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Fatty Acid Content of Egg Yolk Lipids from Hens Fed with Safflower Seed

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Abstract: Ninety-six, 26 week-old White Leghorn laying hens (w-36) were fed commercial diets containing 0, 4, 7 and 10% Safflower Seed (SS) and the diets were iso-caloric and iso-nitrogenous. Hens were randomly assigned to 4 treatment diets, with 3 replicates having 8 layers in each. The experiment was conducted to study the effects of feeding safflower seed on fatty acid composition of yolks. Saturated fatty acid (meristat, palemitat and stearat), ω-7 (palemitoleat) and ω-9 (oleat) in egg yolk were not different among treatments ($p>0.05$), but the ω-6 fatty acid (linoleat and arashidonat) were increased at 7 and 10% SS ($p<0.05$). The linoleic acid was the highest in 7 and 10% SS groups. The ω-3 fatty acid (LNA, EPA and DHA) content was not different among treatments. The ω-6/ω-3 ratio increased in SS fed groups. The mean yolk cholesterol content did not significantly differ among treatments ($p>0.05$).

Key words: Safflower seed, cholesterol, layer, polyunsaturated fatty acid

INTRODUCTION

Hen egg yolk contains around 30% of lipids and is therefore, a rich source of lipid. There are some reports in the literature that deal with the effects of terrestrial sources of polyunsaturated fatty acids (PUFA) on the yolk PUFA and SFA composition. 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). Although, cholesterol level in eggs is 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 (Keys *et al*., 1965; Hegsted *et al*., 1965; Pyorala, 1987; Rudel *et al*., 1990; Hayes *et al*., 1991; Hopkins, 1992) some reports in the literature that deal with the effects of terrestrial sources of Polyunsaturated Fatty Acids (PUFA) on the yolk PUFA and SFA composition. The belief that human health may be improved by a reduction of animal fat consumption has undoubtedly decreased the consumption of (Khosla and Hayes, 1992).

In a review, Stadelman and Pratt (1989) noticed that the lipid content of hen eggs is affected by genetics, age, feeding programs and also by the levels and types of dietary lipids. When hens were fed diets containing oleate (Donaldson, 1967; Pankey and Stadelman, 1969) and linoleate (Guenter *et al*., 1971; Murty and Reiser, 1961), the respective dietary fatty acids were readily incorporated into the egg yolk.

The aim of this research, was to investigate the effect of diets containing safflower seed in order to study the possibility of producing eggs with altered fatty acid composition.

MATERIALS AND METHODS

Animals and diets: Ninety-six White Leghorn w-36 laying hens at 26-week-old were housed in laying cages maintained in an environmentally controlled house at the Behparvar Animal Farm plant. The birds were fed a standard layer diet (16% CP; 2820 kcal ME kg^{-1} diet). The birds were randomly assigned (24 hens per treatment) to 4 experimental diets (0, 4, 7 and 10% safflower seed). The

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Table 1: Percent Ingredients and nutrient compositions of experimental diets

	Composition			
Ingredients (%)	0% SS	4% SS	7% SS	4% SS
Corn (%)	66.43	60.42	60.60	58.11
Soybean meal (%)	20.10	20.05	19.15	18.34
Wheat (%)	1.00	3.66	1.00	1.00
Safflower Seed (SS) (%)	0.00	4.00	7.00	10.00
Limestone	8.50	8.50	8.48	8.49
Fish meal (%)	2.00	2.00	2.00	2.00
Mineral premix	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25
DL-Methionine	0.07	0.08	0.08	0.08
Lysine	0.07	0.07	0.09	0.11
Sodium chloride	0.31	0.32	0.34	0.35
Calculated analysis				
Metabolizable energy, Mcal kg^{-1}		2.820		
Protein (%)		16.100		
Lysine (%)		0.840		
Methionin (%)		0.360		
Met + Cys		0.660		
Nonphytin phosphate (%)		0.390		
Calcium (%)		0.370		
Sodium (%)		0.170		

*Each kg contains Vit. A (8800000 IU); Vit. B1 (1.477 g); Vit. B2 (4 g); Vit.B3 (7.84 g); Vit. B6 (2.46 g); Vit. B12 (0.01 g); Vit. D3 (2500000 IU); Vit. E (11000 IU); Vit. K3 (220 g); Folacin (0.25 g) and Biotin (0.15 g); *Each kg contains Manganese Oxide (74.4 g); Ferric Oxide (75 g) Zinc Oxide (64.675); Cupper Sulphate (6 g); Selenium Pre Mix (0.2 g); Calcium Iodate (0.32 g) and Choline Chloride (200 g)

Table 2: Fatty acid composition of experimental diets

	Experimental diet			
Fatty acid	Control	4% SS	7% SS	10% SS
Percentage of total methyl esters of fatty acids				
$C_{14:0}$	1.09	1.43	1.36	1.55
$C_{16:0}$	30.19	21.09	20.01	18.26
$C_{16:1\ n-7}$	3.52	2.50	2.85	2.85
$C_{18:0}$	8.41	9.94	9.06	8.11
$C_{18:1\ n-9}$	34.59	24.74	22.11	19.51
$C_{18:2\ n-6}$	20.12	38.53	43.25	48.92
$C_{18:3\ n-3}$	0.09	0.12	0.09	0.07
$C_{20:4\ n-6}$	1.35	1.97	2.14	2.93
$C_{20:5\ n-3}$	0.03	0.02	0.02	0.01
$C_{22:6\ n-3}$	0.01	0.01	0.01	0.01

ingredient and nutrient composition of the basal and experimental diets is shown in Table 1. The lipid profile of the experimental diets is shown in Table 2.

The hens were fed the experimental diets from 26-40 weeks of age for 3 periods of each 28 days and the first 2 weeks were for adjustment. Hens were maintained on 16:8 h light:dark cycle and all the diets were iso-caloric and iso-nitrogeneous according to NRC (1994). Feed and Water was supplied *ad libitum*.

Egg production and egg quality measurements: Performance data were collected during the 12 weeks experimental period. Egg production was recorded daily, while feed consumption, egg weight, egg mass and Feed Conversion Ratio (FCR) were recorded every week and weight gain, antibody titter against Newcastle Disease (ND) and Infectious Bursal Disease (IBD) were recorded at the end of experimental period. Egg quality parameters viz. Haugh unit score, yolk colour index (as measured by Roche yolk colour fan), yolk index, egg shape, shell weight, shell thickness and specific gravity were measured on 3 eggs from each replicate for 3 consecutive days in every period. The yolk of eggs was extracted and cholesterol content was determined once every 28 days. Blood samples were collected in non-heparinsed tubes from 6 hens in each treatment by puncturing the brachial vein at the end of experiment and serum was collected after 8-10 h as per standard procedures and stored for subsequent analysis.

Chemical analysis: Eggs were collected for chemical analysis during the last 3 days of each period (each 28 days), weighed and cracked; thereafter, yolks were separated. Three random samples of yolk from each replicate from each treatment were combined together. Three samples of yolks for each diet were freeze-dried and stored at -20°C before the fatty acid analysis performed. Total lipid was extracted from egg yolk according to the method of Folch *et al.* (1957). Approximately, 0.5 g of yolk was weighed into a test tube with 20 mL of Hexan: methanol (2:1 v v^{-1}) and methylated with 5% Boron trifluoride methanol complex (BF_3) in methanolic solution (Morrison and Smith, 1964). The FAME (Fatty Acid Methyl Ester) were separated and quantified at Department of Chemistry of Birjand University by gas chromatography using Shimadzu GC-16A chromatograph equipped with a CPSil 88 capillary column (Fused silica capillary column; length, 100 m; I.D., 0.25 mm), film and a flame ionization detector. The operating conditions of the gas chromatograph were as follows. The initial temperature was 130°C for 5 min, then increased by 3°C min^{-1}-217°C, from 217-230°C, the temperature was increased by 4°C min^{-1} and then remained at 230°C for 25 min^{-1}. The temperature of the injector and the detector remained stable at 280 and 300°C, respectively. The column head pressure of the conductor gas (helium) was 2.20 g cm^{-2}. Each FA was identified in the form of a methyl ester by comparing the retention times with the standard acquired from Sigma Quimica S.A.

Statistical analysis: All percentage data were normal and did not need arcsine transformation. The experimental design used was a completly randomized design. Data were analyzed by using the General Linear Models (GLM) procedure of SAS® software (SAS Institute Inc., 1996). Mean separation was accomplished using Duncan's (1995) multiple range test. A probability value of <0.05 was considered significant.

RESULTS AND DISCUSSION

The Safflower Seed (SS) levels in diet did not affect (p>0.05) production parameters such as feed intake, egg production, egg weight, egg mass and FCR, which were 93.57 g, 89.98%, 57.47 g, 52.59 kg and 1.79, respectively. But the maximum gain in body weight was observed in 4% SS (140), which was statistically significant with control (p<0.05).

Haugh unit, yolk index, yolk colour index, shell thickness, egg shape (86.03, 41.13, 5.78, 34.21 mm and 76.92) and eggshell deformation were unaffected by feeding different levels of safflower seed (p>0.05). Specific gravity of eggs reduced in SS treatments and the lowest specific gravity of eggs was observed in 10% SS (1.076 g cm^{-3} at the end), which was statistically significant with control group (p<0.05).

Yolk weight expressed as a percentage of egg weight and antibody titer against IBD and ND were not different (27.14, 8.67 and 6329.3, respectively) among treatments (p>0.05).

The lowest level of cholesterol in egg, yolk and blood cholesterol was observed in 10% SS (190.65 mg egg^{-1}, 12.3 mg g^{-1} of yolk and 134.33 mg $dlit^{-1}$).

The FA composition of total yolk lipids reflected that of the laying hen diets (Table 3). There were no statistical differences in the degree of saturated fatty acid content of yolk among treatments. Saturated fatty acids that we studied in this experiment, involved: meristic acid, palmitic acid and stearic acid. These acids were not statistically different among treatments (p>0.05), but the lowest contents of meristat, palmitat and stearat were observed in 10% SS (0.75, 31.51 and 8.48, respectively) (Table 3).

Sum of SFAs (saturated fatty acid) reduced in groups fed different levels of SS and the lowest SFA was observed in 10% SS (42.98), but this reduction was not statistically significant (p>0.05). Wheeler *et al.* (1959) reported reduction in SFAs in yolk when they used different levels of safflower in diets of hens.

The content of ω-7 fatty acids such as palmitoleic acid in egg yolk was not different (p>0.05) (Table 3 and 4). The trend with oleic acid (ω-9 fatty acids) was not clear and the MUFA or monounsaturated fatty acids (sum of oleat and palemitoleat) content was not affected (p>0.05) (Table 4). But Wheeler *et al.* (1959) reported that the MUFA in yolk was reduced with feeding different levels of safflower.

After feeding the hens for 4 weeks with different levels of SS, the linoleic acid increased with 7 and 10% SS (11.95 and 12.46, respectively) but at 4% SS (11.34) no difference was observed with control diet (10.24) (p>0.05) (Table 3 and Fig. 1). Similar results, were reported by others (Wheeler *et al.*, 1959; Wang *et al.*, 1990).

Table 3: Fatty acids profile in yolk lipids from eggs feeding different levels of Safflower Seed (SS)

	Experimental diet			
Fatty acid	Control	4% SS	7% SS	10%SS
Percentage of total methyl esters of fatty acids				
$C_{14:0}$	0.860	0.870	0.920	0.750
$C_{16:0}$	34.920	34.650	33.350	31.510
$C_{16:1\ n-7}$	2.990	2.230	2.720	3.090
$C_{18:0}$	8.890	9.770	10.000	8.480
$C_{18:1\ n-9}$	39.960	38.650	38.910	39.450
$C_{18:2\ n-6}$	10.240^{b}	11.340^{ab}	11.950^{a}	12.460^{a}
$C_{18:3\ n-3}$	0.500	0.510	0.510	0.490
$C_{20:4\ n-6}$	1.340	1.820	1.960	1.680
$C_{20:5\ n-3}$	0.157	0.147	0.147	0.137
$C_{22:6\ n-3}$	0.031	0.026	0.030	0.032

Values having different superscript within each column were significantly different (p<0.05)

Table 4: Lipids, cholesterol and fatty acids in comparison with the saturation (%) in the yolk lipids of eggs from feeding different levels of Safflower Seed (SS)

	Experimental diet			
Fatty acid	Control	4% SS	7% SS	10%SS
Percentage of total methyl esters of fatty acids				
SFA*	44.98	45.11	44.26	42.98
PUFA	55.02	54.88	55.74	57.02
MUFA	42.95	40.88	41.63	42.54
ω-7	2.99	2.23	2.72	3.09
ω-9	39.96	38.65	38.91	39.45
ω-6	12.38^{b}	13.16^{a}	13.91^{a}	14.11^{a}
ω-3	0.69	0.68	0.68	0.65
SFA/PUFA	0.82^{a}	0.82^{a}	0.79^{ab}	0.74^{b}
SFA/MUFA	1.05	1.10	1.06	1.01
ω-6/ω-3	18.01^{b}	19.41^{b}	20.51^{ab}	21.77^{a}

SFA; Saturated Fatty Acid; PUFA: Polyunsaturated Fatty Acid; MUFA: Monounsaturated Fatty Acid; ω-7: ω-7 fatty acids and ω-6/ω-3 the ratio of ω-6/ω-3 fatty acids; Values having different superscript within each column were significantly different (p<0.05)

Arachidonic acid content in egg yolk did not vary significantly among treatments (p>0.05). But the ω-6 fatty acids in egg yolk increased after feeding different levels of SS (p<0.05) (Table 4). Similarly, Wheeler *et al.* (1959) reported that with increased levels of safflower in laying hen diets, the n-6 fatty acids in egg increased.

After the first period and end of experiments, the diets containing different levels of SS did not affect content of linolenic, eicosapentaenoic and docosahexaenoic acids (Table 3). Therefore, the ω-3 fatty aids of yolk did not vary significantly with feeding different levels of SS (Table 4).

The ratio of SFA/PUFA of egg yolk decreased with feeding different levels of SS. The lowest ratio of SFA/MUFA was observed in 10% SS treatment (0.74) and this reduction was statistically significant (p<0.05), but 4,

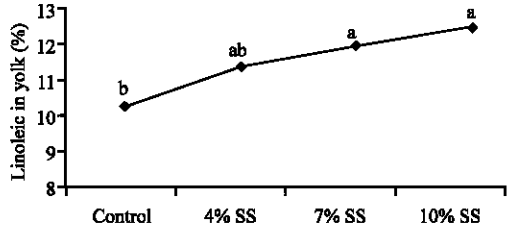

Fig. 1: The effect of different levels of safflower seed on linoleic acid in egg yolk

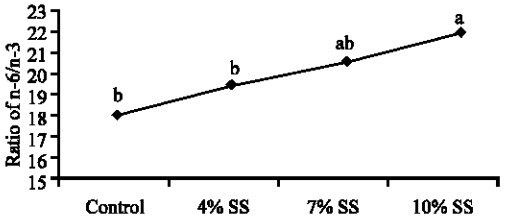

Fig. 2: The effect of different levels of safflower seed on n-6/n-3 in egg yolk

7% SS did not differ with control diet. Also, the ratio of SFA/MUFA did not vary between SS treatments and control. However, with increase of SS levels, the percentage of SFA was decreased but these differences were statistically not significant ($p>0.05$).

The diets containing different levels of SS presented a large amount of n-6 fatty acid and a small amount of n-3 fatty acids and the ratio of ω-6/ω-3 was increased at different SS treatments and the largest ratio was observed in 10% SS ($p<0.05$) (Table 4 and Fig. 2).

CONCLUSION

After 14 weeks of feeding with different levels of safflower seed, there was no significant effect on production parameters, egg quality, egg and blood cholesterol, except body weight gain and specific gravity. The SFA, ω-7, ω-9 and ω-3 fatty acids in egg content did not vary with feeding different levels of SS ($p>0.05$). The ω-6 fatty acids of egg yolk especially linoleic acid increased with increasing the SS levels in diet and this increase was statistically significant ($p<0.05$). Also, after 4 weeks of feeding with SS, the SFA/PUFA in egg yolks reduced but the ω-6/ω-3 statistically increased ($p<0.05$). The greatest amount in ω-6/ω-3 ratios was observed in 10% SS treatments (21.77).

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