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Effects of dietary fish oil and flax seed on cholesterol and fatty acid composition of egg yolk and blood parameters of laying hens

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Abstract

This study was conducted to determine the effects of the supplementation of different levels of fish oil (FO) and flax seed (FS) in the diets of layers on the content of egg yolk fatty acid, cholesterol, blood parameters, egg production and egg quality criteria. In the experiment, a total of 120 Isa-White laying hens of 34 weeks of age were used. Hens were divided randomly into five groups and fed different diets containing no FO and FS; 1.5% FO; 4.32% FS; 1.5% FO+4.32% FS and 8.64% FS for eight weeks. A significant decrease in yolk cholesterol content (mg/g yolk) was found in the eggs from hens fed the diets containing 1.5 % FO and 8.64% FS as compared with the control. Adding FO and FS to diets significantly increased the total omega-3 fatty acids in eggs at 28 (phase 1) and 56 (phase 2) days of the trial. By contrast, the addition of both FO and FS to diets had no effect on total omega-6 fatty acids in eggs in phase 1. But dietary 1.5% FO, 4.32% FS and 1.5% FO+4.32% FS supplementation decreased significantly the total omega-6 fatty acids compared to the controls in phase 2. The linolenic acid content of eggs was the highest in eggs from hens fed the diet with added FS, while docosahexaenoic acid content was the highest in eggs from hens fed diets with added FO. Dietary supplementation of FO and FS did not significantly affect the concentrations of serum trigliceride and high density lipoprotein. The serum cholesterol level of hens fed diets containing 1.5% FO+4.32% FS and 8.64% FS was lower than the control group. The addition of FO and FS to diets did not cause any negative effect on some egg quality criteria such as egg weight, yolk weight, yolk ratio, albumen weight, albumen ratio, shell weight, shell ratio, shell strength and shell thickness. The egg production of hens fed a diet containing 4.32% FS was significantly higher than the controls. Feed intake and feed conversion were not affected by all treatments.

Keywords: Omega-3 fatty acids, cholesterol, eggs, flax seed, fish oil
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Introduction

The egg is one of the most complete foods from a nutritional point of view. However, consumers refrain from egg consumption due to the relatively high cholesterol content of eggs and the perception that cholesterol rich foods lead to coronary heart disease (CHD) and atheriosclerosis (Zeidler, 1998). Risk factors for CHD include hypertension, obesity and elevated blood cholesterol concentrations. The occurrence of CHD was higher in humans who have high blood cholesterol levels. Nutrition, genetics, age and sex affect blood cholesterol levels in humans. During the past 20 years public concern has focused on the relationship between dietary cholesterol and the development of CHD. However, dietary fat type and fatty acid composition of fats consumed, are more important than the amount of dietary cholesterol consumed (Leskanich & Noble, 1997; Simopoulos, 2000b). According to the results of many studies, it was confirmed that saturated fatty acids and trans fatty acids cause negative effects on human health, but polyunsaturated fatty acids (PUFA) have a positive effect on human health as regards CHD (Blanch & Grashorn, 1995; Bhatnagar & Durrington, 2003; Erkkila *et al*., 2003; Meyer *et al*., 2003). In recent years, consumer demands for more healthy foods stimulated the interest in modifying the fatty acid profile of eggs. Over the past 20 years many studies and clinical investigations revealed that omega-3 PUFA, particularly eicosapentaenoic (EPA) and docosahexaenoic (DHA) exert beneficial effects on human health. Omega-3 PUFA are essential for normal growth and development and many play an important role in the prevention and treatment of CHD, hypertension, inflammatory, autoimmune disorders and cancer (Meluzzi *et al*., 1997a; Lewis *et al*., 2000; Simopoulos, 2000a). Eggs and poultry meat rich in omega-3 fatty acids can be produced by using feed ingredients rich in omega-3 fatty acids. In general, studies have been conducted to determine the effects of different omega-3 fatty acids sources in diets on the cholesterol and fatty acid composition of egg yolk and

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meat (Caston & Leeson, 1990; Cherian & Sim, 1991; Hammershøj, 1997; Coetzee & Hoffman, 2002; Komprda *et al*., 2003). However, studies with regard to the effects of omega-3 fatty acids on blood cholesterol are lacking. In addition, in many studies the most important criteria such as egg yolk cholesterol and fatty acid content of eggs, blood cholesterol, the performance of layers and egg quality criteria were not fully determined (Caston & Leeson, 1990; Cherian & Sim, 1991; Jiang *et al*., 1991; Herber & Van Elswyk, 1996; Hammershøj, 1997; Niemiec *et al*., 1997).

In this study, the effects were studied of adding fish oil and flax seed to layers diets on the cholesterol and fatty acid content of egg yolk, blood parameters, the performance of layers and egg quality criteria.

Materials and Methods

One hundred and twenty 34-week old Isa-White laying hens were randomly allocated to five experimental treatments. Each treatment consisted of six replications, with a replication consisting of four hens in a cage. The cages were kept in an open-sided layer house and the experiment lasted for eight weeks. The experimental diets were: A control diet (C) containing no fish oil (FO) or flax seed (FS); treatment FO containing 1.5% fish oil; treatment FS1 containing 4.32% flax seed; treatment FO+FS with 1.5% fish oil +4.32% flax seed and treatment FS2 containing 8.64% flax seed. The 4.32 and 8.64% FS were added to provide 1.5 and 3.0% flax seed oil to the respective diets. The experimental diets were formulated to be iso-energetic (11.5 MJ ME /kg DM) and isonitrogenous (16.5% crude protein). The ingredient and chemical composition of the experiment diets are presented in Table 1 and their fatty acid composition in Table 2.

Table 1 The ingredient and chemical composition of the experimental diets (as fed)

	C	FO	FS1	FO+FS	FS2
Ingredients (kg/1000 kg)					
Maize	604.8	539.0	526.8	497.3	484.5
Soybean meal	191.2	183.3	177.8	156.6	153.6
Cotton seed meal	95.8	100	76.4	125	97.7
Wheaten bran	7.4	56.5	80.3	59.7	84.1
Flax seed	-	-	43.2	43.2	86.4
Sawdust	10	6.5	5.6	0.3	-
Fish oil	-	15	-	15	-
Limestone	67.3	78.3	69.0	80.0	73.9
Dicalcium phosphate	16.9	14.6	14.1	16.1	13.1
Salt	2.8	2.9	2.8	2.8	2.7
Mineral Premix*	2	2	2	2	2
Vitamin Premix**	1.0	1.0	1	1	1
DL-Methionine (0.98)	0.8	1	1	1.0	1
Total	1000	1000	1000	1000	1000
Composition, g/kg (analysed)					
Dry matter	906.3	911.6	909.4	915.5	914.3
Crude protein	164.9	163.1	164.1	162.8	166.0
Ether-extract	30.3	42.6	44.4	57.5	58.5
Crude cellulose	43.8	43.4	42.0	43.4	47.7
Crude ash	108.9	116.3	108.8	121.9	126.9
Apparent metabolisable energy, MJ/kg feed	11.47	11.43	11.45	11.57	11.57
Total calcium	35.6	39.0	38.0	38.7	39.1
Total phosphorus	7.4	7.2	7.2	7.7	7.4
Lysine (calculated)	7.9	7.9	7.7	7.9	7.7
Met.+Cys. (calculated)	5.7	5.7	5.7	5.7	5.7

C: Control: without fish oil and flax seed; FO: with 1.5% fish oil; FS1: with 4.32% flax seed; FO+FS: with 1.5% fish oil + 4.32% flax seed; FS2: with 8.64% flax seed

*Mineral premix (mg/kg diet): Mn - 80; Fe - 60; Zn - 60; Cu - 5; Co - 0.2; I - 1; Se - 0.15

**Vitamin premix (/kg diet): Vitamin A - 12 000 IU; vitamin D_3 - 2 000 IU; vitamin E - 35 IU; vitamin K_3 - 5 mg; vitamin B_1 - 3 mg; choline chloride - 350 mg; vitamin B_2 - 6 mg; niacin - 20 mg; calcium D-pantothenate - 6 mg ; vitamin B_6 - 5 mg; vitamin B_{12} - 0.015 mg; folic acid - 0.75 mg; D-biotine - 0.045 mg; vitamin C - 50 mg

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Table 2 The fatty acid composition of the control and experimental diets (percentage of total fatty acids)

Fatty acid	C	FO	FS1	FO+FS	FS2
$C_{14:0}$ myristic	0.55	2.66	0.50	2.68	0.12
$C_{16:0}$ palmitic	10.59	13.32	9.78	10.39	8.14
$C_{16:1}$ palmitoleic	0.14	3.05	0.15	2.29	0.15
$C_{18:0}$ stearic	2.29	2.94	3.35	3.29	3.48
$C_{18:1}$ oleic	26.51	23.06	21.99	21.43	20.86
$C_{18:2n-6}$ linoleic	57.57	39.29	41.38	31.81	34.00
$C_{18:3n-3}$ linolenic	2.35	2.74	22.85	18.87	33.25
$C_{18:4n-3}$ stearidonic	-	0.87	-	0.61	-
$C_{20:4n-6}$ arachidonic	-	0.27	-	0.30	-
$C_{20:5n-3}$ eicosapentaenoioic	-	3.53	-	2.70	-
$C_{22:5n-3}$ docosapentaenoic	-	0.49	-	0.34	-
$C_{22:6n-3}$ docosahexaenoic	-	6.78	-	5.25	-
∑ Saturated	13.43	18.92	13.63	15.59	11.74
∑ Unsaturated	86.57	80.08	86.37	83.64	88.26
∑ omega-3	2.35	14.68	22.85	25.64	56.90
∑ omega-6	57.57	39.56	41.38	32.11	14.62
∑ omega-6/omega-3	24.50	2.69	1.81	1.25	0.26

C: Control: without fish oil and flax seed; FO: with 1.5% fish oil; FS1: with 4.32% flax seed; FO+FS: with 1.5% fish oil + 4.32% flax seed; FS2: with 8.64% flax seed

To determine cholesterol and fatty acid content of egg yolk, six eggs were collected after 28 days (phase 1) and after 56 days (phase 2) from each treatment. In order to determine serum cholesterol, triacylglycerol and high density lipoprotein (HDL) concentrations, a total of 30 blood samples (six samples per group) were taken from the wing vein, after feed withdrawal for 12 h. After coagulation, the blood was centrifuged at 2000 rpm to obtain the serum. The serum parameters were analysed by auto analyzer (Opera technicon®), using commercial kits (Sigma Diagnostic® Kits). A direct saponification procedure was applied for the analysis of cholesterol in egg samples (Poyraz, 1987). Lipids were extracted from eggs using the method of the AOAC (1990). The fatty acid methyl esters were prepared from lipid samples according to Joseph & Ackman (1992) and from subsequent fatty acid profiles obtained by gas liquid chromatography (GLC). The fatty acid methyl esters were analysed using a 50 x 0.25 mm inside diameter WCOT fused silica CP-Sil 88 capillary column installed in a Hewlett Packard 5890 GLC with flame ionization detector. Egg production was recorded daily and calculated as hen-day (%). Feed consumption (g/hen/day) and feed conversion as kg feed consumed/kg eggs were recorded at 28 and 56 days of the trial. Egg quality criteria (egg weight, yolk weight, yolk ratio, albumen weight, albumen ratio, shell weight, shell ratio, shell strength and shell thickness) were measured at 28 days and 56 days of the trial, using 18 eggs from each experimental unit. Mortality was recorded daily. The standard techniques for the Proximate analysis were used to determine the nutrient concentrations in the diets (Naumann & Bassler, 1993). The experimental diets were analysed also for starch, sugar, total calcium and phosphorus, according to VDLUFA method (Naumann & Bassler, 1993). Metabolisable energy content of the diets was calculated based on their chemical composition (Anonymous, 1991). The data were analysed statistically using the General Linear Models procedure of SAS (1985). Significant differences between treatment means were separated using the Duncan's multiple range test with a 5% probability.

Results and Discussion

The cholesterol contents in both the egg yolk and eggs of layers decreased significantly ($P < 0.05$) with increasing age (Table 3). The finding that cholesterol content of egg yolk in layers decreased with increasing age agrees with the findings of previous research (Oltjen & Dinius, 1975; Gissel *et al*., 1976; Brendl *et al*., 1979; Basmacıoğlu & Ergül, 2000). It was previously reported that the cholesterol content in eggs increased with age and this increase resulted from an increase in both egg and yolk weights (Oltjen & Dinius, 1975; Basmacıoğlu & Ergül, 2000).

A significant decrease in yolk cholesterol concentration (mg/g yolk) was found in the eggs from the hens receiving the diets containing 1.5% FO and 8.64% FS as compared with the control. Lin & Pratt (1992) reported that when omega-3 fatty acid rich 3% menhaden oil was added to the diet, yolk cholesterol

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concentration (mg/g yolk) decreased by 15%. In addition, it was reported that fish oil (Meluzzi *et al*. 1997b) or flax seed (Caston & Leeson, 1990) did not effect the egg yolk cholesterol concentration (mg/egg). Our results (Table 3) agree with these findings.

Table 3 The effect of adding fish oil (FO) and flax seed (FS) to the diet on the concentration of cholesterol in the egg yolk

	Egg yolk cholesterol	
Treatments	mg/g yolk	mg/egg
C	13.71 [ab]	205.42
FO	12.56 [c]	198.75
FS1	13.93 [a]	219.20
FO +FS	13.51 [abc]	211.07
FS2	12.79 [c]	202.00
SEM	0.335	7.558
Phase 1	14.15 [a]	218.28 [a]
Phase 2	12.46 [b]	196.29 [b]
SEM	0.212	4.780
	Significance	
Treatment	*	NS
Phase	**	**
Treatment x Phase	**	NS

C: Control: without fish oil and flax seed; FO: with 1.5% fish oil; FS1: with 4.32% flax seed; FO+FS: with 1.5% fish oil + 4.32% flax seed; FS2: with 8.64% flax seed
[a,b,c] Means in the same column with the same superscript do not differ (P > 0.05)
** P < 0.01; *:P < 0.05; NS - not significant (P > 0.05)
SEM = standard error of the mean

Table 4 The effect of including fish oil (FO) and flax seed (FS) in a layer diet on the fatty acid composition of egg yolk at day 28 of the trial

Fatty acids[1]	C	FO	FS1	FO+FS	FS2	SEM	Significance
$C_{14:0}$	0.33 [c]	0.48 [b]	0.26 [d]	0.53 [a]	0.22 [d]	0.016	**
$C_{16:0}$	26.33 [a]	25.80 [ab]	24.13 [c]	25.07 [bc]	22.06 [d]	0.375	**
$C_{16:1}$	3.19 [ab]	3.57 [a]	3.01 [bc]	3.28 [ab]	2.65 [c]	0.123	**
$C_{18:0}$	9.01 [a]	8.22 [b]	9.05 [a]	8.96 [a]	9.46 [a]	0.199	**
$C_{18:1}$	38.29	39.30	37.50	35.54	37.72	0.863	NS
$C_{18:2n-6}$	17.22	16.24	18.02	17.74	17.65	0.665	NS
$C_{18:3n-3}$	0.62 [d]	0.71 [d]	3.49 [c]	4.00 [b]	5.89 [a]	0.124	**
$C_{18:4n-3}$	0.20 [a]	0.16 [b]	0.16 [b]	0.13 [b]	0.15 [b]	0.012	**
$C_{20:4n-6}$	2.25 [a]	1.13 [d]	1.52 [b]	0.91 [e]	1.36 [c]	0.042	**
$C_{20:5n-3}$	-	0.18 [b]	0.06 [d]	0.26 [a]	0.12 [c]	0.009	**
$C_{22:5n-3}$	0.07 [b]	0.19 [a]	0.17 [a]	0.21 [a]	0.22 [a]	0.015	**
$C_{22:6n-3}$	0.65 [d]	3.29 [a]	1.70 [c]	2.90 [b]	1.98 [c]	0.108	**
∑ saturated	35.67 [a]	34.50 [b]	33.44 [c]	34.56 [b]	31.74 [d]	0.375	**
∑ omega-3	1.54 [e]	4.53 [d]	5.58 [c]	7.50 [b]	8.36 [a]	0.204	**
∑ omega-6	19.47	17.37	19.54	18.65	19.01	0.688	NS
∑ omega-6/omega-3	12.64 [a]	3.83 [b]	3.50 [b]	2.49 [c]	2.27 [c]	0.289	**

C: Control: without fish oil and flax seed; FO: with 1.5% fish oil; FS1: with 4.32% flax seed; FO+FS: with 1.5% fish oil + 4.32% flax seed; FS2: with 8.64% flax seed
[1] % of total fatty acid; [a,e] Row means with common superscripts do not differ (P > 0.05)
**P < 0.01; *P < 0.05; NS - not significant (P > 0.05)

Fatty acid composition of eggs obtained at the end of the first phase is given in Table 4, while that obtained at the end of the second phase of the trial is summarized in Table 5. Adding FO and FS to the diets significantly increased the amount of total omega-3 fatty acids in both phases. The total saturated fatty acids decreased in both phases of the experiment, and was significant (P < 0.05) except for layers fed the 1.5% FO in phase 1. Total omega-3 fatty acid concentrations increased more in eggs obtained from layers fed both FO

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and FS than those fed either FO or FS. A five fold increase ($P < 0.05$) in the concentration of DHA in eggs was recorded when the hens received the diet containing 1.5% FO, compared to the control diet. These results were similar to those reported by Caston & Leeson (1990), Cherian & Sim (1991), Hargis *et al*. (1991), Scheideler & Froning (1996), Gonzalez-Esquerra & Leeson (2000) and Meluzzi *et al*. (2000). The highest levels of linolenic acid, which is also a omega-3 fatty acid, were detected in eggs of layers fed the diets containing FS. Due to the increase in omega-3 fatty acids and decrease in omega-6 fatty acids in eggs, the ratio of omega-6/omega-3 in eggs was reduced from 12.64 to 2.27 at day 28 of the trial and from 14.56 to 2.28 at day 56.

Adding FO and FS to the diets did not affect the total omega-6 fatty acid content of yolk at day 28 of the trial. Dietary supplementation of 1.5% FO, 4.32% FS and 1.5% FO+4.32% FS decreased ($P < 0.05$) the concentration of total omega-6 fatty acids of yolk compared to controls at day 56 of the trial. Jiang *et al*. (1991) found that there were no significant changes in the total omega-6 fatty acid content of yolk at a dietary inclusion of 15% FS, compared to their control.

Table 5 The effect of including fish oil (FO) and flax seed (FS) in a layer diet on the fatty acid composition of egg yolk at day 56 of trial

Fatty acids [1]	C	FO	FS1	FO+FS	FS2	SEM	Significance
$C_{14:0}$	0.31 [b]	0.48 [a]	0.23 [c]	0.45 [a]	0.22 [c]	0.013	**
$C_{16:0}$	25.92 [a]	25.48 [a]	23.12 [b]	23.91 [b]	21.33 [c]	0.302	**
$C_{16:1}$	2.95 [b]	3.47 [a]	3.06 [b]	3.17 [b]	2.50 [c]	0.084	**
$C_{18:0}$	9.19	8.92	8.53	8.68	9.17	0.197	NS
$C_{18:1}$	37.46 [ab]	39.14 [ab]	40.37 [a]	36.21 [b]	36.20 [b]	1.003	*
$C_{18:2n-6}$	19.08 [a]	16.29 [b]	17.16 [b]	17.04 [b]	19.44 [a]	0.581	**
$C_{18:3n-3}$	0.56 [c]	0.71 [c]	3.23 [b]	3.60 [b]	6.66 [a]	0.180	**
$C_{18:4n-3}$	0.21 [a]	0.15 [bc]	0.14 [c]	0.14 [c]	0.18 [ab]	0.012	**
$C_{20:4n-6}$	2.33 [a]	1.29 [c]	1.52 [b]	1.04 [d]	1.40 [bc]	0.053	**
$C_{20:5n-3}$	-	0.19 [a]	0.07 [c]	0.22 [a]	0.13 [b]	0.013	**
$C_{22:5n-3}$	0.07 [b]	0.19 [a]	0.22 [a]	0.23 [a]	0.24 [a]	0.021	**
$C_{22:6n-3}$	0.63 [c]	3.42 [a]	1.90 [b]	3.24 [a]	1.95 [b]	0.095	**
∑ saturated	35.42 [a]	34.88 [a]	31.88 [c]	33.04 [b]	30.72 [d]	0.384	**
∑ omega-3	1.47 [e]	4.66 [d]	5.56 [c]	7.43 [b]	9.16 [a]	0.219	**
∑ omega-6	21.41 [a]	17.58 [b]	18.68 [b]	18.08 [b]	20.84 [a]	0.595	**
∑ omega-6/omega-3	14.56 [a]	3.77 [b]	3.36 [b]	2.43 [c]	2.28 [c]	0.202	**

C: Control: without fish oil and flax seed; FO: with 1.5% fish oil; FS1: with 4.32% flax seed; FO+FS: with 1.5% fish oil + 4.32% flax seed; FS2: with 8.64% flax seed

[1] % of total fatty acid; [a,e] Row means with common superscripts do not differ ($P > 0.05$)

**$P < 0.01$; *$P < 0.05$; NS - not significant ($P > 0.05$)

Table 6 The effect of including fish oil (FO) and flax seed (FS) in a layer diet on serum parameters (mg/dL) of the hens

Treatments	Trigliceride	Cholesterol	HDL
C	1467.67	127.33 [a]	38.00
FO	1401.17	119.67 [ab]	35.17
FS1	1337.00	117.83 [ab]	37.50
FO+FS	1417.83	105.50 [b]	39.00
FS2	1348.00	99.67 [b]	40.83
SEM	111.55	6.719	2.21
		Significance	
Treatment	NS	*	NS

C: Control: without fish oil and flax seed; FO: with 1.5% fish oil; FS1: with 4.32% flax seed; FO+FS: with 1.5% fish oil + 4.32% flax seed; FS2: with 8.64% flax seed

[a,b] Column means with common superscripts do not differ ($P > 0.05$)

*$P < 0.05$; NS - not significant ($P > 0.05$)

SEM = standard error of the means

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Inclusion of FO and FS in the diet did not affect (P > 0.05) the triacylglycerol and HDL cholesterol concentrations of the serum of the hens. Supplementation with 1.5% FO+4.32 FS and 8.64% FS to diets decreased (P < 0.05) serum cholesterol concentration (Table 6). Our finding was similar to that of Van Elswyk *et al*. (1994) who demonstrated that dietary FO supplementation at a 3.0% inclusion level resulted in a decreased serum cholesterol concentration in hens.

Table 7 The effect of including fish oil (FO) and flax seed (FS) in the diet on egg production (%), feed intake (g/hen/day) and feed conversion (g feed/g eggs) of hens

Treatments	Egg production %	Feed intake g/hen/day	Feed conversion g feed/g eggs
C	84.75 [bc]	110.11	2.06
FO	87.35 [ab]	110.49	2.00
FS1	89.28 [a]	110.50	1.95
FO+FS	84.21 [bc]	114.95	2.04
FS2	82.44 [c]	111.23	2.13
SEM	1.149	2.023	0.040
	Significance		
Treatment	**	NS	NS

C: Control: without fish oil and flax seed; FO: with 1.5% fish oil; FS1: with 4.32% flax seed; FO+FS: with 1.5% fish oil + 4.32% flax seed; FS2: with 8.64% flax seed
[a,b, c:] Column means with common superscripts do not differ (P > 0.05)
**P < 0.01, NS - not significant (P > 0.05)

Table 8 The effect of including fish oil (FO) and flax seed (FS) in a diet on egg quality criteria

Treatments	Egg weight g	Yolk weight g	Yolk ratio %	Albumen weight g	Albumen ratio %	Shell weight g	Shell ratio %	Shell strength kg/cm^2	Shell thickness mm
C	63.21	15.71	24.85	41.18	65.18	6.32	10.00	2.64	0.410
FO	62.46	15.76	25.23	40.38	64.65	6.32	10.12	2.44	0.416
FS1	62.69	15.88	25.33	40.74	64.99	6.07	9.68	2.62	0.396
FO+F1	64.37	16.04	24.92	41.93	65.14	6.40	9.94	2.53	0.411
FS2	63.41	15.98	25.20	41.34	65.19	6.09	9.60	2.44	0.408
SEM	1.54	0.336	0.461	0.848	0.499	0.128	0.169	0.212	0.006
Phase 1	62.27 [b]	15.47 [b]	24.84	40.53	65.09	6.27	10.07 [a]	2.65	0.411
Phase 2	64.19 [a]	16.28 [a]	25.36	41.70	64.96	6.21	9.67 [b]	2.41	0.406
SEM	0.667	0.213	0.292	0.537	0.316	0.451	0.107	0.134	0.004
				Significance					
Treatment	NS	NS	NS	NS	NS	NS	NS	NS	NS
Phase	*	**	NS	NS	NS	NS	*	NS	NS
Treatment x Phase	NS	NS	NS	NS	NS	NS	NS	NS	NS

C: Control: without fish oil and flax seed; FO: with 1.5% fish oil; FS1: with 4.32% flax seed; FO+FS: with 1.5% fish oil + 4.32% flax seed; FS2: with 8.64% flax seed
[a,b] Column with common superscript do not differ (P > 0.05)
**P < 0.01; *P < 0.05; NS - not significant (P > 0.05)

While adding FO to the diet did not cause a significant difference in egg production, 4.32% FS supplementation increased egg production (P < 0.01) compared to hens receiving 8.64% FS, 4.32% FS+ 1.5% FO and the control (Table 7). This finding is similar to that of Hargis *et al*. (1991) who determined that FO was not effective in egg production. Jiang *et al*. (1991), who included FS at a 15% level and Yannokopoulos *et al*. (1999), who used FS at levels of 5, 10 and 15% reported that FS did not effect egg production. However, Scheideler & Froning (1996) reported that 1.5% FO and 5, 10 and 15% FS increased egg production significantly (P < 0.05). Feed intake and feed conversion were not affected by any of the treatments. From the egg quality criteria determined, only egg and yolk weights increased significantly with the age of the hen (Table 8).

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Feeding dietary FO and FS did not affect ($P > 0.05$) the determined egg quality criteria. This finding is similar to those of other researchers (Voght & Harnisch, 1978; Roland, 1979; Hurtwitz, 1987; Basmacıoğlu & Ergül, 2000). Yannakopoulos *et al*. (1999) reported that FS used in laying hen diets did not affect egg quality criteria except for egg and yolk weights. Only one mortality occurred, and that amongst the hens receiving the 1.5 FO+ 4.32 FS diet.

Conclusions

The results of the present study show that adding FO and FS to the diets of laying hens resulted in up to a 6 fold increase in total omega-3 fatty acids concentration of yolk and a decrease in omega-6/omega-3 ratio of yolk compared to the control. In addition, these supplements did not affect the performance of hens and egg quality criteria adversely. Therefore, it is possible to obtain yolk containing satisfactory amounts of beneficial fatty acids by supplementing the diets with fish oil and flax seed.

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