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Fatty acid profile of egg yolk lipids from hens fed diets rich in n-3 fatty acids

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Abstract

The effects of diet and of strain on lipid, cholesterol and fatty acid compositions of egg yolk were studied. Two hen strains (Red Lohman—RL and White Lohman—WL) and four experimental diets with different fatty acid composition were used. No significant ($P<0.01$) effects of diets and strain on the cholesterol or lipid contents of egg yolk were obtained. The fatty acid composition of yolk lipids was affected ($P>0.05$) by the experimental diets. The major effects of the diets were observed in fatty acids C16:0, C18:0, C18:1n9, C18:2n6, C20:4n6, C20:5n3 and C22:6n3. The addition of oils to the diets fed to hens allowed the production of eggs with higher n3/n6 and PUFA/SFA fatty acid ratios than the eggs from control hens. It was concluded that the amounts of saturated and unsaturated fatty acids in egg yolk could be altered by dietary manipulation.

Keywords: Egg yolk; Lipids; Cholesterol; Fatty acid; Vegetable oils

1. Introduction

Hen egg yolk contains around 30% of lipids; therefore it is a rich source of lipid. The belief that human health may be improved by a reduction of animal fat consumption has undoubtedly decreased the consumption of eggs, due to their concentrated fat content (Tullet, 1987).

In recent years, the lipid composition of chicken egg has been an area of primary consumer concern, due to the connection between specific dietary lipids and the development of coronary heart disease and some forms of cancer (Simopoulos & Salem, 1992).

Although cholesterol levels in eggs are indeed high, recent studies show that the nutritional quality of the fat in food products should be evaluated by taking into account not only their cholesterol levels, but also their contents of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. Higher levels of PUFA and MUFA and lower levels of SFA could decrease the negative effects of high cholesterol intakes (Grundy & Denke, 1990; Hayes, Pronczuk, Lindsey, & Diersen-Shade, 1991; Hegsted, McGandy, Myers, & Stare, 1965; Hopkins, 1992; Keys, Anderson, & Grande, 1965; Khosla & Hayes, 1992; Pyorala, 1987; Rudel, Haines, & Sawier, 1990).

In a review, Stadelman and Pratt (1985) noticed that the lipid content of hen eggs is affected by genetics, age, feeding programmes, and also by the levels and types of dietary lipids. When hens were fed diets containing oleate (Donaldson, 1967; Pankey & Stadelman, 1969) and linoleate (Guenter, Braggs, & Kondra, 1971; Murty & Reiser, 1961), the respective dietary fatty acids were readily incorporated into the egg yolk.

The objective of the present work was to investigate the effect of the four diets rich in n-3 fatty acids in order to check the possibility of producing eggs with different fatty acid compositions.

2. Materials and methods

2.1. Animals and diets

At 20 weeks of age, 96 red Lohman (RL) and 96 white Lohman (WL) hens were housed in cages and were assigned (24 hens per group) to four experimental diets.

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Diet 1 contained 58.2% of corn, 10.1% of canola meal and 3.2% of canola oil. Diet 2 contained 59.0% of corn, 2.3% of flax meal and 3.0% of flax oil. Diet 3 contained 58.4% of corn, 26.6% of soybean meal and 3.0% of soybean oil. Diet 4 contained 59.1% of corn, 8.0% of sunflower meal and 2.9% of sunflower oil and the Control diet contained 61.9% of corn, 25.7% of soybean meal and 2.0% of soybean oil (Table 1).

2.2. Sample collection

For the determination of total lipids, cholesterol and fatty acid composition, five eggs from each dietary treatment were randomly selected and analyzed at the end of the 16th week of experimental feeding. The yolk from each egg was separated and held in polyethylene packing (in N_2 atmosphere) at −18 °C. At the beginning of each analysis, the samples were allowed to achieve room temperature and homogenized.

2.3. Analysis

Moisture, ash and protein contents were determined as described by Cunniff (1998). Lipids were extracted from the egg yolks using the Folch, Lees, and Sloane-Stanley (1957) method, and the fatty acid methyl esters (FAME) were prepared by methylation of the triacylglycerols, as described by ISO method 5509 (1978). The FAME were analyzed by a Shimadzu 14A (Japan) gas chromatograph, equipped with flame ionization detector (FID) and fitted with a fused silica capillary column (50 m×0.25 mm i.d. and 0.20 μm of Carbowax 20M). Column temperature was programmed at 2 °C/min from 150 to 245 °C. Injector and detector temperatures were 220 and 240 °C, respectively. The carrier gas was hydrogen (1.2 ml/min) and the make-up gas was nitrogen (30 ml/min). The split used was 1:100. Peak areas were determined by the CG-300 Computing integrator (CG Instruments, Brazil) and FAME identification was done by comparison with retention times of the known standards from Sigma (USA). Data were calculated using the normalized peak area percentages of fatty acids.

The extraction and quantification of cholesterol were carried out by the method published by Al-Hasani, Mlavac, and Carpenter (1993). Samples of egg yolk (10.000±0.001 g) were transferred to a 250 ml flat-bottom flask. The sample was stirred in an ethanol–methanol–isopropanol (90:5:5, v/v/v) solution, in an amount equivalent to 4 ml/g sample, and 1 ml 60% KOH/g sample. The flask containing the mixture was connected to the water-cooled condenser, and refluxed for 1 h. After cooling the digest to room temperature, 100 ml of hexane were added and the mixture was stirred for 10 min. Next, 25 ml of deionized water were added and the mixture was stirred for a further 15 min. The layers were then separated and the hexane layer was collected in an Erlenmeyer flask. An aliquot of 25 ml from the hexane layer was evaporated in a rotatory evaporator at 37 °C. The residue was dissolved in 2 ml of hexane containing 0.2 mg/ml of standard internal 5α-cholestane, and 3 μl were injected into a gas chromatograph. A Shimadzu (Japan) chromatograph model 14A, fitted with flame ionization detector (FID, 300 °C) and a split/splitless injector (260 °C, split 1:150) were used for the cholesterol analysis. Separation was carried out in a fused silica capillary column (25 m; 0.25 mm i.d.), coated with SE-30 (0.25 mm phase thickness), at 300 °C. The carrier gas was hydrogen (1.5 ml/min) and the make-up gas was nitrogen (25 ml/min). Cholesterol identifications were achieved by a comparison between the relative retention time peaks from the samples and the standards from SIGMA. For peak integration, a CG-300 Computing integrator (CG Instruments, Brazil) was used.

Table 1
Compositions of the experimental diets[a]

Ingredients (%)	Diet 1	Diet 2	Diet 3	Diet 4	Control diet
Corn	58.176	58.983	59.179	59.134	61.895
Soybean meal	18.238	24.531	26.582	19.446	25.677
Canola meal	10.113	–	–	–	–
Flaxseed meal	–	2.269	–	–	–
Sunflower meal	–	–	–	8.000	–
Limestone	7.741	8.415	8.348	7.791	7.781
Canola oil	3.200	–	–	–	–
Flaxseed oil	–	3.000	–	–	–
Soybean oil	–	–	3.000	–	1.976
Sunflower oil	–	–	–	2.891	–
Bicalcium phosphate	1.782	1.874	1.983	1.800	1.881
Salt	0.350	0.350	0.350	0.350	0.350
Min + Vit suplemment	0.400	0.400	0.400	0.400	0.400
DL-Met 99%	–	0.151	0.158	0.053	0.040
L-Lys HCl	–	0.027	–	0.135	–
Total	100.000	100.000	100.000	100.000	100.000

[a] Data provided by the Department of Animal Science Laboratory of the State University of Maringá.

3. Statistical analysis

The results were submitted to variance analysis (ANOVA), at 5% significance level, by the Statistica Software (StatSoft, USA, 1996) version 5.0. The mean values were compared by the Tukey test.

4. Results and discussion

Table 2 shows the composition of the diets supplied to hens. It was verified that the canola and sunflower diets had similar compositions, while the soy and flaxseed diets had smaller contents of protein and fat. Diet 1 had low percentages of saturated and polyunsaturated fatty

Table 2
Chemical composition (wet matter %) and fatty acids in relation to the saturation (%) of experimental diets[a]

Diets	1	2	3	4	Control
Moisture	11.3a±0.13	11.5a±0.24	11.5a±0.34	11.4a±0.41	11.9a±0.65
Ash	8.95a±0.38	8.14a±0.98	8.52a±0.19	8.71a±0.12	8.69a±0.73
Crude protein	17.1a±1.09	16.4a±1.63	16.6a±0.91	17.1a±0.59	17.5a±0.91
Crude fat	5.70a±0.76	4.44ab±0.83	5.22a±0.52	5.70a±0.81	4.12b±0.49
Fatty acids					
SFA[b]	11.3b±0.04	14.6d±0.10	17.5a±0.08	14.0c±0.04	16.9a±0.12
MUFA[c]	52.5b±0.13	27.3d±0.04	26.8a±0.11	30.1c±0.15	27.2a±0.22
PUFA[d]	36.2b±0.05	58.1c±0.08	55.8a±0.07	55.9a±0.11	55.9a±0.13
PUFA/SFA	3.19a±0.02	3.98b±0.03	3.19a±0.02	4.01b±0.02	3.30a±0.09

[a] Results expressed as an average of triplicates. Different letters in the same line are significantly different ($P<0.05$) by the Tukey test.
[b] SFA: saturated fatty acid.
[c] MUFA: monounsaturated fatty acid.
[d] PUFA: polyunsaturated fatty acid.

acids. Diet 3 had a high percentage of polyunsaturated fatty acids, as well as a high PUFA/SFA ratio.

Tables 3 and 4 present the fatty acid compositions of the yolks from red and white eggs of Lohman hens submitted to the four treatments. Gas chromatography (GC) analysis of fatty acid methyl esters from the lipids of egg yolk of hens revealed the presence of 21 fatty acids. The fatty acid compositions of the yolk lipids were affected by the experimental diets. The major effects of the diet were observed in fatty acids C16:0, C18:0, C18:1n9, C18:2n6, C20:4n6, C20:5n3 and C22:6n3. Egg lipids from red and white Lohman hens fed four diets showed significantly lower ($P<0.01$) mean values of C16:0 and C18:0 than those in control eggs. However, C16:0 and C18:0 did not significantly vary among the four treatments. Hens fed diets with canola and flaxseed showed significantly higher ($P<0.01$) mean values of C18:1n9 than those hens fed with soybean, sunflower or control diet. The flaxseed diet showed good results in fatty acids C18:3n3, C20:5n3 and C22:6n3, with values of 3.40, 0.18 and 1.55%, respectively. The C20:4n6 content in the yolk did not increase due to the high levels of polyunsaturated fatty acids added to the diets, although this fatty acid can be synthesized from dietary linoleate (Rosenthal, 1987). Cobos, Hoz, Cambero, and Ordónez (1995) also did not observe an increase of the C20:4 n6 content, using diets rich in oleins.

Table 3
Fatty acid profiles of yolk lipids from eggs of Red Lohman (RL) hens after 16 weeks of feeding with diets rich in n-3 fatty acids[a]

FA	Diet 1	Diet 2	Diet 3	Diet 4	Control diet
C14:0	0.30a±0.01	0.29a±0.01	0.29a±0.01	0.28a±0.01	0.27a±0.02
C16:0	22.9a±0.37	22.5a±0.38	23.2a±0.33	23.0a±0.26	25.0b±0.93
C16:1n9	0.94a±0.06	0.94a±0.04	0.83a±0.03	0.82a±0.04	0.70a±0.49
C16:1n7	1.91a±0.07	2.47a±0.04	1.91a±0.08	1.67b±0.10	2.14a±0.13
C17:0	0.18a±0.02	0.16a±0.01	0.18a±0.01	0.20a±0.00	0.17a±0.01
C17:1n10	0.14a±0.01	0.16a±0.01	0.13a±0.00	0.12a±0.00	0.12a±0.01
C18:0	8.83a±0.37	8.87a±0.43	9.46a±0.23	9.00a±0.23	12.4b±2.36
C18:1n9	44.0a±2.03	43.3a±0.44	41.5a±0.76	38.9b±1.35	39.6b±2.83
C18:2n6	15.8a±2.00	13.7a±0.21	17.8b±0.45	21.3c±1.54	14.7ab±1.97
C18:3n6	0.13a±0.03	0.08a±0.02	0.13a±0.01	0.14a±0.02	0.06a±0.02
C18:3n3	0.55a±0.07	3.40b±0.10	0.63a±0.09	0.30c±0.04	0.22c±0.13
C18:4n3	0.08a±0.02	0.06a±0.02	0.05a±0.01	0.06a±0.00	Nd
C20:1n9	0.34a±0.10	0.17b±0.03	0.24ab±0.05	0.37ab±0.16	0.27ab±0.07
C20:2n6	0.26a±0.12	0.21a±0.16	0.24a±0.06	0.36a±0.10	0.22a±0.09
C20:3n6	0.30a±0.19	0.18a±0.05	0.23a±0.08	0.22a±0.05	0.20a±0.04
C20:4n6	1.94a±0.59	1.17b±0.26	1.74a±0.17	1.85a±0.07	2.63a±0.94
C20:5n3	Nd	0.18±0.12	Nd	Nd	Nd
C22:4n6	0.18a±0.03	0.09b±0.04	0.20ab±0.09	0.23a±0.03	0.52c±0.17
C22:5n6	0.40a±0.07	0.08b±0.10	0.38a±0.10	0.59c±0.07	Nd
C22:5n3	0.10a±0.02	0.29b±0.08	0.10a±0.02	0.07a±0.02	Nd
C22:6n3	0.65a±0.15	1.55b±0.09	0.65a±0.04	0.33c±0.07	0.64a±0.07

[a] Results expressed as a percentage of the total fatty acids. Data presented as mean±S.D. of five samples (each treatment), each one in triplicate, $n=15$. Averages followed by different letters in the same line are significantly different ($P<0.05$) by the Tukey test.

Table 4
Fatty acids profile in yolk lipids from eggs of White Lohman (WL) hens after 16 weeks of feeding with diets rich in n-3 fatty acids[a]

FA	Diet 1	Diet 2	Diet 3	Diet 4	Control diet
C14:0	0.27a±0.03	0.27a±0.03	0.24a±0.05	0.25a±0.01	0.27a±0.02
C16:0	22.6a±0.69	22.2a±1.04	21.7a±2.33	22.8a±0.64	25.0b±0.93
C16:1n9	0.67a±0.09	0.58a±0.02	0.57a±0.05	0.61a±0.03	0.70a±0.49
C16:1n7	1.83a±0.26	1.86a±0.16	1.87a±0.55	1.97a±0.14	2.14b±0.13
C17:0	0.16a±0.01	0.17a±0.02	Nd	0.17a±5.35	0.17a±0.01
C17:1n10	0.09a±0.00	0.13a±0.01	Nd	0.08a±0.00	0.12a±0.01
C18:0	14.7a±2.07	11.0b±1.59	11.1b±1.13	15.1a±2.11	12.4ab±2.36
C18:1n9	39.8a±4.03	38.1a±0.76	36.9a±1.30	30.4b±4.83	39.6a±2.83
C18:2n6	13.4a±0.23	12.6a±1.21	19.2b±4.73	22.2b±2.60	14.7a±1.97
C18:3n6	0.10a±0.01	0.20a±0.10	0.10a±0.02	0.15b±0.01	0.06a±0.02
C18:3n3	0.85a±0.66	1.93a±0.67	0.80a±0.64	0.13b±0.01	0.22b±0.13
C18:4n3	Nd	0.07b±0.00	Nd	0.02b±0.01	Nd
C20:1n9	0.49a±0.28	0.83b±0.18	0.52a±0.26	0.23c±0.02	0.27c±0.07
C20:2n6	0.40a±0.34	0.88a±0.36	0.42a±0.26	0.22b±0.01	0.22b±0.09
C20:3n6	0.61a±0.20	0.81a±0.37	0.40ab±0.21	0.19b±0.01	0.20b±0.04
C20:4n6	3.22a±0.48	1.85b±0.15	2.29b±0.33	2.67ab±0.76	2.63ab±0.94
C20:5n3	Nd	2.23b±0.23	Nd	0.22c±0.22	Nd
C22:4n6	0.29a±0.06	0.90b±0.18	0.83b±0.45	0.69ab±0.41	0.52ab±0.17
C22:5n6	0.40a±0.02	0.43ab±0.17	0.78b±0.24	0.81b±0.12	Nd
C22:5n3	0.21a±0.00	0.57b±0.10	0.59ab±0.36	0.33ab±0.22	Nd
C22:6n3	0.91a±0.01	1.79b±0.48	1.59ab±0.54	0.54c±0.16	0.64c±0.07

[a] Results expressed as a percentage of the total fatty acids. Data presented as mean±S.D. of five samples (each treatment), each one in triplicate, $n=15$. Averages followed by different letters in the same line are significantly different ($P<0.05$) by the Tukey test.

Tables 5 and 6 present the fatty acid results in comparison with the saturation for control red and white Lohman eggs. No significant differences ($P>0.01$) of total lipids or cholesterol were found among egg yolks from hens fed different diets. Our results suggest that dietary fatty acids did not affect the cholesterol levels of the eggs. Similar results have been reported by Cobos et al. (1995) and Watkins and Elkin (1992), who observed that the cholesterol concentration in the eggs were not affected by the lipid treatment. It has been hypothesized that the inability to markedly reduce egg cholesterol levels is due to a physiological control mechanism that ultimately causes the cessation of egg production when yolk cholesterol deposition is inadequate for embryo survival (Marks & Washburn, 1977).

A decrease of SFA percentage and an increase of the PUFA percentage were verified in relation to the control eggs. This shows that, in any of the four treatments, there is a better PUFA/SFA ratio in comparison with the values obtained for the control eggs. The diet containing flaxseed presented a small amount of n6 fatty acid and a large amount of n-3. The diet of sunflower base, in spite of having the largest PUFA/SFA ratio, also showed a large n6/n3 ratio. It is possible to affirm that all the

Table 5
Lipids, cholesterol and fatty acids in comparison with the saturation (%) in the yolk lipids of eggs from Red Lohman (RL) hens after 16 weeks of feeding with diets rich in n-3 fatty acids[a]

Diets	1	2	3	4	Control
Lipids (g/100 g yolk)	22.7a±3.01	21.0a±5.52	21.6a±6.40	21.8a±6.58	23.9a±4,25
Cholesterol (mg/g yolk)	10.7a±1.01	10.3a±1.20	10.9a±1.32	10.7a±1.19	10.9a±1.21
SFA[b]	30.9a±1.54	31.9ab±0.58	33.6b±1.61	33.1b±1.60	38.6c±2.38
MUFA[c]	49.1a±2.58	47.2a±0.73	42.7b±2.26	38.5c±2.65	43.9b±2.85
PUFA[d]	20.0a±1.78	21.0a±0.97	23.7b±1.57	28.4c±2.80	17.5d±2.14
n6	18.4a±1.87	15.5b±0 80	21.5c±1.17	26.9d±2.61	16.5ab±2.15
n3	1.64a±0.32	5.44b±0.49	2.3a±0.54	1.58ac±0.40	1.02c±0.15
PUFA/SFA	0.65a±0.06	0.66a±0.04	0.71a±0.06	0.87b±0.02	0.45c±0.07
n6/n3	11.2a±3.56	2.86b±0.34	9.94a±2.13	17.7c±3.20	16.1c±3.76

[a] Results expressed as a percentage of the total fatty acids. Data presented as mean±S.D. of five samples (each treatment) each one in triplicate, $n=15$. Averages followed by different letters in same line are significantly different ($P<0.05$) by the Tukey test.
[b] SFA: saturated fatty acid.
[c] MUFA: monounsaturated fatty acid.
[d] PUFA: polyunsaturated fatty acid; n3 = total of n-3 FA; n6 = total of n-6 FA.

Table 6
Lipids, cholesterol and fatty acids in comparison with the saturation (%) in the yolk lipids of eggs from White Lohman (WL) hens after 16 weeks of feeding with diets rich in n-3 fatty acids[a]

Diets	1	2	3	4	Control
Lipids (g/100 g yolk)	23.9a±8.48	23.0a±7.26	23.2a±6.35	22.6a±7.65	23.9a±4.25
Cholesterol (mg/g yolk)	10.5a±1.01	10.1a±1.20	11.1a±1.32	10.9a±1.19	10.9a±1.21
SFA[b]	32.9a±4.86	34.4a±2.51	33.3a±2.72	35.4ab±4.53	38.6b±2.38
MUFA[c]	43.5a±2.38	41.7a±2.64	40.9a±2.61	34.7b±3.58	43.9ac±2.85
PUFA[d]	25.1a±4.53	25.3a±1.94	25.8a±4.49	30.8b±3.68	17.5c±2.14
n6	20.9a±2.96	18.1a±0.92	23.3b±4.11	27.8c±3.52	16.5a±2.15
n3	3.75a±1.61	6.58b±2.05	2.53a±0.08	2.06a±0.81	1.02c±0.15
PUFA/SFA	0.76a±0.24	0.74a±0.09	0.79a±0.22	0.88a±0.19	0.45b±0.07
n6/n3	5.56a±3.41	2.76a±1.85	10.3b±3.45	12.0ab±6.35	16.1bc±3.76

[a] Results expressed as a percentage of the total fatty acids. Data presented as mean±S.D. of five samples (each treatment) each one in triplicate, $n=15$. Averages followed by different letters in same line are significantly different ($P<0.05$) by the Tukey test.
[b] SFA: saturated fatty acid.
[c] MUFA: monounsaturated fatty acid.
[d] PUFA: polyunsaturated fatty; n3 = total of n-3 FA; n6 = total of n-6 FA.

experimental diets containing oil resulted in eggs of higher quality than the control eggs. The nutritional quality is assessed by the n-3/n-6 ratio and the PUFA/SFA ratio. The decreasing order of nutritional quality of eggs fed diets is: flaxseed > canola > soy and sunflower.

5. Conclusions

After 4 months of treatment with each diet type, it is possible to verify that there is a decrease in the fatty acid concentration of palmitic (C16:0) and stearic (C18:0) acids; there is an increase in the oleic (C18:1n9), linoleic (C18:2n6) and linolenic (C18:3n3) fatty acids in comparison with the yolk of the control eggs. The addition of oils to the diets fed to hens allowed the production of eggs with higher n-3/n-6 and higher PUFA/SFA fatty acids ratios. Therefore, it is possible to conclude that the C16:0, C18:0 C18:1n9 and C18:2n6 fatty acid compositions of yolk lipids may be modified by the diets fed to hen, which could result in an important nutritional benefit.

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