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Received for review June 19, 1989. Accepted November 7, 1989.

Registry No. L-His, 71-00-1; L-Lys, 56-87-1.

Effect of Dietary Fish Oil on ω -3 Fatty Acid Levels in Chicken Eggs and Thigh Flesh

Zhi-Bin Huang,[†] Henry Leibovitz,^{*†} Chong M. Lee,[†] and Richard Millar[§]

Department of Food Science and Nutrition, University of Rhode Island, West Kingston, Rhode Island 02892, Department of Aquatic Product Processing, Shanghai Fisheries College, Shanghai, China, and Department of Animal and Veterinary Science, University of Rhode Island, Kingston, Rhode Island 02881

Incorporation of ω -3 polyunsaturated fatty acids into chicken egg, thigh meat, and adipose tissue was studied by feeding laying hens diets containing up to 3% menhaden oil for 4 weeks. Dietary fish oil increased eicosapentaenoic acid (20:5 ω 3 (EPA)) and docosahexaenoic acid (22:6 ω 3 (DHA)) in the fatty acid distribution of egg yolk. In thigh meat EPA did not increase while the DHA increased with 3% dietary fish oil. The levels of EPA and DHA in adipose tissue increased with increased dietary fish oil. The ratio of EPA to DHA in egg and thigh meat was inversely proportional to the ratio in fish oil. Organoleptic evaluation showed that eggs and thigh meat remained acceptable with up to a 3% dietary fish oil stabilized with 0.1% ethoxyquin.

Early in the 1960s capsules of fish oil and fish oil fatty acid concentrates were manufactured for heart patients (Stansby, 1982). Through long-term studies, scientists discovered that ω -3 polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) lowered ischemic heart disease (Anonymous, 1981; Ahmed and Holub, 1984). It has been reported that these two fatty acids not only alter membrane lipid composition by reducing the production of thromboxane A₂ but also reduce the levels of plasma triglycerides and cholesterol (Peifer, 1967; Sanders, 1985), thus preventing thrombosis and atherosclerosis (Anonymous, 1979; Zapsalis and Beck, 1985). DHA is also known to be a

very important fatty acid in the vertebrate nervous system (Ackman, 1980).

Attempts to produce a secondary source of dietary ω -3 polyunsaturated fatty acids have been made by feeding animals fish meal rich in ω -3 fatty acids. When hens were fed fish oil, the fatty acid composition of egg yolk reflected that of the diet (Navarra et al., 1972). Machlin et al. (1962) found that polyunsaturated fatty acids in egg lipid can be readily increased by increasing the level of polyunsaturated fatty acids in the diet. Other reports indicated that dietary lipids could also influence the fatty acid pattern of chicken muscle lipids (Marion and Woodroof, 1963; Miller et al., 1967a). Feeding laying hens with fish oil increased both egg production and hatchability (Gauglitz et al., 1974). The effective level of fish oil in the diet was found to be 2-6% (Stansby, 1967). The results of other studies indicated that fish oil could also promote the growth of chickens (Dansky, 1962; Edwards et al., 1961).

As for flavor taint, laying hens were fed up to 2.5% fish oil without adversely affecting egg flavor (Holdas and

[†] Department of Aquatic Product Processing, Shanghai Fisheries College.

[‡] Department of Food Science and Nutrition, University of Rhode Island.

[§] Department of Animal and Veterinary Science, University of Rhode Island.

Table I. Approximate Analysis of Country Egg Layer Ration (Agway, Inc., Syracuse, NY)

protein	16.1%
fat	3.9%
carbohydrate	65.3%
fiber	3.4%
ash	11.3%

May, 1966), while with broiler chickens, a flavor taint was observed at the 2% fish oil level (Edwards and May, 1965).

Previous work, however, did not show to what extent dietary EPA and DHA can be incorporated into egg yolk lipids over extended feeding periods. The objective of this study was to determine the extent that dietary ω -3 polyunsaturated fatty acids, mainly EPA and DHA, can be incorporated into chicken egg and tissue in relation to dietary fish oil and feeding duration without adversely affecting the organoleptic quality of eggs and meat.

MATERIALS AND METHODS

Preparation of Diet and Feeding Procedure. Thirty 21-week-old white Leghorn starter pullets (Shaver, 288) were housed in individual cages (29.2 cm wide \times 45.7 cm deep) and kept on a commercial diet (Country Egg Layer Ration, Agway Inc., Syracuse, NY) for 33 days until they were in full egg production. The fat level in the diet was 3.9% (Table I), and its source was a blend of meat and poultry byproduct.

Twenty-four laying hens of equal egg production were selected and randomly assigned to four groups of six layers each. The first three groups were fed the commercial diet to which 1, 2, and 3% menhaden oil were added, respectively. The fourth group was fed the commercial diet without added oil and served as a control. All four groups were fed over a 4-week period.

Freshly prepared menhaden oil was obtained from Standard Products Co., Inc. (Reedville, VA), and stabilized with 0.10% ethoxyquin (antioxidant) from Monsanto (St. Louis, MO) before it was mixed with the diet. A portion of menhaden oil without antioxidant was stored in the freezer for analysis of fatty acid composition.

During the feeding period, eggs were collected everyday and stored at -20°C for analysis. Weight gain, feed consumption, and egg production were recorded during the experimental period. Eggs laid on the last day were stored in the refrigerator for organoleptic evaluation. All experimental chickens were sacrificed at the end of the 4-week feeding period. The thighs of chickens were removed, placed in airtight bags, and stored at -20°C until fatty acid analysis and organoleptic evaluation.

Sample Collection and Analysis of Fatty Acid Methyl Esters. Each week three eggs were randomly selected from each group. Following the procedure of Ansah et al. (1985), eggs were thawed, allowing the egg yolks to separate from the whites easily. Three yolks from each group were homogenized in an Omnimixer (Omni Corp., Waterbury, CT) for 3 min at the highest speed. Adipose tissue was separated from the muscle tissue. Three thighs from each group collected at the end of the experiment were homogenized in the same manner as the egg yolks. The adipose tissue from the thighs in each group was also homogenized by the same procedure.

Lipids were extracted from all homogenized samples following the procedure of Bligh and Dyer (1959). Following the extraction lipids were saponified, and the free fatty acids were methylated following the procedure of Metcalfe et al. (1966).

The analysis of the fatty acid methyl esters was made with a Varian gas chromatograph Model 1700 Varian Aerograph (Walnut Creek, CA) equipped with flame ionization detectors and a cyanosilicone column (10% SP-2330) and a diethylene glycol polyester column (5% DEGS) (Supelco, Inc.). Carrier gas flow rates were 30 mL of N_2 min^{-1} , and detector flow rates were 30 mL of H_2 min^{-1} and 300 mL of air min^{-1} . The column temperature was 200°C , the injector temperature was 225°C , and the detector temperature was 250°C . The identification of individual fatty acids was made by using GLC-10 and PUFA-1 fatty acid methyl ester standards (Supelco, Inc.) to establish relative

Table II. Feed Consumption,^a Weight Gain,^a and Feed Conversion^a of Laying Hens Fed 0-3% Dietary Menhaden Oil for 4 Weeks

oil level, %	feed consumption, kg	weight gain, g	feed conversion, doz. eggs/kg feed
0	3.89	83	0.54
1	4.65	83	0.43
2	4.00	7.5	0.51
3	3.87	0	0.51

^a Means are not significantly different at $p > 0.05$.

Table III. Distribution of Fatty Acids in Country Egg Layer Ration and Menhaden Oil

fatty acid	country egg layer ration	menhaden oil
12:0	0.02	
14:0	0.33	10.26
14:1	0.11	
16:0	13.72	17.96
16:1	1.34	13.92
18:0	3.12	5.08
18:1	26.21	13.93
18:2	51.51	1.58
18:3	3.34	0.90
20:1		4.23
20:2	0.09	
20:3	0.15	
20:4		1.09
22:1		1.06
20:5		15.87
22:4		0.79
22:5		2.26
22:6		6.81

retention times. The relative content of each fatty acid methyl ester is reported as percent area of total fatty acid methyl esters.

Organoleptic Evaluation. Organoleptic evaluation was carried out by a panel of six trained graduate students in the Department of Food Science and Nutrition. They have served on a sensory panel that routinely evaluates fishery products and requires a keen sense of fish-related flavor, including fish oil. The panel was asked to score the intensity of residual fishy odor and taste in eggs and thighs on a 5-point scale (0 = not fishy, 1 = barely, 2 = slightly, 3 = moderately, 4 = apparently, 5 = pronounced). Eggs were either boiled or scrambled, while the thigh was baked in an oven for 1 h at 177°C . Both were prepared without seasonings.

Statistical Analysis. Statistical analyses of experimental results were made using analysis of the variance and Duncan's multiple range test for differences among the experimental means. A probability level of 0.05 was used to show statistically significant differences.

RESULTS AND DISCUSSION

Feed Consumption, Weight Gain, and Feed Conversion. Feed consumption, weight gain, and feed conversion (dozens of eggs per kilogram of feed consumed) of hens in the four groups are shown in Table II. Feed consumption did not change significantly ($p > 0.05$) in relation to dietary oil level. Decreasing weight gain associated with increasing dietary oil was also not significant ($p > 0.05$). This trend may be in response to associated increases in dietary caloric values. Dietary caloric values increased with increased dietary oil. No attempt was made to make the diets isocaloric. Increasing dietary oil meant that hens were able to meet their energy requirement by consuming less feed. Fully grown, mature laying hens are not expected to increase their body mass. The differences between 0 and 3% added menhaden oil in dietary protein to calorie ratios did not have an effect on the hens. Therefore, the 4-week feeding trial was probably not of sufficient duration to observe significant changes in feed consumption, weight gain, and feed conversion.

Table IV. Changes in the Fatty Acid Distribution (%) in Egg Yolks from Laying Hens Fed 0-3% Dietary Menhaden Oil over 4 Weeks^a

fatty acid	% oil over 1 week of feeding				% oil over 2 weeks of feeding				% oil over 3 weeks of feeding				% oil over 4 weeks of feeding			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
14:0	0.29	0.37	0.36	0.62	0.32	0.36	0.46	0.39	0.26	0.42	0.70	0.48	0.37	0.18	0.53	0.56
16:0	22.4	27.3	29.4	32.9	32.7	31.3	35.6	26.7	26.8	23.9	22.5	22.0	25.8	28.2	24.1	27.0
16:1	8.48	4.90	5.21								6.19	4.44	2.85		0.88	0.98
18:0	7.82	7.87	6.85	8.29	7.26	7.70	8.11	8.64	7.73	11.6	10.3	10.8	8.88	8.56	12.8	9.10
18:1	42.1	36.3	33.3	31.2	33.3	39.8	30.1	41.7	43.5	42.8	37.8	41.4	40.5	42.1	39.8	35.9
18:2	16.4	17.5	20.8	20.1	22.4	16.6	17.3	13.4	18.5	12.7	15.9	13.4	16.8	15.7	14.2	16.5
18:3		1.61											0.58	0.17	0.49	
20:1													0.31	0.04	0.10	
20:4	2.02	1.86	1.71	1.36	1.88	1.03	0.96	0.88	1.81	1.79	1.07	0.93	1.96	1.31	1.25	0.87
20:5		0.06		0.45		0.19	0.35	0.48		0.19	0.45	0.56	0.05	0.25	0.40	0.58
22:4	0.19	0.15	0.11	0.08	0.14	0.04	0.09	0.05	0.15	0.13	0.09	0.08	0.16	0.10	0.09	0.05
22:5	0.08	0.26	0.19	0.34	0.07	0.32	0.45	0.42	0.07	0.34	0.47	0.59	0.10	0.33	0.42	0.53
22:6	0.51	1.79	1.85	2.69	0.53	2.02	3.15	3.20	0.53	2.98	3.64	3.71	0.75	2.84	4.21	3.88

^a Mean of duplicate samples.

Table V. Changes in the Egg Yolk Fatty Acid Distribution of EPA^a with Four Levels of Dietary Menhaden Oil and 4 Weeks of Feeding

% oil	% EPA			
	week 1	week 2	week 3	week 4
0	0 ± 0 (a)	0 ± 0 (a)	0 ± 0 (a)	0.03 ± 0.04 (a)
1	0.06 ± 0.07 (a)	0.19 ± 0.01 (b)	0.19 ± 0.03 (a)	0.25 ± 0 (b)
2	0.01 ± 0 (a)	0.35 ± 0.06 (c)	0.45 ± 0.01 (c)	0.40 ± 0 (c)
3	0.50 ± 0.12 (b)	0.48 ± 0.03 (d)	0.56 ± 0.04 (d)	0.59 ± 0.01 (d)

^a Different letters following treatment means within each week indicate a significant difference (*p* < 0.05).

Table VI. Changes in the Egg Yolk Fatty Acid Distribution of DHA^a with Four Levels of Dietary Menhaden Oil and 4 Weeks of Feeding

% oil	% DHA			
	week 1	week 2	week 3	week 4
0	0.51 ± 0.06 (a)	0.53 ± 0.03 (a)	0.53 ± 0.01 (a)	0.75 ± 0.08 (a)
1	1.79 ± 0.09 (b)	2.02 ± 0.03 (b)	2.98 ± 0.24 (b)	2.72 ± 0.06 (b)
2	1.85 ± 0.36 (b)	3.15 ± 0.48 (c)	3.64 ± 0.09 (c)	4.21 ± 0.06 (d)
3	2.69 ± 0.54 (b)	3.20 ± 0.29 (c)	3.71 ± 0.27 (c)	3.83 ± 0.05 (c)

^a Different letters following treatment means within each week indicate a significant difference (*p* < 0.05).

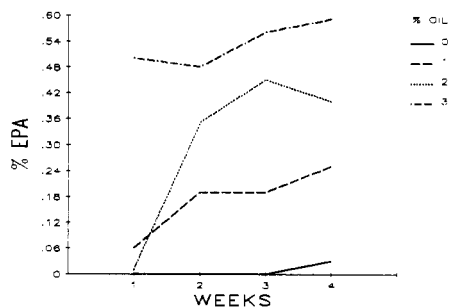


Figure 1. Changes in EPA in egg yolk over 4-week feeding trial.

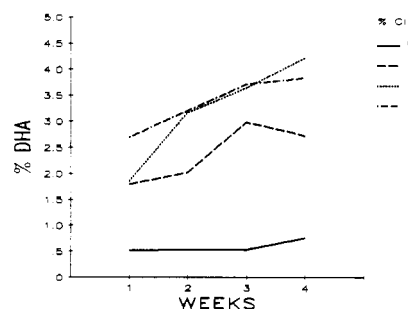


Figure 2. Changes in DHA in egg yolk over 4-week feeding trial.

Distribution of ω-3 Fatty Acids in Egg Yolk, Muscle, and Adipose Tissue. Table III shows the fatty acid distribution of the menhaden oil and of the poultry feed. Eicosapentaenoic acid (20:5ω3) and docosahexaenoic acid (22:6ω3) in the menhaden oil were 15.86 and 6.8%, respectively. EPA and DHA were not found in the poultry feed. Linolenic acid (18:3ω3) was 0.9% in the menhaden oil and 3.3% in the poultry feed. The overall fatty acid distribution in the egg yolk (Table IV) did not reflect that of the menhaden oil, except for the ω-3 fatty acids, as EPA and DHA were incorporated into the distribution. In the fatty acid distribution of egg yolk, EPA increased significantly (*p* < 0.05) with an increase in dietary oil level and time of feeding. DHA also increased signifi-

cantly (*p* < 0.05); however, the total increase was greater than the relative increase of EPA. The ratio of EPA to DHA in egg yolk was inversely proportional to the ratio of menhaden oil. Such a difference between the incorporation of EPA and DHA in eggs may be due to differences in the process by which hens assimilate EPA and DHA.

The incorporation of polyunsaturated fatty acids into egg yolk, especially EPA and DHA (Tables V and VI), increased with an increase in the level of menhaden oil in the diet, as well as with extended feeding (Figures 1 and 2). From the results, it can be concluded that the ω-3 fatty acids in egg yolk were dietary oil dependent for their distribution and accumulated with extended feeding.

Table VII. Distribution of EPA* in Egg Yolk, Thigh Meat, and Adipose Tissue Fatty Acids after 4 Weeks of Feeding

% oil	% EPA		
	egg yolk	thigh meat	adipose tissue
0	0.03 ± 0.04 (a)	0.00 ± 0.00 (a)	0.00 ± 0.00 (a)
1	0.25 ± 0.00 (b)	0.14 ± 0.09 (a)	0.06 ± 0.01 (a)
2	0.40 ± 0.00 (c)	0.27 ± 0.15 (a)	0.26 ± 0.08 (b)
3	0.59 ± 0.01 (d)	0.43 ± 0.38 (a)	0.36 ± 0.04 (b)

* Different letters following treatment means within each sample indicate a significant difference ($p < 0.05$).

Table VIII. Distribution of DHA* in Egg Yolk, Thigh Meat, and Adipose Tissue Fatty Acids after 4 Weeks of Feeding

% oil	% DHA		
	egg yolk	thigh meat	adipose tissue
0	0.75 ± 0.08 (a)	0.36 ± 0.24 (a)	0.00 ± 0.00 (a)
1	2.72 ± 0.06 (b)	0.96 ± 0.08 (a)	0.05 ± 0.01 (a, b)
2	4.21 ± 0.06 (c)	0.92 ± 0.08 (a)	0.11 ± 0.11 (b)
3	3.83 ± 0.05 (d)	1.43 ± 0.30 (b)	0.19 ± 0.05 (c)

* Different letters following treatment means within each sample indicate a significant difference ($p < 0.05$).

The fatty acid analysis showed that the highest content of ω -3 polyunsaturated fatty acid in the egg yolk was DHA and the second was EPA, even though the EPA content in the fish oil was higher than DHA (Table III). In earlier reports, the menhaden oil was fed to chickens, the distribution of these two fatty acids varied in the different organs and tissue of the chicken, namely, DHA > EPA in yolk (Couch and Saloma, 1973), EPA > DHA in adipose lipid, and DHA > EPA in liver and muscle (Miller et al., 1969).

EPA did not increase significantly ($p > 0.05$) in the fatty acid distribution of thigh muscle tissue (Table VII) in hens receiving dietary menhaden oil (Figure 3). DHA did increase significantly in the thigh muscle fatty acid distribution (Table VIII) when fish oil was added to the diet. However, the increase was not significant ($p > 0.05$) between groups receiving 1–3% oil (Figure 4). Given the maturity of the laying hens, it is probable that most of the dietary lipids are being utilized for egg production, and therefore a feeding period longer than 4 weeks might be necessary to obtain significant changes in EPA and further changes in DHA of the thigh muscle fatty acid distribution.

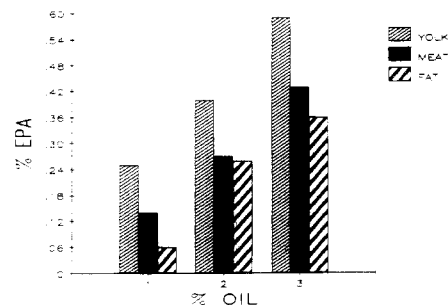
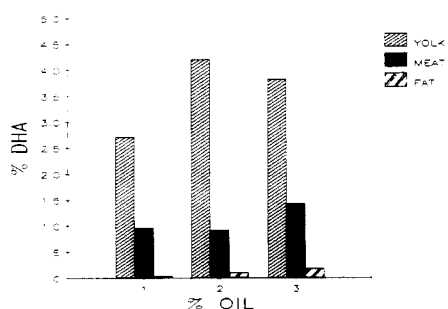
EPA in adipose lipids from hens fed 2 and 3% dietary fish oil was significantly higher ($p < 0.05$) than from hens fed 0 and 1% fish oil. There were no significant differences ($p > 0.05$) in adipose lipids EPA between 0 and 1% or between 2 and 3% dietary fish oil (Figure 3). DHA in adipose lipids from hens fed 1% dietary fish oil was not significantly different ($p > 0.05$) from hens receiving 0 or 2% dietary fish oil. The increase in DHA in adipose tissue from hens fed 2 and 3% fish oil was significantly higher than those fed 0% fish oil ($p < 0.05$) (Figure 4).

Table IV shows that some polyunsaturated fatty acids appeared in the egg yolk of the control group. Our results confirmed the previous report of Smith et al. (1980), which indicated that DHA was found in the eggs although it was absent in the diet. This was also supported by the report of Choudhury et al. (1959) in which the addition of trienoic acid resulted in increases in all polyunsaturated fatty acids having from two to six double bonds. The fatty acid distribution in the poultry feed contained 3.3% linolenic acid. According to the report of Murty and Reiser (1961), linoleic acid is the precursor of arachi-

Table IX. Organoleptic Evaluation of Egg and Thigh Meat of Hens Fed Dietary Menhaden Oil for 4 Weeks

oil level, %	sample	organoleptic score ^a
0	egg (boiled)	0
	egg (fried)	0
	thigh (baked)	0
1	egg (boiled)	0
	egg (fried)	0.16
	thigh (baked)	0
2	egg (boiled)	0
	egg (fried)	0.16
	thigh (baked)	0
3	egg (boiled)	0
	egg (fried)	0.50
	thigh (baked)	0

^a Average score of six judges on a 5-point intensity scale (0 = not fishy, 1 = barely, 2 = slightly, 3 = moderately, 4 = apparently, 5 = pronounced).

**Figure 3. EPA in yolk, thigh meat, and adipose tissue after feeding oil for 4 weeks.****Figure 4. DHA in yolk, thigh meat, and adipose tissue after feeding oil for 4 weeks.**

donic and docosapentaenoic acids, whereas linolenic acid is the precursor of EPA and DHA. To increase EPA and DHA content in the egg yolk, either trienoic acids or lipids rich in EPA and DHA could be added. Although there is a small amount of trienoic acids in menhaden oil, it is rich in EPA and DHA. Therefore, menhaden oil is a good supplemental lipid for the purpose of increasing EPA and DHA in egg yolk. It also increased the levels of these fatty acids in muscle, but not as much as those in egg yolk. Feeding trials longer than 4 weeks may result in further increases in ω -3 fatty acids. Younger growing hens with increasing body mass may show a greater incorporation of EPA and DHA in muscle and adipose tissue in response to dietary menhaden oil. The purpose of this experiment, however, was to incorporate ω -3 fatty acids into the fatty acid distribution of egg yolks by adding menhaden oil to laying hen ration.

Organoleptic Evaluation of Egg and Thigh. The panel scores in Table IX indicated that when menhaden oil was added up to 3% in the diet, the organoleptic scores of eggs and thighs from experimental chickens were still acceptable. In the 1930s Vondell et al. (1939) had reported

that the heavy feeding of fish products did not produce an offending flavor. However, Rhode Island Red birds (Strair) receiving a diet containing 15% rapeseed meal produced eggs having fishy odors. Leeson and Summers (1978) explained that high choline content of both rapeseed and soybean gums may be the contributing factors of fishy odors in eggs from the Rhode Island Red hens. Today, most researchers agree that the presence of polyunsaturated fatty acids in the egg results in a fishy flavor. Miller et al. (1967b) pointed out that marine fatty acids (18:4 ω 3, 20:4 ω 3, 20:5 ω 3, and 22:6 ω 3) deposited in the flesh of broilers highly correlated with the occurrence of the unacceptable flavor.

The results of this study indicated that ω -3 polyunsaturated fatty acids in the egg yolk can be increased without causing a fishy flavor by feeding up to 3% fish oil, stabilized with 0.1% ethoxyquin to prevent rancidity. The lack of fishy flavor in eggs and thigh flesh in this study compared to previous studies could be related to the stabilization of the oil in this study with antioxidant.

Further studies are needed to determine how dietary ω -3 fatty acids are metabolized and assimilated into egg yolk and tissue. The ability of dietary ω -3 polyunsaturated fatty acids to lower cholesterol levels in the egg yolk is under investigation since cholesterol has been implicated in the occurrence of heart disease and atherosclerosis.

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Received for review May 15, 1989. Accepted November 7, 1989.
University of Rhode Island Agriculture Experiment Station Contribution No. 2359.