Effects of Dietary Vitamin E on the Quality of Table Eggs Enriched with n-3 Long-Chain Fatty Acids[1]

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ABSTRACT Because of the proposed cardioprotective benefits of n-3 fatty acids and vitamin E, a trial was carried out to investigate the possibility of enriching eggs with n-3 fatty acid and vitamin E added to the hen's diet. One hundred ninety-two Hy-Line Brown hens, 39-wk-old, were divided into eight groups: four groups received the basal diet supplemented with 3% lard and four doses of dl-α-tocopheryl acetate (0, 50, 100, and 200 ppm), whereas the diets of the other groups were supplemented with 3% of fish oil and the same doses of vitamin E. The performances of the hens and egg weights were not affected either by the type of lipid supplement or by the vitamin level. The treatment with fish oil caused a dramatic increase ($P < 0.01$) of all n-3 fatty acids of the yolk, particularly EPA (19.53 vs. 0.74 mg/egg) and DHA (143.70 vs. 43.66 mg/egg), and an appreciable decrease of arachidonic acid (25.54 vs. 67.72 mg/egg). The different levels of dietary vitamin E slightly affected the fatty acid composition of the yolk. Yolk α-tocopherol increased linearly as dietary dl-α-tocopheryl acetate increased ($P < 0.01$) from the control level of 90.93 μg/g of yolk to 313.84 μg/g of yolk when 200 ppm were added to the hen diets. Twenty-eight days of storage at room temperature (20 to 25 C) did not alter the yolk fatty acid profile, and, moreover, the levels of vitamin E remained still very close to those observed in fresh egg.

(*Key words*: egg, fish oil, lard, vitamin E, n-3 fatty acids)

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INTRODUCTION

Hen eggs contain many essential nutrients for chick embryo development and are considered an excellent food for humans. Eggs are a good source of proteins, vitamins, and lipids of high quality such as phospholipids and polyunsaturated fatty acids. Naber (1979) and Stadelman and Pratt (1989) have described the dietary factors influencing the nutritive value of eggs. Whereas amino acids and total protein are hardly affected by dietary treatments, minerals, fat-soluble vitamins, and lipids, fatty acids are easily modified through feed manipulation. The fatty acid profile of the egg yolk is clearly affected by the fatty acid profile of hen diets, particularly polyunsaturated acids. However, saturated fatty acids are almost unresponsive to dietary strategies (Naber, 1979). The n-3 and n-6 fatty acids play an important role in human and animal bodies. Starting from linoleic (n-6) and linolenic (n-3) acids, n-6 and n-3 series of long-chain polyunsaturated fatty acids (PUFA) are synthesized. In turn, the PUFA are converted to eicosanoids (prostaglandins, tromboxane, and leucotrienes), substances involved in many physiological functions. Eicosapentaenoic acid (EPA; n-3) and docosahexaenoic acid (DHA; n-3) play an important role in reducing blood viscosity and pressure, platelet aggregation, cardiac arrhythmia, and plasma triglyceride level and exert, therefore, a beneficial effect on immune system and in the prevention of cardiovascular diseases (Saynor et al., 1984; Li and Steiner, 1990; Farrell, 1994). These findings have stimulated interest in increasing the levels of the above fatty acids in animal foods by adding fish oil, marine microalgae, or flax seed, common sources of n-3 fatty acids, to the diet. By supplementing hen diets with 3 to 7% fish oil, eggs containing more than 200 mg of n-3 were obtained (Hargis et al., 1991; Farrell, 1994). Huang et al. (1990) and Meluzzi et al. (1997a,b) found that the n-3 content of the yolk is positively related to a dietary addition of fish oil. It is also possible to increase the n-3 content of the yolk by enriching hen diets with flax seed (Caston and Leeson, 1990; Cherian and Sim, 1991) or flax oil (Piva et al., 1995), but the deposition in the yolk concerned mainly linolenic acid (LNA) rather than EPA or DHA. However, long-chain fatty acids are more prone to oxidation because of their double bonds

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Abbreviation Key: DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; LNA = linolenic acid; PUFA = polyunsaturated fatty acids.

and may confer this undesirable trait to the n-3 enriched products. Scheideler et al. (1997) found an increase of oxidation products, measured as TBARs, in eggs of hens fed flax seed. These oxidative phenomena can be prevented or limited by enriching the eggs with some active antioxidants such as vitamin E and β-carotene. These compounds have been shown to have a positive influence on immune system and a protective effect against some human cancers (Bendich and Olson, 1989). In addition, these enriched eggs are a useful contribution to meet the human vitamin requirements. By manipulating hen diets, Jiang et al. (1994) obtained eggs with high levels of vitamin E, retinol, and β-carotene. Some authors have studied the combined influence of dietary vitamin E and vegetable sources of n-3 fatty acids, such as flax seed, on sensory quality of the eggs (Leeson et al., 1998) and egg production (Scheideler and Froning, 1996). However, there is lack of information about the dietary use of vitamin E associated with fish oil. We have tried to fill this gap. In effect, the aim of the present research was to investigate the possibility of fortifying eggs both with n-3 fatty acids and vitamin E added to hen diets and to determine whether the vitamin E addition is dose related and is influenced by the type of lipid used (lard or fish oil). Moreover, the effects of egg storage on fatty acid and vitamin profile were evaluated as well as laying performance.

TABLE 1. Composition and calculated analysis of the basal diet

Ingredients and analysis	Percentage
Yellow corn	56.50
Soybean meal (44% CP)	20.53
Meat meal (48% CP)	5.17
Soft wheat bran	4.50
Sunflower seed meal solvent extracted (40% CP)	3.50
Calcium carbonate	8.13
Dicalcium phosphate	0.67
Sodium bicarbonate	0.03
Vitamin-mineral premix[1]	0.40
Salt	0.25
Choline	0.09
DL-methionine	0.22
Calculated analysis	
Metabolizable energy (kcal/kg)	2,633
Crude protein	18.00
Crude fat	3.12
Crude fiber	3.63
Lysine	0.88
Methionine plus cystine	0.76
Calcium	3.94
Available phosphorus	0.38
Linoleic acid	1.24

[1]Vitamin and mineral premix provided the following per kilogram of feed: vitamin A, 11,000 IU (retinyl acetate); cholecalciferol, 3,000 IU; vitamin E, 40 mg (dl-α-tocopheryl acetate); vitamin K (menadione sodium bisulfate), 3.3 mg; thiamin, 2.5 mg; riboflavin, 6 mg; pantothenic acid, 11 mg; niacin, 30 mg; vitamin C, 100 mg; vitamin B_{12}, 0.02 mg; biotin, 0.05 mg; pyridoxine, 4 mg; folic acid, 1 mg; ethoxyquin 100 mg; manganese, 15 mg; zinc, 50 mg; iron, 30 mg; copper, 6 mg; iodine, 1.5 mg; selenium, 0.2 mg; and cobalt, 0.2 mg.

Assayed concentration of α-tocopherol for the different diets were as follows. For the lard diets—E 0, 33.20 ppm; E 50, 94.04 ppm; E 100, 158.57 ppm; and E 200, 272.95 ppm; and for the fish oil diets—E 0, 43.88 ppm; E 50, 81.31 ppm; E 100, 140.38 ppm; and E 200, 259.94 ppm.

MATERIALS AND METHODS

Animal Care and Dietary Treatments

One hundred ninety-two Hy-Line Brown hens, 39 wk old, were randomly housed in laying cages (four birds per cage) in a windowed poultry house with a light regimen of 16 h light:8 h darkness. Eight groups of 24 hens (six replicates per group) were randomly assigned to one of eight dietary treatments. Four groups received the basal diet (Table 1) supplemented with 3% lard and four levels of dl-α-tocopheryl acetate[3] (0, 50, 100, and 200 ppm), whereas the diets of the other four groups were supplemented with 3% fish oil[4] (herring + redtoby) and the same amounts of the vitamin. The vitamin E content and the fatty acid composition of the eight diets were regularly checked. The mean values are reported in Tables 1 and 2. Feed and water were provided ad libitum. Feed intake was measured weekly, whereas egg production and weight were recorded on a daily basis during the 4-wk trial. After 2 and 4 wk of treatment, four eggs per replicate were collected and individually weighed, and their yolks were pooled. The pooled yolks were stored at –40 C until they were analyzed for content of vitamin E and fatty acid composition. After 4 wk of treatment, a batch of 24 eggs per group was stored at room temperature (20 to 25 C) for 28 d and then were subjected to the same sampling procedure as above.

Analysis of Samples

Fatty Acids. Total lipids were extracted with chloroform:methanol (2:1 vol/vol) from 0.8 g of yolk, according to the procedure of Folch et al. (1957). Total lipids were converted to fatty acid methyl esters by the method of Christopherson and Glass (1969). A Carlo Erba HRGC 5160 with a DP integration system, equipped with a SP 2340 capillary column,[5] was used to determine the fatty acid composition. The concentration of each fatty acid was calculated using the heptadecanoic acid ($C_{17:0}$) as an internal standard. Identification of individual fatty acids was made using PUFA-2[6] fatty acid methyl ester standards to establish the relative retention time.

Vitamin E. One gram of egg yolk was extracted and saponified with 30 mL of ethanol: 50% KOH (1:1 vol/vol) and was kept overnight in the dark under nitrogen gas at room temperature. Twenty milliliters of hexane plus butylated hydroxytoluene (1 g/L) and 20 mL of KH_2PO_4 were added to the flask and carefully mixed for 5 min. After standing 1 h, 5 mL of the upper organic solvent layer was drawn and evaporated with nitrogen

[3]Rovimix 50 supplied by Hoffmann La Roche, Inc., 4070 Basel, Switzerland.
[4]FF Golden Oil supplied by Fiskeres, 9990 Skagen, Denmark.
[5]Carlo Erba, II Strasla Rivoltana, 20090 Rodono (MI), Italy.
[6]PUFA-2, catalog No. 1081. Matreya Inc., Pleasant Gap, PA 16823.

TABLE 2. Fatty acid profile of the diets (%)

	C 14:0	C 16:0	C 16:1 n-9, -7	C 18:0	C 18:1 n-9, -7	C 18:2 n-6	C 18:3 n-3	C 20:4 n-6	C 20:5 n-3	C 22:5 n-3	C 22:6 n-3	Other	Total n-3	Lipid %
Lard diets	1.15	20.26	1.72	7.85	33.18	33.20	1.56	0.18	0.00	0.31	0.00	0.59	1.87	5.05
Fish oil diets	4.19	16.16	4.62	3.60	19.27	28.80	1.62	0.75	8.44	1.20	5.53	5.81	16.79	4.97

gas. The dried material was recovered with 1 mL of ethanol, and 10 μL was injected into a Hewlett Packard HPLC (series 1090), fitted with a Machery-Nagel (C 18-5) column.[7] Samples were eluted with a solution of methanol and water (97:3 vol/vol) and run isocratically at a flow of 1.5 mL/min. α-Tocopherol was read at a wavelength of 292 nm and was quantitatively measured using a solution of α-tocopherol[8] as an external standard.

Statistical Analysis

All data were analyzed by ANOVA using the general linear model (GLM) procedures of the SAS Institute (SAS Institute, 1985). The fixed effects were the amounts of vitamin E added (0, 50, 100, or 200 mg/kg) and the type of lipid supplement (lard or fish oil). The differences between means were determined using the Student Newman-Keuls test.

RESULTS AND DISCUSSION

Production and Egg Parameters

The performance of the hens was not significantly affected by the vitamin dose or by the type of lipid supplement to the diet (Table 3). These data agree with the results of our previous research (Meluzzi et al., 1997b) and with data of Hargis et al. (1991), who reported that dietary menhaden oil (3%) did not modify egg production and weight. The yolk percentage, at 2 and 4 wk of treatment, in groups supplemented with fish oil was not significantly different from nonsupplemented groups (Figure 1). This result is in contrast with those of Van Elswyk et al. (1994) who found significant differences in yolk and egg weight using menhaden oil and animal-vegetable oil. Scheideler and Froning (1996) found a decrease in the yolk size of eggs from hens fed from 5 to 15% of flax seed and 1.5% of fish oil and ascribed the results to the effect of the long-chain fatty acids on the estrogen activity of the hen. Whitehead et al. (1993) observed that the decrease in egg size was accompanied by a decrease of plasma estradiol concentration and postulated a nutritional control of the dietary long-chain fatty acids on the hormonal metabolism of the birds.

Yolk Fatty Acid Composition

Two weeks of treatment with fish oil rich in EPA ($C_{20:5}$) and DHA ($C_{22:6}$) were enough to modify the fatty acid profile of the yolk. By using fish oil in comparison with lard, we observed a dramatic increase in all n-3 fatty acids (Table 4). In detail, LNA ($C_{18:3}$), EPA, docosapentaenoic acid (DPA) ($C_{22:5}$), and DHA increased respectively by 30% and by 10-, 3- and 2.5-fold, so that the total n-3 fatty acids reached 180 mg per egg in fish groups vs. 68 mg/egg in lard groups ($P < 0.01$). The DHA was the most prevalent n-3 long-chain fatty acid of the yolk, but low amounts of LNA, EPA, and DPA were detected. The latter acids are precursors of DHA. They are normally converted to DHA by chain reactions of desaturation and elongation. Hargis et al. (1991) in response to a 2-wk dietary treatment with menhaden oil (3%), obtained eggs containing an average of 190 mg of n-3 fatty acids, of which EPA and DHA composed approximately 89% of the total. Similar results were reported by Meluzzi et al. (1997a,c) by adding refined fish oils at the rate of 2, 3, or 4% to the diet. The arachidonic acid ($C_{20:4}$, n-6) content was significantly lower in fish groups (about 40% less than in the lard groups). This phenomenon is probably due to the greater utilization of Δ-6-desaturase in the n-3 fatty acid pathway with respect to the n-6 pathway, as this enzyme acts in both pathways. High

TABLE 3. Analysis of variance of dietary effects on the performance of hens over 4 wk of treatment

Treatments	Egg weight	Daily feed intake	FCR[1]	Egg production
	(g/egg)	(g/hen/d)	(kg/kg)	(% hen-day)
Lipid supplement (LS)				
Lard	63.26	121.42	2.34	83.38
Fish oil	63.07	121.41	2.33	83.78
Vitmain E dose (VD)				
0	62.97	123.63	2.49	80.95
50	63.23	122.79	2.38	82.54
100	64.08	119.22	2.24	83.93
200	62.37	120.03	2.23	86.92
Error mean square	9.90	63.53	0.11	0.02
df	40	40	40	40
LS	NS	NS	NS	NS
VD	NS	NS	NS	NS
LS*VD	NS	NS	NS	NS

[1]FCR = feed conversion rate.

[7]Hewlett Packard, via G. Di vittoris, 9, 20063 Cernusco sul Naviglis (MI), Italy.

[8]α-Tocopherol (T-3251) supplied by Sigma Chemical Co., St. Louis, MO 63178-9916.

TABLE 4. Analysis of variance of fatty acid profile of egg yolk over 2 wk of treatment (mg per 63 g egg)

	C 14:0	C 16:0	C 16:1 n-9, -7	C 18:0	C 18:1 n-9, -7	C 18:2 n-6	C 20:4 n-6	C 18:3 n-3	C 20:5 n-3	C 22:5 n-3	C 22:6 n-3	Total n-3
Lipid suppplement (LS)												
Lard	15.03^{B}	1,002.36^{A}	122.28	331.75^{A}	1,752.68^{A}	534.53^{A}	65.23^{A}	9.67^{B}	1.74^{B}	3.96^{B}	52.67^{B}	68.04^{B}
Fish oil	20.78^{A}	950.46^{B}	128.70	307.45^{B}	1,462.42^{B}	483.75^{B}	25.63^{B}	12.54^{A}	17.24^{A}	15.71^{A}	135.08^{A}	180.58^{A}
Vitamin E dose (VD)												
0	17.02	956.74	122.33	307.73^{b}	1,559.06	497.81	42.75^{Bb}	10.82	9.70	10.02	95.51	126.05^{ab}
50	18.65	998.68	131.65	327.49^{a}	1,647.10	513.46	44.90^{ABb}	11.31	10.94	10.33	98.78	131.36^{a}
100	17.72	970.93	123.17	321.74^{ab}	1,606.92	515.82	44.79^{ABb}	11.35	8.72	9.63	93.65	123.38^{ab}
200	18.23	979.28	124.89	321.45^{ab}	1,617.72	509.49	49.26^{Aa}	10.92	8.61	9.34	87.58	116.44^{b}
Error mean square	3.14	3,326.06	184.72	314.97	9,183.27	1,044.06	16.08	1.2551	13.42	6.42	112.18	183.04^{ab}
df	40	40	40	40	40	40	40	40	40	40	40	40
LS	<0.01	<0.01	NS	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
VD	NS	NS	NS	<0.05	NS	NS	<0.01	NS	NS	NS	NS	NS
LS*VD	<0.01	NS	NS	NS	NS	<0.01	<0.01	<0.01	NS	NS	NS	<0.05

[a,b]Values in the same column with no common superscript differ significantly ($P < 0.05$).
[A,B]Values in the same column with no common superscript differ significantly ($P < 0.01$).

concentrations of dietary n-3 fatty acids reduce the activity of the enzyme in the n-6 pathway and the conversion of linoleic into arachidonic acid. The decrease in arachidonic acid content could be important for human health, as this acid is a precursor of some proinflammatory eicosanoids (British Nutrition Foundation, 1992). Myristic, palmitic, oleic, and linoleic acids were significantly ($P < 0.01$) influenced by fish oil supplementation (Table 4). The different amounts of dietary vitamin E slightly affected the fatty acid composition of the yolk (Table 4). By analyzing the data separating the groups receiving lard from those receiving fish oil (Figure 2), we observed that the highest level of vitamin E (200 ppm) significantly reduces the total n-3 content of yolk only in lard groups whose diet has a lower content of n-3 fatty acids. Atkin-

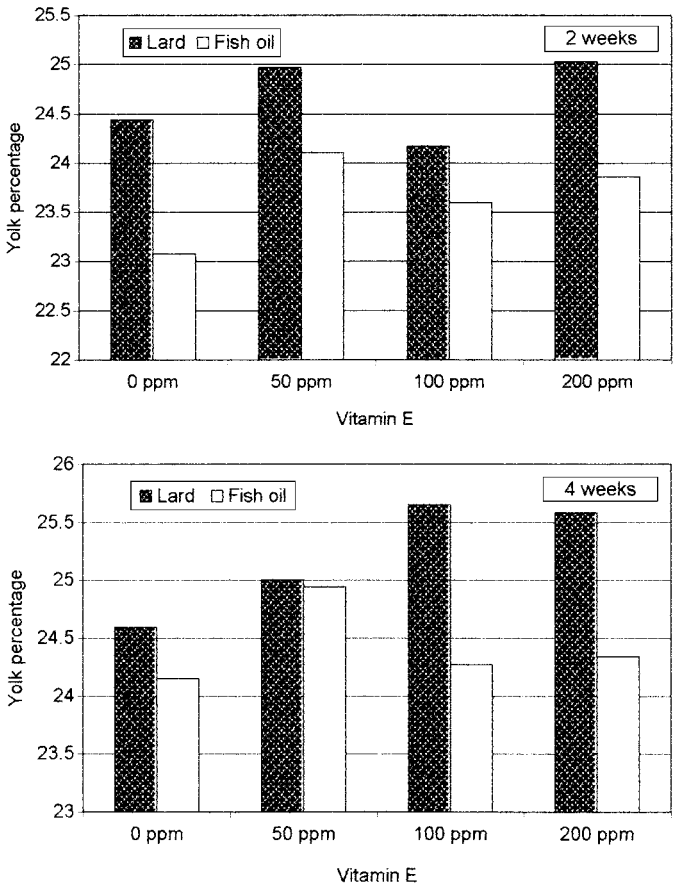

FIGURE 1. Yolk percentage after 2 and 4 wk of treatment.

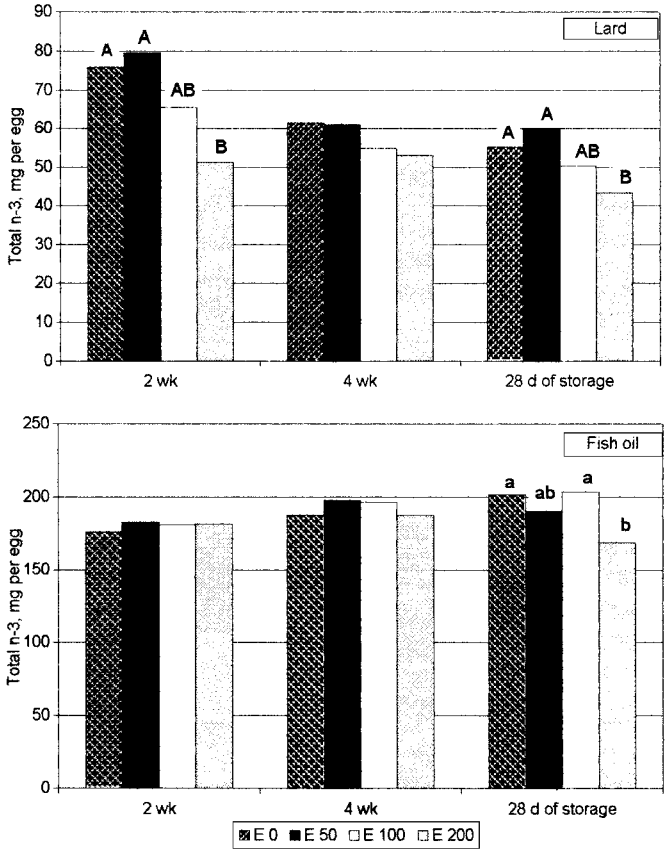

FIGURE 2. Total n-3 fatty acid of eggs (mg per 63 g egg). Bars with different letters are significantly different at $P < 0.01$ if capital letters and $P < 0.05$ if small letters.

TABLE 5. Analysis of variance of fatty acid profile of egg yolk over 4 wk of treatment (mg per 63 g egg)

	C 14:0	C 16:0	C 16:1 n-9, -7	C 18:0	C 18:1 n-9, -7	C 18:2 n-6	C 20:4 n-6	C 18:3 n-3	C 20:5 n-3	C 22:5 n-3	C 22:6 n-3	Total n-3
Lipid suppplement (LS)												
Lard	16.61^{B}	1,079.16^{A}	135.75	351.55^{A}	1,853.10^{A}	552.95^{A}	67.72^{A}	10.16^{B}	0.74^{B}	3.09^{B}	43.66^{B}	57.65^{B}
Fish oil	22.66^{A}	997.76^{B}	142.59	333.35^{B}	1,514.38^{B}	487.69^{B}	25.54^{B}	13.32^{A}	19.53^{A}	16.03^{A}	143.70^{A}	192.58^{A}
Vitamin E dose (VD)												
0	18.43^{b}	1,019.23	132.79	338.68	1,637.21	509.74	42.89	10.75	9.86	10.98	93.15	124.74
50	21.29^{a}	1,087.84	149.31	352.22	1,747.34	533.86	48.04	12.52	10.13	9.26	97.52	129.43
100	19.72^{ab}	1,034.55	139.27	343.29	1,692.07	525.47	47.56	12.14	10.30	9.24	94.16	125.83
200	18.91^{ab}	1,012.21	135.46	335.61	1,658.33	512.21	48.02	11.56	10.24	8.77	89.90	120.47
Error mean square	5.84	8,710.68	340.16	503.43	18,804.1	1,391.59	97.00	3.05	7.43	9.17	135.66	276.12
df	40	40	40	40	40	40	40	40	40	40	40	40
LS	<0.01	<0.01	NS	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
VD	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LS*VD	<0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

^{a,b}Values in the same column with no common superscript differ significantly ($P < 0.05$).
^{A,B}Values in the same column with no common superscript differ significantly ($P < 0.01$).

son et al. (1972) also observed a reduction in EPA and DHA contents of the tissue associated with α-tocopherol treatment.

Over 4 wk of treatment, the previous remarks about fatty acids were confirmed, and the increase of total n-3 fatty acids was more evident in fish oil groups (192 vs. 57 mg/egg). No appreciable differences emerged regarding the effect of dietary vitamin dose on the fatty acids of the yolk (Table 5).

As for the influence of the lipid supplementation, eggs stored 28 d at room temperature (20 to 25 C) showed a fatty acid composition similar to that observed in fresh eggs over 4 wk of treatment (Table 6). Indeed the egg, being a closed system, is very resistant to lipid oxidation because of its natural antioxidant constituents such as vitamin E, avidin, and phosphatine (Scheideler et al., 1997). In the stored eggs with the highest level of vitamin E, the yolk contents of total n-3 and, particularly, of DHA were reduced significantly (about 18%) (Table 6). This reduction could be ascribed to a pro-oxidant effect rather than an antioxidant effect of vitamin E when used at a very high concentration, as suggested by Fennema (1987). According to Leeson et al. (1998) the development of off-flavors from the eggs laid by hens fed high levels of vitamin E and n-3 fatty acids is due to the pro-oxidant effect of the vitamin.

Vitamin E

Table 7 shows data on α-tocopherol content of egg yolk. The type of the dietary lipid supplement did not significantly influence the vitamin E amounts. The vitamin E level remained constant at approximately 3 mg per egg during our study. However, in all fish oil groups a lower content of vitamin E was observed, compared with lard groups. Lynch (1994) also reported that the presence of PUFA reduces tocopherol absorption from the intestine. Also Miller and Huang (1993) reported that breast and thigh vitamin E content are reduced by dietary fish oil. By comparison of the data in Table 7 with those of Figure 2, we could claim that high levels of dietary vitamin E associated with low levels of n-3

TABLE 6. Fatty acid profile of egg yolk over 4 wk of treatment and after 28 d of storage (mg per 63 g egg)

	C 14:0	C 16:0	C 16:1 n-9, -7	C 18:0	C 18:1 n-9, -7	C 18:2 n-6	C 20:4 n-6	C 18:3 n-3	C 20:5 n-3	C 22:5 n-3	C 22:6 n-3	Total n-3
Lipid suppplement (LS)												
Lard	15.68^{B}	1,023.11	128.34	339.52^{a}	1,858.61^{A}	552.50^{A}	69.43^{A}	9.37^{B}	0.00^{B}	0.71^{B}	42.10^{B}	52.19^{B}
Fish oil	22.38^{A}	998.48	146.58	323.29^{b}	1,568.52^{B}	482.36^{B}	24.70^{B}	13.04^{A}	19.29^{A}	15.83^{A}	144.16^{A}	191.29^{A}
Vitamin E dose (VD)												
0	18.61	1,029.46	135.96	331.94	1,714.12	514.82	47.52	11.19	9.88	9.60	97.99^{A}	128.65^{A}
50	19.89	1,026.93	142.73	332.19	1,734.26	508.96	46.21	11.90	9.19	8.18	95.89^{A}	125.16^{A}
100	18.66	1,002.43	136.50	334.54	1,723.07	495.23	46.86	10.96	9.31	8.67	98.00^{A}	126.94^{A}
200	18.95	984.37	134.64	326.89	1,682.80	490.66	47.65	10.79	8.20	6.62	80.59^{B}	106.20^{B}
Error mean square	3.20	3,625.73	192.95	570.89	11,709.03	543.42	17.66	1.75	3.13	7.68	123.78	176.85
df	40	40	40	40	40	40	40	40	40	40	40	40
LS	<0.01	NS	NS	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
VD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.01	<0.01
LS*VD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.05

^{a,b}Values in the same column with no common superscript differ significantly ($P < 0.05$).
^{A,B}Values in the same column with no common superscript differ significantly ($P < 0.01$).

TABLE 7. α-Tocopherol content of egg yolk

	2 wk	4 wk	28 d of storage
	(μg/g of yolk)		
Lipid supplement (LS)			
Lard	178.96	202.17	211.59
Fish oil	163.12	186.62	196.78
Vitamin E dose (VD)			
0	97.33^{Cd}	90.93^{D}	80.94^{D}
50	141.85^{BCc}	136.05^{C}	132.93^{C}
100	179.35^{Bb}	227.48^{B}	226.68^{B}
200	265.63^{Aa}	313.84^{A}	366.53^{A}
Error mean square	1,786.44	1,509.34	1,169.96
df	40	40	40
LS	NS	NS	NS
VD	<0.01	<0.01	<0.01
LS*VD	NS	NS	NS

$^{a–d}$Values in the same column with no common superscript differ significantly ($P < 0.05$).

$^{A–D}$Values in the same column with no common superscript differ significantly ($P < 0.01$).

fatty acid reduce the total n-3 fatty acids deposition in the yolk, whereas high levels of dietary n-3 depress the vitamin E deposition.

The amount of vitamin E in the yolk was strictly related to the amount of α-tocopherol in the diet and increased linearly as dietary dl-α-tocopheryl acetate increased. The highest levels of 3.98 and 4.70 mg for groups receiving 100 and 200 ppm of vitamin E per egg were recorded, respectively. Similar results were obtained by Jiang et al. (1994). After 28 d of storage, the levels of vitamin E remained still very close to those observed in fresh eggs, suggesting that the vitamin was not used to prevent lipid oxidation in the yolk. This hypothesis is supported by the studies of Pike and Peng (1985), who maintained that shell egg yolk lipids are very resistant to oxidative deterioration during extended refrigerated storage (12 to 18 mo).

This experiment confirmed that it is possible to produce designer eggs enriched with n-3 and vitamin E through a targeted manipulation of hen diets. The amount of n-3 contained in only one egg is able to satisfy the updated daily human requirements estimated at approximately 100 to 200 mg/d (Department of Health, 1994). As for the vitamin E, whose human daily requirement depends on the amount of unsaturated fatty acid of the diet, the British Nutrition Foundation (1992) advises a total daily intake of 3.2 to 10.4 mg for men and 2.5 to 8 mg for women. Thus, the consumption of one of these designer eggs can match, according to the estimated requirements, 50 to 100% of the daily requirements just mentioned. Therefore, the vitamin E-enriched egg is a very interesting food, as it is well known that α-tocopherol is a free radical scavenger that has beneficial effects in preventing the onset of cancer and coronary heart diseases.

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