3 fatty acids, thus making eggs more favorable to health-conscious consumers. The lipid stability of *n*−3 fatty acid modified eggs could be improved by increasing their tocopherol concentrations through dietary modification.

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