Kafkas Univ Vet Fak Derg
17 (5): 761-766, 2011
DOI:10.9775/kvfd.2011.4368

RESEARCH ARTICLE

The Effects of Dietary Flaxseed Oil Supplementations on Fatty Acids Composition of The Yolks in Quail *(Coturnix Coturnix Japonica)* Eggs

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Makale Kodu (Article Code): KVFD-2011-4368

Summary

This experiment was carried out to determine effects of Japanese quail diets which is supplemented with different levels of omega-3 fatty acids compositions in yolk. In this study, one hundred-twenty laying quails at 10 weeks of age *(Coturnix coturnix japonica)* were used. They were divided into 4 tretament groups, each group included 30 quails. The diets in treatment groups contained different levels of flaxseed oil 0, 1.0, 2.0, and 3.0%, respectively. At the end of the experiment lasted for 21 days, 7 eggs taken from each group were subjected to analysis. The fatty acid compositions of egg yolks were determined by gas chromatography. Twenty seven different fatty acid components were determined in the compositions of quail egg yolks. No differences were found in oil content among the groups. The increased ingestion of linolenic acid in diet reduced the formation of arachidonic acid in egg yolks of the treatment groups. The ω-3 fatty acid levels were higher in all treatment groups than that of control group. Supplemental flaxseed oil at the level of 3% increased the concentration of ω-3 in egg yolk. At the end of the experiment, the ω-3/ω-6 ratio increased from 0.05 (control) to 0.18 (3.0%). It was observed that when flaxseed oil amounts was increased in quail rations, omega-3 fatty acid amounts in egg yolk was improved.

Keywords: *Quail egg yolk, Omega-3, Fatty acid, Flaxseed oil*

Bıldırcın *(Coturnix Coturnix Japonica)* Rasyonlarına Keten Tohumu Yağı İlavesinin Yumurta Sarısının Yağ Asiti Kompozisyonu Üzerine Etkileri

Özet

Bu araştırmada japon bıldırcını rasyonlarına farklı oranlarda ilave edilen keten tohumu yağının yumurta sarısında bulunan omega-3 yağ asidi kompozisyonları üzerine etkileri incelendi. Bu çalışmada 120 adet 10 haftalık yaşta japon bıldırcını *(Coturnix coturnix japonica)* kullanılmıştır. Her bir grup 30 bıldırcından oluşan, dört gruba bölünmüştür. Denemede, rasyonlara, keten tohumu yağının %0, 1, 2 ve 3 seviyeleri ilave edilmiştir. 21 gün süren denemenin sonunda her alt gruptan yumurta alınarak analiz edilmiştir. Yumurta sarılarının yağ asidi kompozisyonları gaz kromatografisi cihazında belirlenmiştir. Bıldırcın yumurtalarının kompozisyonlarında yirmi yedi farklı yağ asidi belirlenmiştir. Dört gruptan elde edilen yumurtaların ham yağ oranlarında farklılık bulunmamıştır. Rasyonlarda linolenik asitin artması, yumurtadaki araşidonik asitin oranını azaltmıştır. Yemlerine keten tohumu yağı ilave edilen deneme gruplarına ait yumurta sarısı ω-3 yağ asidi seviyesi, kontrol grubundaki yağ asidi seviyesinden daha yüksektir. Rasyonlara keten tohumu yağı ilavesi, yumurtadaki ω-3 yağ asidi oranını artırmıştır. Denemenin sonunda ω-3/ω-6 oranı 0.05'den (kontrol), 0.18'e (3.0%) yükselmiştir. Japon bıldırcınlarında keten tohumu yağı miktarı artırıldığı zaman, yumurtadaki omega-3 yağ asitleri oranı artar.

Anahtar sözcükler: *Japon bıldırcını yumurtası, Omega3, Yağ asidi, Keten tohumu yağı*

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INTRODUCTION

Dietary fats or oil supplies were shown to change the fatty acid composition of egg yolk in laying hens [1]. Fatty acids play important metabolic, structural and functional roles in physiology. ω-3/ω-6 ratio of 5:1 was recommended by researchers. This unsatisfactory ratio in human nutrition leads to falling regulation of ω-3 PUFA derived from endogenous synthesis of docosahexaenoic acid (DHA, C22:6, ω3) and eicosapentaenoic acid (EPA, C20:5, ω3), which are known to be important factors in the prevention of human cardiovascular diseases. A ratio of ω-3/ω-6 in food should contribute to maintenance and improvement of human health [2].

Fish is one of the best known sources of ω-3 PUFA in human diet, as it is rich in EPA and DHA fatty acids. Another sources of ω-3 PUFA are oil seeds, particularly flaxseed, which has a high level of linolenic acid content (50-55%) [3]. Several studies have demonstrated that ω-3 PUFA level in yolk was increased by the addition of flaxseed to poultry diet [4]. The increased level of ω-3 PUFA promotes a qualitative change fatty acid composition in egg yolk. With ω-3/ω-6 ratio increasing contributes to a more beneficial human nutritional [5].

Generally, except for fish, animal originated fats contain triglycerides consist of dominantly palmitic or stearic acids. As known, palmitic acid and stearic acid are long-chain saturated fatty acids. It is attributed these fatty acids are commonly responsible for health problems such as cardio-vascular diseases of humans [6]. Common varieties of flaxseed has high concentrations of polyunsaturated fatty acids, especially linolenic acid [7]. ω-3 fatty acid content of eggs can be significantly increased by feeding flaxseed [8].

Several reports have stated that the ration containing flaxseed oil, decreased the omega-6 fatty acids and omega-6/omega-3 fatty acid ratio significantly, whereas increased the amount of omega-3 fatty acid [9,10].

Enrichment of ω-3 PUFA in eggs of laying hens or quails is a successful strategy to ensure an adequate supply of ω-3 PUFA for the health of population. Production of ω-3 PUFA enriched eggs can be realized by adding common sources of ω-3 PUFA (*i.e.* fish oil, marine algae, or linseed) to the layer diet [11].

Therefore, the aim of this study was to determine the effects of dietary oil, rich in omega-3 fatty acids, on the fatty acids composition of quail egg yolk. Additionally, this investigation aimed to improve the nutritional quality of quail egg yolk by increasing its ω-3/ω-6 ratio.

MATERIAL and METHODS

Animals and Diets

In the periods of 21 days, 120 laying quails *(Coturnix coturnix japonica)* at 10 weeks of age were housed in battery cages and assigned to four experimental diets. Quails were housed 5 per cage. Quails has been kept 40x30x30 cm sized stainless-steel cages. Animals were kept in the cages in a room at 22±2ºC and equal light/dark periods (12/12 h). Experimental diets contained flaxseed oil levels of 0, 1.0%, 2.0%, 3.0%, respectively. The chemical composition of all diets are listed in *Table 1*. Experiment was done Duzgunes of Research Farm in Selçuk University. Flaxseed oil that was used in the experiment was bought from Zade oil factory in Konya.

Sample Collection

Seven eggs from each dietary treatment were randomly selected and analyzed to determine the total lipid and fatty acids composition, at the end of the 21st day of experimental feeding. The yolks from each egg were separated mechanically and held in polyethylene packing (in N_2 atmosphere) at -18°C.

Feed Analysis

The contents of dry matter, ash, crude protein and crude fat were determined according to the methods of AOAC [12]. Feeds were analyzed in Department of Animal Nutriton Laboratuary in Faculty of Veterinary in Selcuk University.

Fatty Acid Analysis

Total lipids were extracted from the egg yolk samples by the method of Folch et al.[13]. Four-grams samples of egg yolk were homogenised with 80 ml of a 2:1 (v/v) mixture of chloroform-methanol, after 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 N_2 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 gas chromatograph (GC) Shimadzu 15-A, equipped with dual flame ionisation detector and a 1.8 m x 3 mm internal diameter packed glass column containing 100/120 Chromosorb WAW coated with 10% SP 2330 in Department of Animal Nutrition in Selcuk University. The injector and detector temperatures were 225 and 245°C, respectively. Column temperature program was 190°C for 35 min then increasing at 30°C/min up to 220°C where it was maintained for 5 min. Nitrogen at a flow rate of 20 ml/min was used as the carrier gas.

The fatty acid compositions were identified by the comparison of retention times with known as external standard mixtures, quantified by a Shimadzu Class-VP software sysytem. The results were 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 results were subjected to variance analysis (ANOVA), at 5% significance level, by the Statistica Software [14] version 5.0. The mean of values were compared by the Tukey test [15].

RESULTS

Fatty acid compositions of quail egg yolks are shown in *Table 2*. Supplementation of different levels of flaxseed oil containing ω-3 to quail layer diets, has led to a dramatically increase of the ω-3 fatty acid composition in the yolks of quail eggs. In this study, flaxseed oil supplementation of layer diets significantly increased ω-3/ω-6 in eggs *(Table 3)*. In addition to these, addition of flaxseed oil to the diet increased the C18:3n-3 and n-3 fatty acid levels in eggs ($P<0.05$). Gas chromatography (GC) analysis of fatty acid methyl esters from the lipids of egg yolk of quails revealed the presence of different 27 fatty acids.

DISCUSSION

The results of the peresent study show that the fatty acid composition of yolks were greatly affected by the level of flaxseed oil in diet. The main responses were obtained from three polyunsaturated fatty acids (C18:3, C20:4, and C22:6) when flaxseed oils were added to layer diets.

The total lipid content of the eggs were also determined in current study. The fat contents of eggs were not influenced by the dietary treatment with flaxseed oil in the study. The lipid content was around 34% in dietary treatments. Similarly, Da Silva et al.[16] found that total lipid of egg of yolk was 34%.

There were significantly differences ($P<0.05$) for DHA, ω-3 levels and ω3/ω6 ratio among all the groups. The results observed in this study for ω-3 content were supported by Balevi et al.[17] and Ferrier et al.[18].

In the present study, the å ω-3 amount in the eggs from the groups which consumed flaxseed oil in their diet were sinificantly higher than that of the control group. Our results were confirmed by Da Silva et al.[16] who reported that when 1.5, 3.0 and 5.0 g/kg flaxseed oil was added to

Table 1. *The Ingridients and chemical compositions of the experimental diets*
Tablo 1. *Deneme rasyonlarının kimyasal kompozisyonları*

Ingredients (%)	0% Flaxseed Oil	1% Flaxseed Oil	2% Flaxseed Oil	3% Flaxseed Oil
Corn	57.18	57.35	56.24	55.28
Soybean meal	33.59	32.62	32.79	32.45
Flaxseed oil	-	1	2	3
Limestone	3.74	3.41	3.35	3.79
Soybean oil	2.2	2.24	2.22	2.13
Dicalcium phosphate	1.29	1.29	1.29	1.3
Salt	0.34	0.34	0.34	0.35
Vitamin	0.11	0.11	0.12	0.10
Mineral	0.21	0.21	0.21	0.22
DL-Methionine	0.14	0.15	0.15	0.16
Mineral vitamin supp	0.28	0.28	0.28	0.28
L-Lys HCl	–	–	0.01	0.02
Ca	0.27	0.28	0.28	0.28
P	0.51	0.51	0.51	0.51
Lysin	0.09	0.08	0.08	0.08
Cystine	0.13	0.13	0.13	0.13
Energy ((kcal/kg)	2900	2900	2900	2900
Total	**100.00**	**100.00**	**100.00**	**100.00**
Dry matter %	89.81	89.65	89.46	89.71
Crude ash %	8.67	8.71	8.38	8.82
Crude protein %	20.18	20.88	20.54	20.34
Crude fat %	4.71	4.74	4.57	4.48

***Table 2.** Fatty acids composition of experimental diets (%)*
***Tablo 2.** Deneme rasyonlarının yağ asidi kompozisyonları (%)*

Fatty Acids	Name of Fatty Acid	0% Flaxseed Oil	1% Flaxseed Oil	2% Flaxseed Oil	3% Flaxseed Oil
C 8:0	Kaprilic acid	-	-	-	-
C 9:0	Nonanoic acid	-	-	-	0.01
C 12:0	Lauric acid	0.00	0.00	-	0.16
C 14:0	Myristic acid	0.89	0.98	1.17	1.06
C 16:0	Palmitic acid	9.07	9.59	10.32	8.07
C 18:0	Stearic acid	2.27	3.17	3.83	2.61
C 20:0	Arashidic acid	-	0.10	-	0.07
C 21:0	Heneikosanoic acid	0.02	0.02	-	0.01
C 22:0	Behenic acid	0.39	0.62	0.94	0.07
Σ SFA		12.64	14.48	16.26	12.06
C 14:1 ω5	Myristoleic acid	0.01	0.01	0.02	0.04
C 16:1 ω7	Palmitoleic acid	0.65	0.97	1.02	0.79
C 18:1 ω9	Oleic acid	31.43	30.73	28.89	31.79
C 20:1 ω9	Gadoleic acid	-	-	-	-
C 22:1 ω9	Erucic acid	0.34	0.15	0.20	0.09
Σ MUFA		32.43	31.86	30.13	32.71
C 16:2 ω4	Palmitoelaidic acid	0.02	-	-	0.03
C 18:2 ω6	Linoleic acid	52.46	44.21	42.97	41.38
C 18:3 ω3	Linolenic acid	2.06	7.29	9.43	12.52
C 20:4 ω6	Arachidonic acid	-	-	-	-
C 20:5 ω3	Eicosapentaenoic acid	-	0.03	-	-
C 22:3 ω3	Docosatrienoic acid	-	-	-	-
C 22:4 ω6	Docosatetraenoic acid	-	-	-	-
C 22:5 ω3	Docosapentaenoic acid	0.27	0.62	0.52	0.46
C 22:6 ω3	Docosahegzaenoic acid	0.12	0.51	0.69	0.84
Σ PUFA		54.93	51.66	53.61	55.23
ω3		2.45	8.45	10.64	13.82
ω6		52.46	44.21	42.97	41.38
ω3/ω6		0.047	0.191	0.248	0.334

the diets of quail eggs, w-3 amounts in eggs were found to be as 1.51, 2.36 and 3.07%, respectively. In general, It has been respected the total contents of C 16:0, C 18:0, C 18:1 and C 18:2 fatty acids are accounted about 90% of the total fatty acids in egg [19]. Similar results were identified in this study for all fatty acid contents of egg yolks. Palmitic acid was the primary saturated fatty acid at level of 23.50-25.26% in egg yolk of groups fed with different diets. These results were similar to research data reported by Manila et al.[20] and Cachaldora et al.[21] for egg.

Cherian et al.[22] reported that Monounsaturated fatty acid content in laying quail egg yolks to be in very wide range of total fatty acids (44.49-46.34%). In this work, the MUFA contents were higher than SFA contents of quail egg yolks, as 48.96, 52.01, 51.99, and 51.91%, respectively.

Gao and Charter [23] verified correlation between ω-3 and Arachidonic acid in their works. In this work, it was observed a decrease for arachidonic acid ($P<0.05$) in egg yolks. Arachidonic acid contents were found in egg yolk of the treatment groups as 1.76, 1.70, 1.44, and 1.15%, respectively. These differences may be attributed to the competition between the two fatty acids families (ω-6 PUFA and ω-3 PUFA) for the Δ6 desaturase enzyme involved in the desaturation process. This enzyme has a large affinity for ω-3 fatty acids and needs a lower quantity of these acids than of the ω-6 series to generate the same amount of product [24]. Thus, the increased ingestion of ω-3 in diet results in a significant decrease in the formation of Arachidonic acid.

Parlat et al.[25] obtained higher levels of ω-3 PUFA in eggs of hens fed with enriched ω-3 diets. In regard to the importance of PUFA in the formation of ω-3 and ω-6 metabolites, a 10:1 ratio of dietary ω-3/ω-6 fatty acids

Table 3. *Total Fatty acid compositions of egg yolks of quail fed diets with different amounts of flaxseed oil (%)*

Tablo 3. *Keten tohumu yağının farklı miktarlarıyla beslenen japon bıldırcınlarının yumurta sarılarındaki yağ asidi kompozisyonları (%)*

Fatty Acids	0% Flaxseed Oil (n=7)	1% Flaxseed Oil (n=7)	2% Flaxseed Oil (n=7)	3% Flaxseed Oil (n=7)	P
C 8:0	0.01±0.00	0.02±0.00	0.01±0.01	0.01±0.01	0.69
C 10:0	0.01±0.00	0.01±0.01	0.00±0.00	0.00±0.00	0.87
C 12:0	0.13±0.00	0.11±0.00	0.30±0.18	0.31±0.18	0.54
C 14:0	0.46±0.01	0.45±0.03	0.45±0.03	0.47±0.02	0.87
C 15:0	$0.03±0.03^{a}$	$0.01±0.01^{b}$	$0.01±0.01^{b}$	$0.01±0.01^{b}$	0.01
C 16:0	$25.03±6.07^{b}$	$25.12±0.19^{a}$	$25.21±0.32^{a}$	$24.89±0.19^{a}$	0.01
C 17:0	$0.08±0.02^{a}$	$0.04±0.02^{b}$	$0.02±0.01^{bc}$	$0.02±0.01^{c}$	0.00
C 18:0	$8.14±1.87^{b}$	$8.09±0.71^{a}$	$6.58±0.81^{a}$	$7.00±0.73^{a}$	0.03
C 21:0	0.17±0.03	0.13±0.01	0.13±0.01	0.13±0.01	0.57
C 22:0	$0.01±0.03^{a}$	$0.00±0.00^{b}$	$0.00±0.00^{b}$	$0.00±0.00^{b}$	0.01
C 24:0	0.57±0.11	0.29±0.06	0.40±0.04	0.42±0.05	0.24
Σ SFA	**34.63**	**34.27**	**33.11**	**33.27**	
C 14:1 ω5	$0.12±0.00^{a}$	$0.09±0.01^{b}$	$0.09±0.01^{b}$	$0.10±0.01^{b}$	0.01
C 16:1 ω7	$5.45±1.27^{b}$	$4.61±0.25^{a}$	$4.62±0.07^{a}$	$4.89±0.08^{a}$	0.21
C 18:1 ω9	$43.09±10.72^{b}$	$47.01±1.22^{a}$	$46.99±0.19^{a}$	$46.61±1.17^{a}$	0.00
C 20:1 ω9	0.24±0.05	0.11±0.02	0.12±0.01	0.12±0.01	0.12
C 22:1 ω9	$0.00±0.03^{a}$	$0.00±0.00^{b}$	$0.01±0.00^{b}$	$0.01±0.00^{b}$	0.01
Σ MUFA	48.96	52.01	51.99	51.91	
C 18:2 ω6	$13.74±3.28^{b}$	$10.67±0.66^{a}$	$11.61±0.44^{ab}$	$11.23±0.70^{a}$	0.04
C 18:3 ω3	$0.24±0.05^{b}$	$0.16±0.02^{b}$	$0.47±0.07^{b}$	$0.94±0.12^{a}$	0.00
C 20:2 ω3	$0.10±0.01^{b}$	$0.17±0.02^{ab}$	$0.18±0.02^{ab}$	$0.18±0.02^{a}$	0.09
C 20:3 ω3	$0.07±0.02^{ab}$	$0.07±0.01^{ab}$	$0.09±0.00^{b}$	$0.12±0.01^{a}$	0.11
C 20:4 ω6	$1.76±0.39^{a}$	$1.70±0.02^{b}$	$1.44±0.05^{c}$	$1.15±0.09^{d}$	0.01
C 20:5 ω3	$0.00±0.03^{a}$	$0.01±0.00^{b}$	$0.01±0.00^{b}$	$0.01±0.00^{b}$	0.01
C 22:2 ω3	$0.01±0.03^{a}$	$0.01±0.00^{b}$	$0.01±0.00^{b}$	$0.01±0.00^{b}$	0.01
C 22:3 ω3	$0.00±0.03^{a}$	$0.00±0.00^{b}$	$0.01±0.00^{b}$	$0.01±0.01^{b}$	0.01
C 22:4 ω6	$0.06±0.03^{a}$	$0.05±0.00^{b}$	$0.05±0.00^{b}$	$0.05±0.01^{b}$	0.01
C 22:5 ω3	0.23±0.04	0.29±0.08	0.35±0.08	0.41±0.08	0.24
C 22:6 ω3*	$0.21±0.03^{d}$	$0.59±0.13^{c}$	$0.69±0.06^{b}$	$0.72±0.07^{a}$	0.00
Σ PUFA	**16.41**	**13.72**	**14.90**	**14.82**	
ω3*	$0.76±0.18^{d}$	$1.12±0.17^{c}$	$1.61±0.19^{b}$	$2.20±0.19^{a}$	0.00
ω6	$15.66±3.73^{a}$	$12.60±0.65^{c}$	$13.29±0.46^{b}$	$12.62±0.75^{c}$	0.29
ω3/ω6*	$0.05±0.02^{d}$	$0.09±0.01^{c}$	$0.12±0.01^{b}$	$0.18±0.02^{a}$	0.03
SFA/PUFA	$2.12±0.49^{b}$	$2.55±0.04^{a}$	$2.25±0.18^{a}$	$2.25±0.08^{a}$	0.00
Total Lipid levels of egg yolks (%)	34.76	33.98	34.37	35.01	0.01

SFA: *Saturated Fatty Acid,* ***MUFA:*** *Monounsaturated Fatty Acid,* ***PUFA:*** *Polyunsaturated Fatty Acid*
**a-d Mean values within the same row sharing a common superscripts are significantly different at P<0.05*

has been recommended in human diets [26]. In the current study ω-3/ω-6 ratio of all the groups ranged from 0.05 to 0.18, whereas ω-3/ω-6 ratio of the control group were lower than that of other treatment groups ($P<0.05$). Harper and Jacobson [27] have reported that the coronary disease were reduced when the diets enriched with ω-3. Simopoulos [28] has outlined that dietary ω-3/ω-6 ratio might be acceptable up to 1:4. In the current study, ω-3/ω-6 ratio were found in egg yolks of the groups as 0.05, 0.09, 0.12, 0.18%, respectively. Our results was supported by Da Silva et al.[16] who found that ω-3/ω-6 ratio was found from 0.05, 0.10, 0.15, 0.22%, respectively.

As conclusion, addition of different amounts of flaxseed oil to the quail diets did not have any negative effects on fatty acid composition in yolk content. The ω-3 fatty acid content and ω-3/ω-6 ratio were improved by addition of the different amounts of flaxseed oil quail to diets. Therefore, it is concluded that ω-3 fatty acid compositions of egg yolk oil may be modified by the diets of quail, which could result in a beneficial nutritional effect for human health.

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