Yolk Fatty Acid Composition and Cholesterol Content in Response to Level and Form of Dietary Flaxseed

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The effect of the level and form of dietary flaxseed on the fatty acid composition and cholesterol content of hen egg has been investigated. Dietary flaxseed notably altered yolk fatty acid composition by increasing total polyunsaturated fatty acids while decreasing monounsaturated fatty acids. α-Linolenic acid was linearly increased in response to levels of ground flaxseed, but whole flaxseed resulted in reduced α-linolenic acid availability for yolk deposition. Similar deposition profiles were exhibited by docosapentaenoic and docosahexaenoic acids. These alterations in yolk composition resulted in n−6 to n−3 fatty acid ratio values as low as 2.92 and 1.94 for 5 and 10% ground flaxseed and 2.49 for 10% whole flaxseed feeding, respectively. Dietary flaxseed had no effect on the cholesterol level of eggs, which showed a mean value of 188 mg/egg or 1247 mg/100 g of yolk.

Keywords: *Flaxseed; cholesterol; n−3 fatty acids; yolk; egg*

INTRODUCTION

Extensive early research has indicated that foods high in cholesterol are directly related with a rise in blood cholesterol, which in turn has long been associated with increased incidence of coronary heart disease in humans (Renaud and De Lorgeril, 1994). As a result, a public consensus that limiting dietary cholesterol contributes to good health has emerged in the past two decades, whereas consumption of eggs has experienced a considerable decline. Because consumers regard eggs as a high-cholesterol dietary product, marketing trends in the egg industry have centered on improving the health quality of eggs through reduction in yolk cholesterol. Several genetic, management, nutrition, and pharmacological approaches have been explored, but most have met with only marginal success (Hargis, 1988). A number of interesting approaches have dealt with supplementing the hens' diet with several kinds of fat and plant sterols. These approaches look promising, but their results contradict each other and, thus, their efficacy in lowering yolk cholesterol is still questionable (Naber, 1983; Adams et al., 1989; Hargis et al., 1990).

Another way to improve the health quality of eggs is through alteration of their fatty acid composition. Recent metabolic and epidemiological research has demonstrated that the cholesterogenic effects of foods should not be evaluated only on the basis of their cholesterol content but rather on the relationship between cholesterol and unsaturated fat in the diet (Grundy and Denke, 1990; Hayes et al., 1991; Hopkins, 1992). Studies on humans with diets rich in egg cholesterol have shown that blood cholesterol scarcely increases when the ratio of the polyunsaturated to saturated fatty acids in the diet is relatively high (Schonfeld et al., 1982; Edington, 1987; Edington et al., 1989). Incorporation of polyunsaturated fatty acids into yolk could counteract, thus, the cholesterogenic effects of eggs, providing a viable means of increasing consumer acceptance.

Dietary manipulation of the fatty acid composition of yolk is not a new concept. Modification of yolk fatty acids through feeding hens with different fats has been reported previously (Cruickshank, 1934). Later, other investigators (Reiser, 1950; Wheeler et al., 1959; Murty and Reiser, 1961; Navarro et al., 1972; Couch and Saloma, 1973) unequivocally demonstrated alteration in yolk fatty acids using dietary fish meal or oil and seeds or seed oil. The renewed interest in the dietary enrichment of eggs with specific fatty acids stems from recent reports of the numerous protective effects of the n−3 fatty acids against cardiovascular diseases, cancer, and rheumatoid arthritis (Leaf and Weber, 1988; Fernandes and Venkatraman, 1993). Metabolism of n−3 fatty acids results in production of eicosanoids with greatly reduced capacity to induce inflammatory responses or to stimulate aggregation of platelets (Harris, 1989; Kinsella et al., 1990; Kromhout, 1992). Thus, public interest in n−3 fatty acid-enriched eggs has been demonstrated (Marshall et al., 1994).

Currently, ground flaxseed, a feedstuff of plant origin, and menhaden oil, a fish byproduct, are the only rich sources of n−3 fatty acids available to the poultry industry for diet formulation. Both have been shown to dramatically increase the n−3 fatty acid content of eggs (Caston and Leeson, 1990; Jiang et al., 1991a, 1992; Hargis and Van Elswyk, 1993). An unfortunate drawback to use of fish oils as feed ingredients, in general, is that they are easily susceptible to oxidation (Frankel, 1984). In contrast, flaxseed is fairly resistant to oxidation (Aymond and Van Elswyk, 1995).

Most previous studies on flaxseed use in laying hen diets have dealt with ground flaxseed (Caston and Leeson, 1990; Cherian and Sim, 1991; Lee et al., 1991; Jiang et al., 1991a, 1992). However, feeding whole flaxseed eliminates seed grinding costs and has reduced potential for lipid oxidation during long feed storage as compared with ground flaxseed. Two recent investigations have examined the use of whole flaxseed, but their results contradict each other as far as the efficacy of

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whole versus ground flaxseed in the modification of the n−3 fatty acid composition is concerned (Aymond and Van Elswyk, 1995; Scheideler and Froning, 1996).

The objectives of the current study were to determine the effect of dietary flaxseed on the fatty acid and cholesterol composition of egg yolk. Determination of the level and form of the dietary flaxseed that could successfully achieve the desired fatty acid composition of yolk while maintaining an acceptable egg cholesterol level and requiring minimum changes in management practices would be of value.

MATERIALS AND METHODS

Instrumentation. A capillary column gas chromatographic system (model GC-15A, Shimadzu Corp., Kyoto, Japan) equipped with a flame ionization detector, an automatic sampler, model AOC-17, and a model Class-VP chromatography data system was used in this study. A temperature-regulated water bath (type 3044, Kottermann, Germany), a vortex mixer (model G-560E, Scientific Industries, Inc., Bohemia, NY), a centrifuge (model Centra-MP4; IEC, Needman Heights, MA), a magnetic stirrer plate (Fisher Scientific, PA), solvent dispensers (models P1000 and P5000, Gilson, Villiers-le-Bel, France), and sample preparation tubes, 16 × 12.5 mm, with Teflon-lined screw caps (Corning, NY) were used for sample treatment.

Chemicals. Reference fatty acid methyl ester and cholesterol standards were from Sigma Chemical Co. (St. Louis, MO). All other analytical grade reagents and solvents were from Merck (Darmstadt, Germany).

Hens and Diets. Forty-eight Lohman laying hens, 35 weeks of age, were randomly distributed in 12 pens with four birds per pen. The birds were allotted to four dietary treatments with each treatment replicated three times. Dietary treatments included a typical corn−soybean-based layer ration, which did not contain flaxseed and served as the control diet, and three rations enriched with 5% ground, 10% ground, and 10% whole flaxseed, respectively. All diets were formulated to meet the requirements for nutrient and energy content for laying hens (National Research Council, 1984), stored in airtight containers, and given to hens ad libitum daily. Egg sample collection commenced 4 weeks after feeding the dietary treatment and lasted 2 weeks.

Egg Sampling. All eggs collected during the 2-week trial were stored at 4 °C, pending analysis for fatty acid and cholesterol content. The day of analysis, three eggs from each pen, totaling nine eggs per dietary treatment, were randomly selected. The eggs were broken, yolks were separated, and adhering albumen was removed by rolling on a paper towel. Yolks were weighed, and ∼1 g aliquots were sampled from each yolk for cholesterol analysis. Following sampling, all yolk quantities within each dietary treatment were pooled and mixed well with a wire whisk, and three aliquots were taken from each of the four pools for fatty acid analysis.

Yolk Fatty Acid Analysis. Fatty acids were extracted and converted to methyl esters according to the one-step extraction−transesterification procedure described by Sukhija and Palmquist (1988). Separation and quantification of the fatty acid methyl esters were carried out by gas chromatography on a fused silica capillary column, 30 m × 0.32 mm i.d., coated with cyanopropyl polysiloxane (phase type 007-23) with film thickness of 0.25 μm (Quadrex Scientific, Surrey, U.K.). Column temperature was set initially at 70 °C and maintained for 0.3 min; it was then increased to 180 °C at a rate of 30 °C/min and held there for 10 min; finally, column temperature was elevated to 225 °C at a rate of 5 °C/min and held there for 3 min. The temperature of both the injection port and the flame ionization detector was set at 240 °C. Helium carrier gas flow was set at 1 mL/min, hydrogen at 30 mL/min, and air at 300 mL/min. Injection of the 1-μL samples was performed with a split ratio of 20:1. Identification of individual fatty acids was based on comparisons of retention times of unknown peaks to authentic fatty acid methyl ester standards. The relative content of each fatty acid methyl ester was reported as percent area of total fatty acid methyl esters.

Table 1. Fatty Acid Composition of Flaxseed

type of fatty acid	fatty acid concn in flaxseed, wt %	type of fatty acid	fatty acid concn in flaxseed, wt %
$C_{14:0}$	0.03	$C_{18:1}$	6.00
$C_{16:0}$	2.33	$C_{18:2(n-6)}$	5.34
$C_{16:1}$	0.03	$C_{18:3(n-3)}$	18.01
$C_{18:0}$	1.33	≥C_{20}	0.03

Cholesterol Analysis. Determination of cholesterol in egg yolk was carried out by a direct saponification gas chromatographic method developed previously (Botsoglou et al., 1998) and routinely applied in our laboratory. The performance characteristics of this method are excellent, showing an overall recovery of 98.8%, quite acceptable linearity ($r = 0.9964$), and an overall precision better than 2.0%. According to this method, yolk sample (0.2 g) was mixed with 5 mL of methanolic potassium hydroxide solution (0.5 M) and heated in an 80 °C bath for 15 min. Following heating, the mixture was cooled, and 1 mL of water was added. Extraction of cholesterol was carried out using 5 mL of hexane. A 1-μL aliquot from the extract was submitted to gas chromatographic analysis on a fused silica capillary column, 15 m × 0.32 mmi.d., coated with SPB-1 (Supelco Inc, Bellefonte, PA) with film thickness of 1.0 μm. The column temperature was programmed from 250 to 275 °C at 2 °C/min and held there for 12 min. Injection port temperature was set at 300 °C, whereas the flame ionization detector temperature was set at 300 °C. Helium carrier gas was set at 2 mL/min, hydrogen at 30 mL/min, and air at 300 mL/min. All analyses were performed with a split ratio of 20:1.

Statistical Analysis. The experimental data were analyzed statistically using analysis of variance. Significant differences among treatments were tested using Duncan's test (Duncan, 1955). Significance is reported at $P < 0.05$.

RESULTS AND DISCUSSION

Flaxseed is one of the most concentrated sources of α-linolenic acid ($C_{18:3(n-3)}$) available in natural plant feedstuffs for poultry. Flaxseed contains as much as 35% crude fat, of which nearly 50% is α-linolenic acid (Table 1). Incorporation of flaxseed into hens' diet notably altered egg fatty acid composition; total polyunsaturated fatty acids (PUFA) were significantly increased, whereas monounsaturated fatty acids (MUFA) were significantly decreased (Table 2).

The decrease in MUFA observed in the eggs of the flaxseed diets was mainly contributed by oleic acid ($C_{18:1(n-9)}$). This decrease was less pronounced in treatments when a significantly higher n−6 to n−3 fatty acid ratio occurred. Therefore, the n−6 to n−3 fatty acid ratio might be an important marker for the production of oleic acid (Garg et al., 1988). The decrease in MUFA noted in this study lends support to previous investigations (Jiang et al., 1991a; Ahn et al., 1995), which are inconsistent, however, with other reports on this subject (Hargis and Van Elswyk, 1993; Herber and Van Elswyk, 1996). Recently, MUFA have been a subject of interest due to their possible hypolipidemic and antithrombotic effects (Grundy, 1987).

The increase in PUFA observed in the eggs of all flaxseed diets was due to both n−6 and n−3 fatty acids. However, the increase in n−6 fatty acids was represented solely by linoleic acid ($C_{18:2(n-6)}$), whereas that of the n−3 fatty acids was mainly contributed by α-linolenic acid. It might also be of interest to note that the increase of linoleic and α-linolenic acid in yolk total lipids was in place of palmitoleic ($C_{16:1}$) acid and oleic

Table 2. Dietary Effect of Flaxseed on Yolk Fatty Acid Composition

type of fatty acid	yolk fatty acid composition (% of total fatty acids)			
	control	5% ground flaxseed	10% ground flaxseed	10% whole flaxseed
$C_{14:0}$	0.36 ± 0.00	0.31 ± 0.01	0.26 ± 0.00	0.30 ± 0.03
$C_{16:0}$	24.41 ± 0.13	22.58 ± 0.16	22.11 ± 0.15	22.27 ± 0.14
$C_{16:1}$	3.65 ± 0.15	3.17 ± 0.02	2.97 ± 0.10	3.05 ± 0.05
$C_{18:0}$	9.07 ± 0.05	8.86 ± 0.18	9.04 ± 0.27	9.30 ± 0.16
$C_{18:1}$	41.26 ± 0.06	39.72 ± 0.28	37.20 ± 0.16	38.05 ± 0.18
$C_{18:2(n-6)}$	15.43 ± 0.09	16.14 ± 0.11	16.69 ± 0.17	16.50 ± 0.17
$C_{20:0}$	0.00 ± 0.00	0.00 ± 0.00	0.12 ± 0.01	0.11 ± 0.00
$C_{18:3(n-3)}$	0.91 ± 0.03	3.14 ± 0.03	5.19 ± 0.07	4.11 ± 0.07
$C_{20:1(n-9)}$	0.35 ± 0.03	0.26 ± 0.02	0.27 ± 0.00	0.31 ± 0.02
$C_{20:2(n-6)}$	0.00 ± 0.00	0.12 ± 0.00	0.19 ± 0.02	0.15 ± 0.02
$C_{20:4(n-6)}$	1.94 ± 0.03	1.52 ± 0.06	1.48 ± 0.04	1.55 ± 0.09
$C_{20:5(n-3)}$	0.00 ± 0.00	0.14 ± 0.01	0.21 ± 0.01	0.17 ± 0.00
$C_{22:4(n-6)}$	0.26 ± 0.02	0.12 ± 0.02	0.00 ± 0.00	0.13 ± 0.01
$C_{22:5(n-3)}$	0.10 ± 0.01	0.30 ± 0.02	0.54 ± 0.04	0.37 ± 0.03
$C_{22:6(n-3)}$	1.33 ± 0.04	2.54 ± 0.05	3.53 ± 0.06	2.72 ± 0.15
Σ_{SFA}	33.84	31.75	31.53	31.98
Σ_{MUFA}	45.26	43.15	40.44	41.41
Σ_{PUFA}	19.97	24.02	27.83	25.70
$\Sigma_{n-6PUFA}$	17.63	17.90	18.36	18.33
$\Sigma_{n-3PUFA}$	2.34	6.12	9.47	7.37
$\Sigma_{n-6}/\Sigma_{n-3}$	7.53	2.92	1.94	2.49

acid, which corroborates a previous publication (Chen et al., 1965).

Egg α-linolenic acid increased linearly as the ground flaxseed increased from 5 to 10% in the diet. In contrast, use of whole flaxseed in the diet resulted in reduced α-linolenic acid availability for yolk deposition as compared to ground flaxseed at the same level. Grinding of flaxseed before feed formulation appeared to increase the deposition of yolk α-linolenic acid. Nevertheless, the availability of α-linolenic acid from 10% whole flaxseed was better than that from 5% ground flaxseed. It seems that whole flaxseed, which has a relatively soft seed coat, could not be adequately pulped within the hen gizzard. This observation lends support to the results of Aymond and Van Elswyk (1995), who also reported greater deposition of n−3 fatty acids in eggs from a different breed of laying hens (White Leghorn) fed 15% ground versus whole flaxseed, but it is in contrast to the results of Scheideler and Froning (1996), who reported no significant difference between ground and whole flaxseed with White Leghorn hens.

The deposition profiles of α-linolenic acid and linoleic acid presented in Table 2 are consistent with previous investigations. Caston and Leeson (1990) have reported values of 14.6% for linoleic acid and 4.6% for α-linolenic acid in eggs from hens fed 10% flaxseed, which are comparable to the values of 16.7% for linoleic and 5.2% for α-linolenic acid found in this study from hens fed 10% flaxseed. Jiang et al. (1991a), after feeding hens with 15% ground flaxseed, reported a level of 6.9% for α-linolenic acid, a level that cannot be compared with but could be accounted for by the results of this study.

Apart from α-linolenic acid, moderate levels of eicosapentaenoic ($C_{20:5(n-3)}$) and docosapentaenoic ($C_{22:5(n-3)}$) acids and high levels of docosahexaenoic acid ($C_{22:6(n-3)}$) were also produced in eggs following flaxseed feeding. The production of these fatty acids was at the expense of oleic acid and arachidonic ($C_{20:4(n-6)}$) acid. Considering that eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids do not occur in flaxseed (Table 1), their increasing production in response to the level of the flaxseed incorporation into diets indicated that there should be an efficient conversion mechanism for the desaturation and chain elongation of the amount of α-linolenic acid initially present in flaxseed. Nettleton (1991) has reported that α-linolenic acid can serve as precursor for the in vivo production of these n−3 fatty acids; in hen liver, α-linolenic acid can be desaturated−elongated to eicosapentaenoic and docosahexaenoic acids, whereas dietary linoleic acid is converted to arachidonic acid. Brenner et al. (1969) have also reported that the efficient conversion of α-linolenic acid is probably limited by the competition with linoleic acid for Δ^6-desaturase, the enzyme essential for the first step in the desaturation of these n−3 and n−6 fatty acids. These authors further reported that α-linolenic acid is the preferred substrate over linoleic acid, thus limiting the biosynthesis of arachidonic acid from linoleic acid, a mechanism which is in accordance with the results of the present study.

Although the alteration in yolk fatty acid composition caused by the flaxseed diets used in this study differs quantitatively from that reported in some investigations (Caston and Leeson, 1990; Cherian and Sim, 1991; Jiang et al., 1991a), the large increase in n−3 fatty acids and decrease in n−6 fatty acids were the same. The ratio of n−6 to n−3 PUFA in yolk total lipids was significantly reduced by flaxseed feeding, consistent with aforementioned studies. The ratio values of 2.92 and 1.94 for 5 and 10% ground flaxseed and 2.49 for 10% whole flaxseed feeding (Table 2) confirm that use of dietary flaxseed can help to ensure a reliable and consistent egg product in the designer food market (Hargis and Van Elswyk, 1993).

Unlike fatty acids, yolk cholesterol was not responsive to dietary manipulation. Results showed that cholesterol levels were not influenced by dietary flaxseed in any of the treatments investigated (Table 3). This

Table 3. Effect of Dietary Flaxseed on the Cholesterol Content of Egg

control diet		5% ground flaxseed diet		10% ground flaxseed diet		10% whole flaxseed diet	
mg/100 g of yolk	mg/egg	mg/100 g of yolk	mg/egg	mg/100 g of yolk	mg/egg	mg/100 g of yolk	mg/egg
1142.7	195.4 (17.1)[a]	1220.2	192.8 (15.8)	1288.3	188.1 (14.6)	1232.6	201.6 (17.8)
1200.6	194.5 (16.2)	1305.0	184.0 (14.1)	1169.9	194.2 (16.6)	1331.1	183.7 (13.8)
1190.2	194.0 (16.3)	1131.4	194.6 (17.2)	1282.6	184.7 (14.4)	1258.8	186.3 (14.8)
1259.5	186.4 (14.8)	1185.3	193.2 (16.3)	1314.1	186.6 (14.2)	1254.0	188.1 (15.0)
1237.1	186.8 (15.1)	1225.8	190.0 (15.5)	1326.1	183.0 (13.8)	1224.3	191.0 (15.6)
1289.4	183.1 (14.2)	1305.6	185.4 (14.2)	1250.0	187.5 (15.0)	1292.9	182.3 (14.1)
1241.7	187.5 (15.1)	1258.1	186.2 (14.8)	1229.6	186.9 (15.2)	1219.6	192.7 (15.8)
1320.6	179.6 (13.6)	1262.4	188.1 (14.9)	1275.3	186.2 (14.6)	1234.4	190.1 (15.4)
1253.3	188.0 (15.0)	1266.9	183.7 (14.5)	1182.9	194.0 (16.4)	1272.2	183.2 (14.4)
1237.2 ± 50.5	188.4 ± 5.1 (15.5 ± 1.0)	1240.1 ± 53.2	187.7 ± 3.9 (15.2 ± 1.0)	1257.6 ± 51.5	187.9 ± 3.6 (15.0 ± 0.9)	1246.6 ± 52.2	188.8 ± 5.7 (15.2 ± 1.1)

[a] Values in parentheses represent yolk weight in grams.

finding is in contrast to investigations reporting increase (Naber, 1983) or decrease (Adams et al., 1989; Oh et al., 1991) of egg cholesterol through use of fat or fatty acid-enriched diets. It is consistent, however, with other studies reporting that egg cholesterol is fairly resistant to dietary reduction (Hargis et al., 1990; Scheideler and Froning, 1996; Grimes et al., 1996).

Table 3 shows that the cholesterol content per egg ranged from 179.6 to 201.6 mg for yolk weights of 13.6 and 17.8 g, respectively, with an overall mean of ~188 mg of cholesterol/egg. These results indicate that even the larger egg (74.2 g of whole egg, corresponding to 17.8 g of yolk weight) in this study contained much lower cholesterol than the value of 274 mg/egg initially estimated by the USDA (1976) and still lower cholesterol than the value of 213 mg/egg recently reset for a 50-g egg (USDA, 1989). This discrepancy is generally difficult to explain, although the accuracy of several analytical methods to determine cholesterol concentrations has been questioned by many authors (Beyer and Jensen, 1989; Ulbrecht and Reich, 1992). Jiang et al. (1991b) compared colorimetric, enzymatic, gas chromatographic, and liquid chromatographic techniques to determine cholesterol in eggs and found values of 282.8, 204.6, 194.6, and 194.0 mg/egg, respectively. Marshall et al. (1989), using gas chromatography for the analysis of cholesterol in a variety of mixed diets, have found values representing 75% of those obtained by calculating contents from food composition tables, whereas the gas chromatography results were 50% of the values obtained by a colorimetric technique. It is obvious that different analytical techniques can provide different results.

Table 3 further shows that the cholesterol concentration in yolk ranged from 1320.6 to 1132.6 mg/100 g of yolk for yolk weights of 13.6 and 17.8 g, respectively. It is apparent that the two popular ways to express cholesterol values, milligrams per egg or milligrams per 100 g of yolk, exhibit opposite trends. In terms of milligrams per egg, smaller eggs have a lower cholesterol value than larger eggs. On the other hand, smaller eggs show a higher cholesterol concentration than the larger eggs when the concentration is expressed in milligrams per 100 g of yolk. This is in agreement with the hypothesis that there might be a cholesterol-sensing mechanism that controls cholesterol deposition in yolk so as to provide adequate cholesterol for embryo survival (Marks and Washburn, 1977). The lack of a standard to express cholesterol values coupled with the inadequacy of the analytical methods seems to be a major reason for the conflicting results in the literature as far as the effect of the supplementation of hen diets with n−3 fatty acids on the cholesterol content of the produced eggs is concerned.

In conclusion, the results of this study show that dietary flaxseed notably alters yolk fatty acid composition by increasing total PUFA while decreasing the MUFA. α-Linolenic acid is linearly increased in response to levels of ground flaxseed, but whole flaxseed results in reduced α-linolenic acid deposition. This alteration in yolk composition results in n−6 to n−3 fatty acid ratio values of 2.92 and 1.94 for 5 and 10% ground flaxseed, respectively, and 2.49 for 10% whole flaxseed feeding. Dietary flaxseed appears to exert no effect on the cholesterol level of eggs.

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