Asian Journal of Chemistry Vol. 22, No. 2 (2010), 1445-1452

Effects of Dietary Fats or Oils Supplementations on Fatty Acid Composition of Yolk of Brown Eggs

SINAN SEFA PARLAT†, OZCAN BARIS CITIL* and ISKENDER YILDIRIM†
Department of Animal Nutrition and Nutritional Disorders,
Faculty of Veterinary Medicine, Selçuk University 42100 Konya, Turkey
Fax: (90)(332)2410063; Tel: (90)(332)2232674
E-mail: ocitil@selcuk.edu.tr

In this study, 140 ATAK-S brown egg layers were used. They were divided into 7 groups, each including 20 hens. The diets of the groups were 3 % soybean oil, sunflower oil, corn oil, palm oil, sufflower oil, sheep's tallow fat and sheep's fail fat, respectively. The fatty acid compositions of brown egg yolk were determined using gas chromatography. Twenty seven different fatty acids were determinated in the compositions of brown egg yolk. At the end of experiment, while the lowest total polyunsaturated fatty acid (PUFA) level was found in sunflower oil group's egg yolk, the highest level was found in sheep's tail fat groups. It was shown that the fatty acid profile played an important role on fatty acid composition of egg yolks. Supplemental soybean oil increased the concentration of ω-3 of egg yolk (2.14 %).

Key Words: Egg, Fatty acid composition, ω-3 Fatty acid.

INTRODUCTION

Oils or fats have commonly been used as energy sources in the diets for laying hens. Fatty acid composition is affected by diets[1]. Studies have shown that type of dietary lipids of the laying hen can drastically alter the lipid profile of the egg yolk[2].

Long chain ω-3 polyunsaturated fatty acid cannot be synthesized by humans and must be obtained from the diet[3]. The synthesis of polyunsaturated ω-3 fatty acid docosahexaenoic acid (DHA) from linolenic acid is even more restricted than that of eicosapentaenoic acid (EPA). It is generally assumed that linoleic acid linoleic acid reduces EPA synthesis because of the competition between linolenic acid and linoleic acid for the common desaturation and elongation enzymes[4]. The conversion of dietary linolenic acid into EPA is limited because the efficacy of the synthesis of ω-3 polyunsaturated fatty acids decreases down the linolenic acid conversion cascade. The polyunsaturated fatty acids of the linoleic acid (ω-6) and linolenic acid (ω-3) families have been recognized as important nutrients for growth and reproduction in poultry[5]. Polyunsaturated ω-3 fatty acid docosahexaenoic acids

†Department of Animal Science, Agricultural Faculty, Selcuk University, 42075 Konya, Turkey.

(DHAs) and eicosapentaenoic acids (EPAs) are essential for the development of foetal brain[6] and play important roles in physiology, especially during foetal and infant growth, in particular in the formation of the central nervous system and retina[7].

Due to the imbalanced ω-6/ω-3 PUFA ratio of the western diet today, dietary saturated fatty acid and ω-6 PUFA are promoters of chronic diseases, such as arteriosclerosis, essential hypertension, obesity, diabetes and possibly some forms of cancers[8]. Johansen *et al.*[9] reported that supplementation with ω-3 fatty acids significantly decreased hemostatic markers of atherosclerosis. Several scientific studies have shown that ω-3 fatty acids have benefits for lowering CHD risk. It has been also suggested that ω-3/ω-6 ratio of 10 or less results in reduction in fatal CHD risk[10]. Thus, PUFAs, especially the longer chain ω-3 and ω-6 PUFAs, have been considered essential fatty acids and have been shown to have curative, fat glycemic control[11]. World Health Organization (WHO) is now recommending a ratio between 3:1 and 4:1 for n-6 to ω-3 fatty acids in the diet and the best way to achieve this is by the identification of foods with an adequate ω-3 and ω-6 PUFA composition.

Nettleton[12] reported positive effects from the intake of monounsaturated fatty acid (MUFA), such as oleic acid and of ω-3 fatty acid on health, with reduced triglyceride concentration in blood. 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[13]. The objective of the present study is to compare the nutritionally important fatty acid compositions between egg yolks from laying hens fed with a different fatty acid sources diets. The levels of ω-3/ω-6 ratios and PUFA, MUFA were compared among the fatty acid composition of egg yolks obtained from brown layer fed with the diets.

EXPERIMENTAL

Animals and diets: At 56 weeks of age, 140 ATAK-S brown egg layers were housed in cages and were assigned (20 laying hens each group) to seven experimental diets. The diets of groups were based on 3 % soybean oil, 3 % sunflower oil, 3 % corn oil, 3 % palm oil, 3 % sufflower oil, 3 % sheep's tallow fat and 3 % sheep's tail fat, respectively. The experiment lasted 84 days. The ingredients and chemical composition of diets are listed in Table-1 and the fatty acid composition of the various oil or fat sources used in the experiment are given in Table-2.

Sample collection: For the determination of fatty acid composition, seven eggs from each dietary treatmant were randomly selected and analyzed at the end of the 84 days of experimental feeding. The yolk from each egg was separeted and held in polyethylene (PE) packing (in nitrogen atmosphere) at -18 ºC. At the beginning of each analysis, the samples were allowed to achieve at room temperature and homogenized.

TABLE-1
COMPOSITIONS OF THE EXPERIMENTAL DIETS

Ingredients	Using
Corn	60.55
Soyben meal (47 % CP)	25.10
Oil/Fat	3.00
Calcium Carbonate ($CaCO_3$)	8.74
Dicalcium phosphate (DCP 20 %)	1.91
Salt (NaCl)	0.35
Min + Vit premix	0.25
DL-Methionine	0.10
Total	100.00

TABLE-2
FATTY ACID COMPOSITION OF DIETARY FATS OR OILS (%)

Fatty acids	Soybean oil	Sunflower oil	Corn oil	Palm oil	Sufflower oil	Sheep's tallow fat	Sheep's tail fat
C 8:0	-	-	-	-	-	-	0.01
C 9:0	-	-	-	0.01	-	0.08	0.21
C 12:0	0.00	0.00	-	0.16	0.00	0.82	0.27
C 14:0	0.13	0.06	0.17	1.06	0.12	2.84	4.89
C 16:0	9.07	6.59	15.32	41.07	6.81	28.41	24.68
C 18:0	2.27	2.17	1.83	2.61	1.12	21.99	9.19
C 20:0	-	0.10	-	0.07	0.18	0.10	1.06
C 21:0	0.02	0.02	-	0,01	0.02	0.17	0.04
C 22:0	0.39	0.62	0.94	0.07	0.27	0.21	0.15
Σ SFA	**11.91**	**9.57**	**18.80**	**45.08**	**8.59**	**55.08**	**43.60**
C 14:1 ω5	0.01	0.01	-	0.04	-	0.07	0.10
C 16:1 ω7	0.65	0.19	-	0.02	0.37	2.51	2.64
C 18:1 ω9	20.43	20.73	22.89	40.79	33.42	37.37	50.91
C 20:1 ω9	-	-	-	-	-	-	-
C 22:1 ω9	0.34	0.15	0.50	0.09	0.15	0.12	-
Σ MUFA	**21.39**	**1.09**	**23.39**	**40.94**	**3.94**	**39.51**	**50.33**
C 16:2 ω4	0.02	-	-	0.03	-	0.26	1.94
C 18:2 ω6	62.41	69.08	56.89	13.46	57.40	4.92	3.86
C 18:3 ω3	4.26	0.25	0.84	0.52	0.10	0.13	0.06
C 20:4 ω6	-	-	-	-	-	-	0.06
C 20:5 ω3	-	0.03	-	-	-	-	-
C 22:3 ω3	-	-	-	-	-	-	0.05
C 22:4 ω6	-	-	-	-	-	-	-
C 22:5 ω3	-	-	0.52	-	0.04	0.05	0.10
C 22:6 ω3	-	-	-	-	-	0.03	-
Σ PUFA	**66.70**	**69.35**	**57.81**	**13.98**	**57.47**	**5.41**	**6.07**
ω3	4.26	0.28	1.36	0.52	0.13	0.23	0.22
ω6	62.42	69.08	56.45	13.44	57.34	4.92	3.91
ω3/ω6	0.07	0.00	0.02	0.04	0,00	0.05	0.06

Fatty acid analysis: Total lipid was extracted from the egg yolk samples by the method[14]. 4 g samples of egg yolk were homogenized with 80 mL of a 2:1 (v/v) mixture of chloroform-methanol, after which 4 mL 0.88 % NaCl was added. The liquid was mixed and left to stand for 2 h to allow phase separation. The chloroform-methanol extract was evaporated to dryness in a water bath at 50 °C under nitrogen flow. The lipid extracts were then converted to fatty acid methyl esters by using boron-trifluoride-methylation solution (catalogue no. 3-3021). The fatty acid methyl esters (FAMEs) were separated and analyzed by Shimadzu 15-A gas chromatograph, equipped with dual flame ionization detector and a 1.8 m × 3 mm internal diameter packed glass column containing 100/120 Chromosorb WAW coated with 10 % SP 2330. The injector and detector temperatures were 225 and 245 °C, respectively. Column temperature program was 190 ºC for 35 min then increasing at 30 ºC/min up to 220 ºC where it was maintained for 5 min. Nitrogen at a flow rate of 20 mL/min was used as the carrier gas. Conditions were chosen to separate fatty acids of carbon chain length 8 to 24. The fatty acids were identified by comparison of retention times with known external standard mixtures, quantified by a Shimadzu C-R4A integrator and the results expressed as percentage distribution of fatty acid methyl esters. All the chemicals used for the gas chromatography analysis procedure were obtained from Supelco Inc. (Bellefonte, PA, U.S.A.).

Statistical analysis: The experiment was based on a completely randomized design. The data were analyzed by means of one-way ANOVA ($p < 0.05$). When analysis of variance indicated a significant treatment the means were compared by Duncan's multiple range tests. The data were expressed as means ± standard error.

RESULTS AND DISCUSSION

Brown egg yolk contains around 30 % of lipids, thus it is a rich source of lipid[15]. The fat content and the composition of fatty acids in egg lipids have been implicated in human health[16]. The fatty acid profiles of the hen diets were altered by the inclusion of different types of dietary fat or oil. The total lipid content determined in the egg yolk throughout the different oil sources. Fat content isn't influenced by different oil sources. Similarly, Silva *et al.*[17] found that lipid content of egg yolk was at a level (34.4 %).

Table-3 shows that the effects of dietary fats or oils on yolk fatty acid composition. In this study, all fatty acids were identified. The results of this experiment showed egg yolk fatty acid content were influenced ($p < 0.05$) by dietary inclusion of different fat or oil sources. There were no significantly differences among the groups for egg yolks fat contents.

The major effects of these fat or oil sources were observed for C16:0, C18:0, C18:1n9, C18:2n6, C20:4ω6, C20:5ω3 and C22:6ω3, respectively. Milinsk[18] reported that the addition of different fat or oil sources to the diets of hens showed that the production of eggs with high ω-3/ω-6 and PUFA/SFA ratios. The fatty acid compositions of the egg yolk lipids directly depended on the types and concentrations of dietary fat or oil sources[19].

TABLE-3
TOTAL FATTY ACID COMPOSITIONS OF YOLK OF BROWN EGG (%)

Fatty acids	Soybean oil	Sunflower oil	Corn oil	Palm oil	Sufflower oil	Sheep's tallow fat	Sheep's tail fat
C 12:0	*	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	*	0.01 ± 0.01	0.01 ± 0.01
C 13:0	0.14 ± 0.03b**	0.08 ± 0.02c	0.24 ± 0.11a	0.24 ± 0.02a	0.10 ± 0.04bc	0.02 ± 0.02d	0.02 ± 0.02d
C 14:0	0.24 ± 0.03c	0.36 ± 0.06a	0.28 ± 0.02bc	0.33 ± 0.03ab	0.23 ± 0.01c	0.38 ± 0.01a	0.39 ± 0.00a
C 15:0	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	*	0.02 ± 0.01	0.01 ± 0.01
C 16:0	25.13 ± 1.41a	25.07 ± 0.98a	24.25 ± 0.56a	25.26 ± 0.08a	23.50 ± 0.17a	24.57 ± 0.02a	24.52 ± 0.34a
C 18:0	8.27 ± 0.36a	6.41 ± 0.53b	6.73 ± 0.93ab	5.97 ± 0.84bc	7.32 ± 0.23a	6.10 ± 0.03b	5.66 ± 0.22c
C 20:0	0.02 ± 0.01d	0.14 ± 0.08b	0.07 ± 0.01c	0.06 ± 0.04c	0.15 ± 0.03b	0.31 ± 0.16a	0.38 ± 0.08a
C 21:0	0.14 ± 0.03	*	*	0.19 ± 0.05	0.15 ± 0.01	0.10 ± 0.01	0.08 ± 0.01
C 22:0	1.42 ± 0.03a	0.09 ± 0.01d	0.09 ± 0.01d	1.03 ± 0.27c	1.56 ± 0.22a	1.20 ± 0.11b	1.26 ± 0.15b
C 24:0	*	*	*	*	*	0.02 ± 0.01	0.03 ± 0.01
Σ SFA	**35.41**	**32.19**	**31.77**	**33.11**	**33.03**	**32.98**	**32.58**
C 14:1 ω5	0.05 ± 0.04cd	0.03 ± 0.01d	0.02 ± 0.01	0.03 ± 0.01d	0.07 ± 0.06bc	0.03 ± 0.01d	0.13 ± 0.01a
C 16:1 ω7	2.97 ± 0.17bc	2.49 ± 0.21d	2.59 ± 0.07d	2.85 ± 0.04cd	3.29 ± 0.28b	4.03 ± 0.03a.	4.34 ± 0.11a
C 17:1 ω9	0.04 ± 0.04d	0.05 ± 0.05d	0.10 ± 0.02c	0.14 ± 0.01b	0.04 ± 0.01d	0.27 ± 0.07a	0.25 ± 0.06a
C 18:1 ω9	41.65 ± 0.99b	42.10 ± 0.52b	43.77 ± 1.05b	48.07 ± 0.62ab	44.38 ± 4.37b	50.10 ± 1.60a	51.18 ± 1.56a
C 20:1 ω9	0.09 ± 0.08	0.23 ± 0.02	0.31 ± 0.03	0.20 ± 0.06	*	0.32 ± 0.06	*
C 22:1 ω9	0.70 ± 0.08b	1.19 ± 0.02a	0.98 ± 0.11ab	0.49 ± 0.16c	0.27 ± 0.01e	0.32 ± 0.03d	0.05 ± 0.02f
Σ MUFA	**45.10**	**46.03**	**47.66**	**51.63**	**48.01**	**54.62**	**55.68**
C 14:2	*	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	*	*	*
C 16:2 ω6	0.15 ± 0.05	0.07 ± 0.03	0.06 ± 0.01	0.13 ± 0.03	*	0.37 ± 0.07	0.42 ± 0.05
C 18:2 ω6	17.13 ± 2.16b	20.56 ± 1.07a	19.50 ± 1.43ab	13.52 ± 0.16c	17.38 ± 3.70b	10.80 ± 1.77de	9.81 ± 1.27e
C 18:3 ω3	0.64 ± 0.19a	0.16 ± 0.11e	0.24 ± 0.01c	0.31 ± 0.04b	0.18 ± 0.02de	0.06 ± 0.04g	0.10 ± 0.02f
C 20:4 ω6	0.07 ± 0.05d	0.33 ± 0.04b	0.41 ± 0.03a	0.22 ± 0.15c	0.08 ± 0.03d	0.05 ± 0.02e	0.04 ± 0.01e
C 20:5 ω3	0.40 ± 0.22a	0.05 ± 0.04c	0.01 ± 0.01d	0.04 ± 0.04c	0.17 ± 0.10b	0.04 ± 0.03c	0.02 ± 0.01cd
C 22:2 ω3	0.01 ± 0.01	*	*	0.04 ± 0.02	*	0.03 ± 0.01	0.03 ± 0.01
C 22:3 ω3	0.18 ± 0.06	*	*	*	*	0.21 ± 0.04	0.20 ± 0.06
C 22:4 ω6	0.18 ± 0.02	0.11 ± 0.01	*	0.40 ± 0.31	0.86 ± 0.03	0.65 ± 0.03	0.63 ± 0.16
C 22:5 ω3	0.34 ± 0.17a	0.14 ± 0.01c	0.08 ± 0.01d	0.29 ± 0.17b	0.31 ± 0.01ab	0.03 ± 0.01e	0.08 ± 0.02d
C 22:6 ω3	0.67 ± 0.10a	0.39 ± 0.07b	0.28 ± 0.04d	0.34 ± 0.12c	0.02 ± 0.02e	0.30 ± 0.04cd	0.44 ± 0.11b
Σ PUFA	**19.50**	**21.78**	**20.57**	**15.27**	**18.98**	**12.40**	**11.74**
ω3	2.14	0.72	0.60	0.98	0.66	0.53	0.84
ω6	17.29	20.99	19.91	14.15	18.31	11.51	10.50
ω3/ω6	0.13	0.04	0.03	0.07	0.04	0.05	0.08

*ND = Not determined. **a-f = Mean values within the same row sharing a common superscripts are not significantly different at $p < 0.05$.

Dietary treatments influenced the fatty acid composition of the yolk in diverse ways, depending on type of fat source ($p < 0.05$). Changes in the fatty acid composition were generally proportional to the respective fatty acid patterns of dietary fats. However, C 12:0, C 21:0, C 22:0 and C 22:1 fatty acids were not influenced by dietary treatments. These results are in agreement with Celebi and Macit[20], who reported that dietary sheep's tallow fat changed the linoleic acid, arachidonic and ω-6 fatty acids in egg yolk.

Oleic acid was identified as a primary MUFA in the egg yolks for all diets (41, 65-51, 18 %). Oleic acid was predominant fatty acid in egg yolks of hens fed. In the experiment of Cachaldora *et al.*[21], oleic acid was the major fatty acid of egg yolks, because oleic acid was the predominant fatty acid in palm oil, sheep's tallow fat and sheep's tail fat diets. Palmitoleic acid was the second most abundant MUFA (2.49-4.34 %) in the present study.

In general, the total contents of C 16:0, C 18:0, C 18:1 and C 18:2 fatty acids accounted for close to 90 % of the total fatty acids[22]. Similar results were identified in this study for all egg yolk. Palmitic acid was the primary saturated fatty acid 23.50-25.26 % for egg yolk in feeeding different diets. These results were similar to reported research data for egg[23] and feeding regimens[24].

Polyunsaturated fatty acid content in egg yolk have been reported to be in very wide range: 13.0-37.1 % of total fatty acids[23] to 17.2-18.9[25]. In this work, the SFA contents were generally much higer than PUFA in soybean oil, sunflower oil, corn oil, palm oil, sufflower oil, sheep's tallow fat and sheep's tail fat diets, 19.5, 21.78, 20.57, 15.27, 18.98, 12.40, 11.74 %, respectively. Monounsaturated fatty acid contents of egg yolks were higher than SFA in soybean oil, sunflower oil, corn oil, palm oil, sufflower oil, sheep's tallow fat and sheep's tail fat diets, 35.41, 32.19, 31.77, 33.11, 33.03, 32.98, 32.58 %, respectively. In soybenan oil diet, a high of C 18:3 (0.64 %), C 20:5 (0.40 %) and C 22:6 (0.67) increased the ω-3 content and a low level of linoleic acid lowered the PUFA contents of egg yolks. In general, MUFA is higher than SFA and PUFA[19-26]. Similar results were identified in this study for MUFA in all groups (45.10-55.68 %).

The diet containing soybean oil, which has a high ratio of ω-3 (0.64), increased the ω-3 content of egg yolk lipids and lowered the MUFA contents of egg yolk lipids. The diet containing sheep's tail fat, which has a low level of C 18:2, lowered the PUFA contents of egg yolk lipids. Variations in fatty acid composition might be related to the changes in nutritional habits of egg yolk[27]. The high ω-3 content (2.14 %) in yolk of brown egg of soybean oil group may be attributed to these researchers' report.

In this research, the amount of linoleic acid which can be determined in palm oil, sheep's tallow fat and sheeps's tail fat diets is less than the amount of linoleic acid which is derived from the eggs of hens fed by these diets. Oleic acid is higher than the linoleic acid in palm oil, sheep's tallow fat and sheep's tail fat groups. Moreover, it could be seen that the amount of the fatty acids of the eggs of hens fed

by these diets is higher than the amount of oleic acid which is derived from eggs of hens fed by the other diets. Similar results for fatty acid composition of egg yolk[19] and different fat or oil sources have also been reported in the literature[26].

The long chain ω-3 and ω-6 fatty acids commonly called PUFAs and their ratios are also (ω-3/ω-6) considered to be important[28]. In the present study, the ω-3/ω-6 ratios were 0.13, 0.04, 0.03, 0.07, 0.04, 0.05 and 0.08 % in soybean oil, sunflower oil, corn oil, palm oil, sufflower oil, sheep's tallow fat and sheep's tail fat yolk, respectively. Dyerberg[29] noted that an increase in the ratio of ω-3/ω-6 PUFA increases the availability of ω-3 PUFAs, which are beneficial for human health. ω-6 content was higher than ω-3 content in all egg yolks. Unsaturated fatty acids constituted a significant component 64.59-68.23 % in all egg yolks. With a high ratio of UFAs, the egg yolks is desirable for human nutrition[30].

In the report of HMSO[31], it was suggested that the minimum ratio of PUFA/SFA was 0.45. Celebi *et al.*[20] reported that this ratio of egg yolk was 0.78 in tallow supplemental, 0.96 in sunflower oil supplemental, 0.74 in flaxseed oil. In this study, this ratio in egg yolk was found about 0.50.

Soybean oil addition to the diet increased linearly egg yolk contents of C 18:3, DHA, EPA and total ω-3 fatty acid content of egg yolk. Bean and Leeson[32] reported that ω-3 enriched brown egg could provide a greater proportion of a person's daily requirement of ω-3 fatty acids.

The results of this experiment showed that dietary fatty acid profile played an important role on fatty acid composition of egg yolks. The amount of all fatty acids were affected by the addition of different dietary oils or fats. In this experiment, the fatty acid composition of the egg yolks reflected the fatty acid profiles of the diets. That is, it is possible to say that changing of fatty acid composition of egg yolk. For example, supplemental soybean oil increased the concentration of ω-3 of egg yolk (2.14 %).

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(*Received*: 1 June 2009; *Accepted*: 30 October 2009) AJC-8002