

Lipid fatty acid composition and oxidative susceptibility in eggs of hens fed a fish fat diet supplemented with vitamin E, C, or synthetic antioxidant

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ABSTRACT

Hy-Line laying hens from weeks 24 to 67 of age were fed a 0.3% fish fat diet with or without vitamin E, ascorbic acid, or synthetic antioxidants. Lipid composition, cholesterol and α -tocopherol levels and degree of fat oxidation (TBA-RS) in yolk during storage were investigated.

Dietary fish fat led to an increased n-3 PUFA content in yolk lipids (from 14.4 to 21.5 mg kg⁻¹) and to a lower n-6: n-3 PUFA ratio (from 7.75 to 4.97) without any effect on the level of cholesterol in eggs. The supplements tested exerted no influence on the composition of egg fatty acids. The dietary vitamin E supplement increased α -tocopherol in egg yolk from 24.2 to 68.3 mg kg⁻¹ and improved fat stability during storage for 15 and 42 days. Similar stabilizing properties were observed after supplementing the feed with synthetic antioxidants (BHT, BHA and EQ) and to a lesser degree when vitamin C was added.

KEY WORDS: laying hens, fish fat, yolk, fatty acids, antioxidants, TBA-RS

INTRODUCTION

Linoleic acid (C_{18:2 n-6}) is essential for hens as a precursor of n-6 polyunsaturated fatty acids, PUFA, eg. arachidonic acid (C_{20:4 n-6}) and their derivatives: prostaglandins, prostacyclins and tromboxans, substances of great biological importance. Some vegetable oils contain significant amounts of α -linolenic acid (C_{18:3 n-3}), which is a precursor of n-3 PUFA with a longer carbon chain. Human nutritionists have shown that these acids in the diet reduce the risk of atherosclerosis by

decreasing blood aggregation and viscosity (Simopolous, 1991; Temple, 1996), inhibit the growth of some cancers (Pandalai et al., 1996) and enhance cerebral development (Neuringer et al., 1998). Because α -linolenic acid-based synthesis of eicosapentaenoic (EPA, $C_{20:5\ n-3}$), docosapentaenoic (DPA, $C_{22:5\ n-3}$) and docosahexaenoic (DHA, $C_{22:6\ n-3}$) acids in hens as a result of elongation and desaturation depends on many factors, the proportion of these acids in egg lipids is largely dependent on nutrition (Hargis and Van Elswyk, 1993). An effective way of increasing the level of these long-chain n-3 PUFA in egg yolk lipids is to add them to feeds in the form of cold-water sea fish fat that is rich in EPA, DPH and DHA (Evans et al., 1986; Farrell, 1998; Koreleski et al., 1998; Ryś et al., 1998). The fat of freshwater and warm-water sea fish is less abundant in these acids (Chetty et al., 1989).

The elevation of unsaturated fatty acids in animal products may in theory increase the susceptibility of yolk lipids to oxidation and the oxidative process is inhibited by antioxidants (Aymond and Van Elswyk, 1995; Cherian et al., 1996). Natural antioxidants include vitamin E, which increases the level of egg yolk tocopherols when added to feed (Meluzzi et al., 2000). Tocopherols and derived tocotrienols scavenging reactive hydroxyl radicals and single oxygen protect cells from damage (Diplock, 1991). Vitamin C dietary supplementation might enhance the antioxidant protection afforded by vitamin E (Sheehy et al., 1997) probably regenerating α -tocopherol from α -tocopheroxyl radical in biological membranes.

The aim of the present experiment was to investigate the effects of supplementing dietary fish fat and natural (vitamin E and C) or synthetic antioxidants (a mixture of butyl-hydroxyanisole BHA, butyl-hydroxytoluene BHT and ethoxyquin EQ) on the level of polyunsaturated fatty acids in lipids and the cholesterol content of egg yolks. The oxidative susceptibility of yolk fat (TBA-RS) during storage was also studied.

MATERIAL AND METHODS

Birds and diets

The experiment comprised 45 Hy-Line layers at an initial age of 24 weeks. Hens were randomly allocated to 5 feeding groups with 9 subgroups of 9 layers caged individually. The experiment was completed at 67 weeks of age. Hens were fed a basal diet containing ground cereals, soyabean and rapeseed meal, meat-and-bone meal and non-stabilized blended fat (Table 1). Non-stabilized blended fat of animal origin (lard and poultry fats) contained fatty acids (%): palmitic (C_{16}) 24.4, stearic (C_{18}) 20.47, oleic ($C_{18:1}$) 40.97, linoleic ($C_{18:2}$) 4.19 and linolenic ($C_{18:3}$) 0.94. The vitamin-mineral premix without vitamins E and C and antioxidant was manufactured at the BASF Premix Plant in Kutno (Poland).

TABLE 1

Composition of diets, g kg⁻¹

Components	Groups				
	I control	II	III	IV	V
Ground maize	200		200		
Ground wheat	339		315		
Ground barley	102		102		
Extracted soyabean meal (43.0% CP)	127		127		
Extracted rapeseed meal (32.7% CP)	40		40		
Meat-and-bone meal (46.0% CP, 8.0% ether extract)	50		50		
Lucerne meal	20		20		
Fodder limestone	81		81		
Dicalcium phosphate	2		2		
NaCl	3		3		
Vitamin-mineral premix ¹	5		5		
DL-Methionine	1		1		
Non-stabilized blended fat	30		27		
Fish fat	-		3		
Vitamin E	-	-	+	-	-
Vitamin C	-	-	-	+	-
Synthetic antioxidant	-	-	-	-	+
Crude protein	165.3		165.3		
Metabolizable energy MJ · kg ⁻¹	11.33		11.34		
Ether extract	51.2		51.2		
Met	3.61		3.61		
Lys	7.10		7.10		
Ca	35.3		35.3		
P available	3.51		3.51		

¹ premix (without vitamin E) supplied per 1 kg of diet, respectively: (IU) vit. A 10000, vit. D₃ 2000, (mg) vit. K₃ 2, vit. B₁ 1, vit. B₂ 4, B₆ 1.5, folic acid 5.0, nicotinic acid 25, D-calcium panthotenate 8, choline chloride 25, (µg) vit. B₁₂ 10, biotin 100, (mg) Mn 100, Zn 50, Cu 8, Fe 50, J 0.8, Se 0.2, Co 0.2

The basal diet (group I - control) was supplemented with 0.3% Lyso fish fat (groups II, III, IV and V) while reducing the proportion of blended fat by the same amount. This made the experimental diets contain similar amounts of protein and ether extract as the basal diet (Table 1). The Lyso fat pharmacological PUFA additive was characterized by a high concentration of n-3 PUFA, i.e. EPA (11.04%), DPA (1.62%) and DHA (15.94%) in the lipid fraction as well as by a reduced content of substances that have a characteristic fishy smell. In terms of 1 kg dietary mixture, fish fat was added together with 330 mg of EPA, 48 mg of DPA and 470 mg of DHA. The experimental diets were supplemented with nature-identical antioxidants, i.e. vitamin E in the form of 5% Lutavit E 50 (50 mg α-tocopherol

acetate per kg; group III) and 42% vitamin C Lutavit C (2-dimonophosphate L-ascorbic acid calcium salt, 50 mg equivalent of ascorbic acid per kg; group IV). The mixtures were manufactured every month to limit the period between production and feeding to less than 30 days. For comparison, addition of dietary synthetic antioxidant (Fra OX C from Franklin Product Internat.), which supplied 45 mg of BHT, 5 mg of BHA and 25 mg of EQ per kg of diet, was also used. It was added to blended fat in an amount of 250 mg per kg (group V).

Throughout the experiment, the number and weight of eggs laid, feed intake and mortality were recorded and performance indices calculated.

Sample collection

One egg from each hen was collected at 31, 37, 47, 57 and 67 weeks of age to determine the fatty acid content of yolk lipids and one egg at 31, 38 and 50 weeks of age to measure cholesterol in yolk. The measurements were done directly after the eggs were collected. At 31 weeks of age, 8 eggs were taken from each group to determine the concentration of TBA-RS by analysing 4 eggs at 15 days and 4 eggs at 42 days of cold storage at 4-8°C. Additionally, 5 eggs were taken from each group at 31 weeks of age to determine vitamin E in yolk.

Chemical analyses

Crude protein and other nutrients in dietary components and mixtures were determined according to AOAC methods (1990). Lysine and methionine from acid hydrolysates, after preoxidation of methionine to methionine sulphone, were estimated in a colour reaction with ninhydrin reagent, using a Beckman-System Gold 126 AA automatic analyser.

The vitamin E content of yolk fat was determined by a modified method of Manz and Philipp (1981) using an HPLC Spectra System TSP and Supelcosil LC-NH₂-NP 5 µm, 250 × 4.6 mm column in a liquid phase of n-hexane and 1,4-dioxane (97:3). For estimation of fatty acid composition, yolk lipids were isolated according to Folch et al. (1957). Fatty acids were saponificated (0.5 N NaOH in methanol) and esterified (boron-trifluoride-methanol solution) and were determined as methyl esters by gas chromatography using a Varian 3400 GC and CP-Wax 58, 25 × 0.53 mm column.

The cholesterol content of yolk was determined enzymatically with a Cormay kit after extraction with alcohol-ether (3:1) solvent. The fat oxidation rate expressed as TBA-RS was determined using thiobarbituric acid (Salih et al., 1987) with some modifications (Pikul et al., 1989).

Metabolizable energy of the mixtures was calculated by adding the ME value of individual feeds, calculated using the content of basic nutrients and regression equations according to the European Table (1989).

Statistical analysis

Data were subjected to statistical analysis using one-way factorial analysis of variance. The significance of differences between means was determined by Duncan's multiple range test.

RESULTS

During the experiment, the performance of the hens was high, with the laying rate averaging 89.6% and egg weight 63.3 g; feed conversion rate 2.1 kg feed per kg of eggs (Table 2). After fish fat without antioxidants was added to the diet, a significant reduction (group I vs group II) of feed intake was noted ($P \leq 0.05$). A tendency (statistically not confirmed) for numerical reduction of egg weight and laying rate was also observed together with improved feed conversion per kg of eggs laid. For the supplement of vitamin E or synthetic antioxidant, a tendency towards a lower laying rate, greater egg mass and decreased feed conversion was observed (groups III and V vs group II). The difference in feed intake when vitamin E was supplemented to the diet was statistically significant (group III vs group II) ($P \leq 0.05$).

TABLE 2

The performance of experimental hens

Group	Laying rate %	Egg weight g	Daily feed intake g	Feed conversion per 1 kg of eggs kg
I	93.0 ^b	63.2	123 ^{bB}	2.12
II	90.9 ^{ab}	62.4	119 ^{aA}	2.09
III	87.8 ^a	64.0	124 ^{bB}	2.21
IV	89.3 ^{ab}	62.9	120 ^{aAB}	2.14
V	87.1 ^a	63.9	121 ^{abAB}	2.20
SEM	0.821	0.354	0.583	0.0205

^{A,B} - values with different letters are highly significantly different ($P \leq 0.1$)

^{a,b} - values with different letters are not significantly different ($P \leq 0.05$)

Composition of egg yolk lipids

The fatty acid profile in egg yolk lipids analysed on successive laying dates (at 31, 37, 47, 57 and 67 weeks of age) did not undergo any directional changes. This is why the effect of experimental factors on the composition of yolk fat in this paper is discussed based on the mean content of fatty acids during the whole sample collection period (Table 3).

The proportions of saturated, mono-, di- and tri-unsaturated fatty acids and total PUFA in yolk lipids were not differentiated in the group fed the control diet and the 0.3% fish fat diet (Table 3). Likewise, the SFA to PUFA ratio did not change.

The addition of fish fat to the feed (group I vs II) increased ($P \leq 0.05$) the proportion of n-3 PUFA containing 20 or more carbon atoms in the chain, i.e. EPA, DPA and DHA and total n-3 PUFA. This was accompanied by decreases in the proportion of n-6 PUFA, i.e. arachidonic ($C_{20:4, n-6}$), significant at $P \leq 0.05$, and of total n-6 acids ($P \leq 0.01$). Changes in the fatty acid composition of yolk lipids after fish fat supplementation caused the n-6 PUFA to n-3 PUFA ratio to decrease from 7.75 to 4.97, the difference being significant at $P \leq 0.01$.

The addition of vitamins E and C and of the synthetic antioxidant to the fish fat diet (group II vs III, IV, V) did not affect the configuration of fatty acids in yolk (Table 3).

Vitamin E and cholesterol

Dietary supplementation of vitamin E increased the content of this vitamin in egg yolk (group III), the difference in relation to other groups being significant at $P \leq 0.05$ (Table 4). No effect of dietary fish fat and antioxidants was observed on the cholesterol level or content in yolk.

Yolk fat oxidation

TBA values in eggs stored for 15 days were higher in groups I and II than in the other groups ($P \leq 0.05$). The lowest concentration of TBA-RS was found in eggs of hens from groups V and III (Table 4), which received synthetic antioxidant or vitamin E in their feeds, followed by group IV (vitamin C).

TABLE 4

Cholesterol and vitamin E content and TBA value in egg yolk

Group	Vitamin E mg 1 kg ⁻¹ of yolks	Cholesterol		TBA-RS content	
		mg 1 g ⁻¹ of yolk	mg in whole yolk	after 15 days of storage	after 42 days of storage
				mg 1 kg ⁻¹ of yolks	
I	24.2 ^{aA}	16.1	264	1.205 ^{bB}	1.320 ^{cAB}
II	30.7 ^{aAB}	15.7	270	1.344 ^{bB}	1.488 ^{cB}
III	68.3 ^{bb}	15.5	262	0.792 ^{aA}	0.801 ^{aA}
IV	30.8 ^{aAB}	16.5	268	0.914 ^{aAB}	1.215 ^{bcAB}
V	25.0 ^{aA}	15.8	268	0.779 ^{aA}	0.922 ^{abA}
SEM	5.280	0.212	4.261	0.0703	0.0793

^{A,B} - values with different letters are highly significantly different ($P \leq 0.1$)

^{a,b,c} - values with different letters are significantly different ($P \leq 0.05$)

TABLE 3
Average fatty acid composition of egg yolk lipids, g kg⁻¹

Group	n-3 PUFA					n-6 PUFA					n-6:		SFA:					
	C _{18:3}	C _{18:4}	C _{20:5} EPA	C _{22:5} DPA	C _{22:6} DHA	Total n-3	C _{18:2}	C _{18:3}	C _{20:2}	C _{20:4}	C _{22:2}	C _{22:4}	Total n-6	n-3 ratio	SFA	PUFA	UFA ratio	
I	2.7	1.3	0.12 ^A	0.95 ^A	9.3 ^A	14.4 ^A	89.4	0.54	1.0	18.5 ^B	0.74	1.4 ^B	111.6 ^B	7.75 ^B	317.0	552.2	126.0	0.467
II	2.9	1.3	0.38 ^B	1.25 ^B	15.7 ^B	21.5 ^B	87.2	0.50	1.0	15.6 ^A	0.84	1.2 ^{AB}	106.4 ^A	4.93 ^A	314.8	554.7	127.5	0.461
III	2.7	1.45	0.42 ^B	1.3 ^B	15.9 ^B	21.8 ^B	86.9	0.50	1.0	15.7 ^A	0.80	1.2 ^A	106.1 ^A	4.89 ^A	312.6	556.7	128.5	0.457
IV	2.4	1.35	0.45 ^B	1.3 ^B	16.3 ^B	21.7 ^B	84.4	0.46	1.05	16.5 ^B	0.88	1.25 ^{AB}	104.5 ^A	4.81 ^A	310.8	559.5	126.2	0.453
V	2.7	1.35	0.45 ^B	1.35 ^B	16.6 ^B	22.4 ^B	86.9	0.46	1.1	15.3 ^A	0.84	1.2 ^A	105.8 ^A	4.72 ^A	314.1	554.8	128.1	0.460
SEM	0.080	0.035	0.028	0.042	0.628	0.693	0.870	0.017	0.027	0.286	0.061	0.026	0.958	0.0256	1.43	1.31	0.940	0.0033

^{A,B} - values with different letters are highly significantly different (P≤0.01)

After an extended period of storage (42 days) the highest TBA-RS concentration was found in the yolk of eggs from groups fed dietary fish fat with no antioxidants (group II), followed by group I (control) and group IV (supplemented with ascorbic acid). The lowest value was obtained in eggs of hens receiving a dietary supplement of vitamin E (group III), followed by synthetic antioxidant (group V). Differences between group III and group II were significant at $P \leq 0.01$, and in relation to groups I (control) and IV at $P \leq 0.05$. The yolks of eggs from the hens supplemented with vitamin C (group IV) were observed to have a lower TBA-RS content than in groups I and II, but differences were not statistically significant.

DISCUSSION

The preferences of poultry consumers are changing and in the case of eggs, demand has increased for products with a higher n-3 PUFA content in yolk lipids (Lewis et al., 2000).

The introduction of 0.3% fish fat to the hen diets caused the n-3 PUFA proportion in yolk lipids to increase by 49% and n-6 PUFA to decrease by 5%. As a consequence of these changes, the n-6 PUFA to n-3 PUFA ratio favourably decreased from 7.7 to 5. The present results are in good agreement with the findings of other authors who reported a favourable effect of dietary fish fat on the n-3 PUFA content in egg yolk lipids (Evans et al., 1986; Farrell, 1998; Koreleski et al., 1998; Ryś et al., 1998). In earlier studies in which a 0.3% proportion of dietary fish fat (Lyso) was used, there were additionally no adverse effects on the sensory characteristics of eggs, especially aroma and flavour (Koreleski et al., 1998). Miller et al. (1967) take the view that the unfavourable effect of fish fat on the flavour of poultry products is determined by the presence of aldehydes, ketones, mercaptans and trimethylamine. Wessels et al. (1973) related poorer meat aroma to EPA and DHA oxidation products. Lyso fat is largely free from substances like this.

The difference in TBA-RS content in stored eggs from hens fed basal and fish-fat supplemented diets was 11-12 %, but the difference was statistically not confirmed. It shows that a 0.3% level of fish fat supplementation to the diet did not significantly increase oxidation processes in egg yolk lipids. Higher values of TBA-RS in eggs from those diets as compared with diets supplemented with antioxidants may, first of all, suggest oxidation of fatty acids from non-stabilized blended fat, native fats in diet components, and fatty acids originating from synthesis in the body, and only in part being attributed to fish fat.

The dietary supplements of vitamins E and C and synthetic antioxidants had no influence on the composition of fatty acids in egg yolk. Indirectly, this may indicate that the processes of fat oxidation in air-dry feed mixtures until 30 days of storage followed a similar pattern, no matter if antioxidants were added or not

(Matyka, 2000). The effect of the tested supplements on the oxidation processes that may take place during the passage of feed through the digestive tract are not known but under a low level of digesta oxygenation, seems small.

A visible result of using the vitamin E supplement in feed (50 mg kg^{-1}) was the increased level of α -tocopherol in egg yolk from 24.2 to 68.3 mg kg^{-1} . Similarly in the studies of Chen et al. (1998), increased supplementation of α -tocopherol to the feed in the range of 0 to 120 mg kg^{-1} corresponded with the elevated content of vitamin E in eggs from 25 to 75 mg kg^{-1} of yolk.

Cholesterol plays an important role in the synthesis of steroid hormones, in the metabolism of vitamin D_3 and as an agent conditioning the suitability of eggs for hatching. All this means that the cholesterol level in eggs is difficult to manipulate through nutrition (Hargis, 1988). Based on a review of the literature Griffin (1992) proves that inhibiting endogenous cholesterol synthesis in hens and reducing the rate of lipoprotein synthesis in the liver has little effect on the composition of lipoproteins deposited in the egg. In the present experiment, no effect of dietary fish fat on the level or content of cholesterol in egg yolk was found (Table 4).

PUFA-rich fats show increased susceptibility to oxidation, and the resultant products are an undesirable contaminant of food for poultry (Zduńczyk et al., 2001) and humans (Robey et al., 1994). Natural antioxidants include tocopherols and ascorbic acid (Nagórniak-Stasiak et al., 1997), because their presence prevents the formation of free radicals or helps oxidation products transform into compounds of low toxicity.

In eggs that were stored for two weeks, TBA-RS values indicate beneficial effects of supplemental synthetic antioxidants and vitamin E in the feed on yolk fat stability. After prolonged storage periods (six weeks), egg stability was affected most by vitamin E and to a smaller degree by the supplement of synthetic antioxidants. The effect of ascorbic acid was lower and only manifested itself during the shorter storage period. The tests of stored eggs confirmed the antioxidative properties of vitamin E supplemented in the amount of 50 mg kg^{-1} to the feed and deposited in yolk in the amount of 68 mg kg^{-1} . The antioxidative action of 25 , 45 and 50 mg kg^{-1} of α -tocopherol in egg yolk was ascertained by Chen et al. (1998), but when the concentration was higher (75 mg kg^{-1}) they observed yolk fat to be more susceptible to oxidation. A favourable antioxidative action of a high level of total tocopherols in feed (467 and 867 mg kg^{-1}), containing linseed, was reported by Qi and Sim (1998). In light of the literature data quoted above, the level of 68 mg kg^{-1} vitamin E in egg shown in the present experiment is considered beneficial for inhibiting yolk fat oxidation.

The properties of synthetic antioxidants with regard to stabilizing fodder fats and vitamins in feeds have been well documented. In the present study, the antioxidants added to the layer diets were found to decrease TBA number in yolk fat during egg storage for 2 and 6 weeks. These data demonstrate the beneficial effect

of synthetic antioxidants on yolk fat stabilization. Although the mechanism of such BHT, BHA and EQ action in the body is not clear, the present findings show that they may also affect the inhibition of the oxidation process in egg yolk.

CONCLUSIONS

The present research has confirmed the effect of fish fat in layer diets on increasing total n-3 PUFA and shown the beneficial reduction of the n-6 to n-3 ratio in yolk lipids without affecting the cholesterol level. Supplementation of the diet with vitamin E led to an increased α -tocopherol level in yolk and to improved egg fat stability during storage, as measured by TBA-RS content. Improved yolk fat stability was also noted after the diet was supplemented with synthetic antioxidants, and to a lesser extent during a short period, when vitamin C was added.

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STRESZCZENIE

Skład i podatność na utlenianie lipidów żółtka jaja kur otrzymujących w paszy tłuszcz rybny oraz dodatek witaminy E, C lub syntetycznych przeciwutleniaczy

Kury nioski Hy-Line od 24 do 67 tygodnia życia żywiono dietą z udziałem 0,3% tłuszczu rybnego, bez dodatku lub z dodatkiem witaminy E, kwasu askorbinowego lub mieszaniny syntetycznych antyoksydantów. Badano skład frakcji lipidowej, poziom cholesterolu i α -tokoferolu w żółtku jaj oraz stopień oksydacji tłuszczu żółtka w czasie przechowywania (TBA).

Tłuszcz rybny w diecie spowodował zwiększenie udziału sumy n-3 PUFA w lipidach żółtka z 14,4 do 21,5 mg kg⁻¹ oraz obniżenie stosunku PUFA n-6: n-3 z 7,75 do 4,97, bez wpływu na poziom cholesterolu w jajach. Badane dodatki nie wpłynęły na skład kwasów tłuszczowych jaja. Dodatek witaminy E do paszy zwiększył poziom α -tokoferolu w żółtku jaj z 24,2 do 68,3 mg kg⁻¹ oraz zwiększył stabilność tłuszczu w czasie przechowywania przez 15 i 42 dni. Podobne właściwości stabilizujące stwierdzono po dodaniu do paszy mieszaniny syntetycznych antyoksydantów (BHT, BHA i EQ), a w mniejszym stopniu witaminy C.