Journal of Animal & Plant Sciences, 34(2): 2024, Page: 445-453
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2024.2.0730

EFFECT OF DIETARY INCLUSION OF MARGARINE ON LAYING PERFORMANCE, EGG QUALITY, EGG YOLK FATTY ACIDS, AND SERUM LIPID METABOLITES IN LAYING HENS

D. M. Karagöz[1,a,*], M. N. Oğuz[1,b], F. K. Oğuz[1,c], K. E. Buğdaycı[1,d], B. A. Baydur[2,e] and Umair Ahsan[3,4,f]

[1]Department of Animal Nutrition, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, 15030, Burdur, Turkiye
[2]Hurma Veterinary Clinic, 07000, Antalya, Turkiye, bilgehanaktemur@gmail.com
[3]Department of Plant and Animal Production, Burdur Vocational School of Food, Agriculture and Livestock, Burdur Mehmet Akif Ersoy University, 15030, Burdur, Turkiye
[4] Center for Agriculture, Livestock and Food Research, Burdur Mehmet Akif Ersoy University, 15030, Burdur, Turkiye
ORCID ID: ; 0000-0002-1179-1317; [b]0000-0001-8802-3423; [c]0000-0002-9077-8531; [d]0000-0002-1715-6904; [e]0000-0002-4435-587X; [f]0000-0003-4741-3745
[*]Corresponding Author Email: dmarituluk@mehmetakif.edu.tr, derya_merve_arituluk@hotmail.com

ABSTRACT

In this study, feed consumption, feed conversion ratio, egg production, egg quality, and some serum lipid metabolites were evaluated in laying hens fed diets containing different inclusion levels of margarines with different degrees of saturation. A total of sixty 18-wk-old ATAK-S laying hens were distributed to 5 experimental groups, each consisting of 6 replicates with 2 laying hens in each replicate. In the experiment, two inclusion levels (5 and 10%) of margarine with two different degrees of saturation (17 and 35%) containing 60% crude fat were used. Control group was fed diet without the inclusion of margarine. Other groups were fed diets with 5 or 10% of 17% (M17-5 and M17-10) or 35% (M35-5 and M35-10) saturated margarines. All the groups were fed isocaloric and isonitrogenous diets. The experiment lasted for eight weeks. Feeding margarines at different inclusion levels had no effect on daily feed consumption, egg yield, feed conversion rates (dozen eggs/kg feed and kg eggs/kg feed), and serum triglyceride and cholesterol levels of laying hens. In addition, yolk color, albumen index, shape index, egg weight, Haugh unit, and yolk index were not affected across the groups. However, the addition of 35% saturated margarine with regardless of the inclusion level increased the total unsaturated fatty acids in the egg yolk of laying hens. In conclusion, egg yolk fatty acids can be manipulated by different dietary inclusion levels of margarines at saturation points.

Keywords: egg, laying hen, lipid, vegetable oil, margarine

Published first online January 29, 2024 Published final March 31, 2024

INTRODUCTION

Margarine is obtained by the hydrogenation of vegetable oils (Li *et al*., 2019) that adds hydrogen atoms to the unsaturated bonds yielding a more saturated oil with triglycerides. Hydrogenation greatly modifies the physical and chemical properties of oils (Puprasit *et al*., 2020). However, this technique produces trans fatty acids (Li *et al*., 2019) known to adversely affect human health in terms of cardiovascular diseases, obesity, and cancer (Hu *et al*., 2017). Therefore, partial replacement of dietary saturated fatty acids (SFA) with their polyunsaturated counterparts (PUFA) is a cornerstone of numerous nutritional recommendations for protection against coronary heart disease (Virtanen, 2018; Karadağoğlu *et al*., 2019).

Oil improves the feed intake, palatability, immunity, and reduce the morbidity in poultry (Özdoğan and Sarı, 2001; Palmquist, 2009; Gopi *et al*., 2014; Stevanovic *et al*., 2018). Additionally, dietary fats are known to significantly alter the fatty acid composition of egg yolk (Rowghani *et al*., 2017; Skrtic *et al*., 2008) thereby allowing consumers to consume low-cholesterol eggs to control their blood cholesterol levels (Hoan and Khoa, 2016). Therefore, appropriate amount and type of dietary fat is significant for laying performance, lipid metabolism, and egg quality of laying hens (Gao *et al*., 2021).

It is widely accepted that margarine has adverse effects on human health due to the trans fatty acids that occur during the production of margarine (Hu *et al*., 2017). Studies involving animals on this subject could be more extensive since most studies have reported the use

of partially hydrogenated oils (older-type margarine). In new-generation margarine, some oil is fully hydrogenated (fully solidified) and mixed with non-hydrogenated (liquid) oil to adjust its softness. To the best of our knowledge, no study has been conducted so far on the use of new-generation margarine in laying hen diets. Therefore, this study was aimed to investigate the effects of different inclusion levels of dietary margarine containing two different saturated fatty acid contents on laying performance, egg quality, yolk fatty acids, and serum lipid metabolites of laying hens.

MATERIALS AND METHODS

Ethical statement: This study was conducted at Burdur Mehmet Akif Ersoy University, Turkiye as the coordinates 37°41'28" South latitude, 30°20'35" West longitude pursuant to the guidelines of the institutional Committee for Ethical Use of Animals approved vide protocol no. 93773921-11.

Animals, experimental design, and diets: The study was executed as a completely randomized design consisting of five experimental groups, each comprising of 6 replicates having 2 laying hens/replicate. One group serving as control was fed diet without the inclusion of margarine. Other groups were fed diets with 5 or 10% of 17% (M17-5 and M17-10) or 35% (M35-5 and M35-10) saturated margarines. The nutritional composition of margarines used has been summarized in Table 1. All the groups were fed isocaloric and isonitrogenous diets (Table 2) to meet or exceed the nutrient requirements in line with the recommendations of NRC (1994). Laying hens were housed in 2-storey and 2-compartment rearing cages designed for laying hens. The trial lasted for eight weeks. During the experiment, a 17-h L:7-h D lighting program was followed. Each replicate was subjected to group feeding. Laying hens were allowed ad libitum access to feed and water.

Productive performance and egg quality: Feed consumption was recorded on weekly basis. The amount of feed consumed to produce one kg and a dozen eggs were calculated. Laying hens were weighed at the commencement and at the end of the of the experiment. Live weight change was computed using the difference method.

Egg production was recorded continuously, eggs were weighed and stored at 15°C. External and internal egg quality was measured every two weeks using following methods:

Egg size was measured in terms of width and length using a tripod micrometer. Yolk color, eggshell thickness, eggshell weight, shape index, albumen index, yolk index, and Haugh unit (HU) of 18 eggs from each group were evaluated. Shell thickness (mm) was measured from 3 different parts (upper and lower ends, and middle) using a micrometer. Egg yolk color was measured using the Roche Yolk Color Fan (The CIE standard colorimetric system, F. Hoffman-La Roche Ltd., Basel, Switzerland). Other egg quality measurements were calculated using the following equations:

$$\textbf{Shape index}\ (\%) = \frac{\textbf{egg width}}{\textbf{egg length}} \times 100$$

$$\textbf{Albumen index}\ (\%) = \frac{\textbf{albumen height} \times 2}{\textbf{albumen length} + \textbf{albumen width}} \times 100$$

$$\textbf{Yolk index}\ (\%) = \frac{\textbf{yolk height}}{\textbf{yolk diameter}} \times 100$$

$$\textbf{Haugh unit} = \log\left(H + 7.57 - 1.7\,W^{0.37}\right) \times 100$$

Where:
H: albumen height
W: egg weight

Fatty acid composition of egg yolk and diets: Fatty acid composition of diets and egg yolks was measured from 12 eggs in each group using gas chromatography. Eggs were randomly selected, broken, and yolks were separated. Fat from diets and egg yolks was extracted by mixing with 20 ml solution of chloroform and methanol (2:1). Then, the organic phase was taken and evaporated. Fatty acid methyl esters (FAMEs) were prepared by saponification with sodium hydroxide followed by mixing with the solution of boron trifluoride in 35% methyl alcohol (methylated boron trifluoride). FAMEs were separated by the addition of n-hexane, vortexed, condensed, and filtration of the condensate. The condensate was injected into the GC-MS system (AGILENT 5975C&7890A GC, Hewlett Packard, Santa Clara, CA, US) fitted with 100 m × 0.25 mm × 0.2 µm column (HP-88 capillary column, Agilent J&W, Santa Clara, CA, US) under following conditions: initial temperature 60°C increased to 175°C with an increase in temperature of 13°C per minute followed by 4°C increments to 215°C that was maintained for 35 minutes. Finally, injector and detector temperatures were increased to 250ºC (Bardakçı and Seçilmiş, 2006). Fatty acid composition of diets is presented in Table 3.

Serum cholesterol and triglyceride levels: At the end of the experiment, blood was collected from the wing vein, allowed to clot, and serum was separated by centrifugation at 3000 rpm for 10 minutes. Serum triglyceride and cholesterol were analyzed using commercial kits (Roche Diagnostics International Ltd., Risch-Rotkreuz, Switzerland) followed by the measurement of these metabolites in the plate reader (Roche COBAS Integra 800; Roche Diagnostics International Ltd., Risch-Rotkreuz, Switzerland).

Statistical analysis: Data were analyzed by one-way ANOVA using the statistical software package SPSS (version 22.0, Armonk, NY, US) to assess the effect of diets containing two different types and doses of

margarine on laying performance, egg production, egg quality, fatty acid profile of egg yolk, and serum cholesterol and triglyceride levels of laying hens. Following statistical model presented in equation 1 was applied:

$Y_{ij} = \mu + s_i + e_{ij}$ ..... (1)

Where:

Y_{ij} = phenotypic value of the trait for the *j*th group of laying hens belonging to *i*th type and inclusion levels of margarine in laying hen diets;

μ = mean value of the trait for a given population;

s_i = effect of *i*th type and inclusion levels of margarine in laying hens;

e_{ij} = effect of experimental error.

Duncan's multiple range test was applied as post-hoc test to separate the significantly different means. Significance of the results was assumed at 95% probability. Results were shown as mean ± SEM.

RESULTS

Laying performance and serum metabolites: This study showed that the addition of margarines at different inclusion levels had no effect on daily feed consumption, feed conversion rates (dozen eggs/ kg feed and kg eggs/ kg feed) during the experimental period. While weekly egg yield was not different among the groups in 1-4 weeks, it was lower in control and M17-5 groups at 5-8 weeks than in other groups (Table 4). Body weight of laying hens fed two doses of 35% saturated margarine was greater in comparison with other groups ($P \leq 0.001$) (Table 5). Serum triglyceride and cholesterol levels were not different among the groups (Table 6).

Egg quality: Egg quality of laying hens fed diets with different inclusion levels of 17 or 35% saturated margarines remained unaffected in comparison with control group (Table 7).

Egg yolk fatty acids: Laying hens fed diets containing 5 or 10 % of 17% saturated margarine had greater C6:0 ($P \leq 0.001$) in yolk in comparison with those receiving 5 or 10% of 35% saturated margarine (Table 8). Laying hens in M35-10 group had greater ($P \leq 0.001$) C12:0 and C14:0 in yolk in comparison with other dietary treatments. Myristoleic acid (C14:1) was greater in egg yolk of laying hens in M35-10 group compared to those in M17-5, M17-10, and M35-5 groups ($P \leq 0.001$). Inclusion of 5 or 10% of 17% saturated margarine in laying hen diets increased the pentadecanoic acid (C15:0) in yolk in comparison with other groups ($P \leq 0.001$). Egg yolk of laying hens in M35-5 group had lower C16:0 concentration than M17-5 and M17-10 groups ($P = 0.028$). The concentration of C17:0 was greater ($P = 0.005$) in the yolk of control group compared to those of M35-5 and M35-10 groups. The concentration of C18:0 was lower in control, M17-5, and M17-10 groups than other groups ($P \leq 0.001$). Oleic acid (C18:1 n-9) was higher in the yolk of laying hens fed 35% saturated margarine (5% or 10%) ($P \leq 0.001$). Laying hens fed diets containing 5% of 17% saturated margarine had greater alpha-linolenic acid (C18:3 n-3) concentration in the yolk in comparison with M17-10 and M35-10 groups ($P \leq 0.001$). Inclusion of 5 or 10% of 35% saturated margarine in laying hen diets increased gamma linolenic acid (C18:3 n-6) in yolk in comparison with other groups ($P \leq 0.001$). Laying hens fed control diet exhibited greater C20:0 concentration in yolk than those fed diets containing different margarines at different inclusion rates ($P \leq 0.001$). Laying hens in M17-5 group showed greater C20:1 concentration in yolk than those in other groups ($P \leq 0.001$). Laying hens in control and M17-10 groups had greater C20:4 n-6 in comparison with other experimental groups ($P \leq 0.001$). A lower concentration of behenic acid (C22:0) was noted in the yolk of laying hens in M17-5 group compared to other groups ($P \leq 0.001$). Total monounsaturated fatty acids (MUFA) were greater in M35-10 group than other groups ($P \leq 0.001$). Total PUFA were greater in laying hens in control and M35-5 groups than other groups ($P \leq 0.001$). Total UFA concentration and UFA/SFA ratio were greater in M35-5 and M35-10 groups in comparison with other groups ($P \leq 0.001$). Total SFA concentration was lower in M35-5 and M35-10 groups in comparison with other groups ($P \leq 0.001$).

Table 1. Nutritional composition of sunflower oil and margarines used in the experiment (per 100 g, as fed basis).

Item	Sunflower oil	Margarine-M17	Margarine-M35
Gross energy (kJ/kcal)	3387/819	2220/540	2220/540
Crude fat, g	91	60	60
Saturated fat, g	7.5	17	35
Carbohydrate, g	0	0.1	0.3
Protein, g	0	0.1	0.1
Vitamin A, µg	0	600	600
Vitamin D, µg	0	2.5	2.5

Table 2. Composition of experimental diets (%, as fed basis).

Item	Control	M17-5[1]	M17-10[2]	M35-5[3]	M35-10[4]
Sunflower oil	2.5	1.2	0	1.2	0
Margarine	0	5	10	5	10
Corn	66	61.2	56.5	61.2	56.5
Sunflower Meal	7	7	7	7	7
Full-fat soybean meal	14	15.1	16	15.1	16
Dicalcium phosphate	1	1	1	1	1
DL-Methionine	0.1	0.1	0.1	0.1	0.1
Limestone	8.75	8.75	8.75	8.75	8.75
L-Lysin hydrochloride	0.1	0.1	0.1	0.1	0.1
Salt	0.3	0.3	0.3	0.3	0.3
Vitamin-mineral premix*	0.25	0.25	0.25	0.25	0.25
Calculated nutrients					
Crude protein	14.80	15.00	15.10	15.00	15.10
Metabolizable energy (kcal/kg)	2900	2896	2899	2896	2899
Crude fat	4.33	5.69	7.15	5.69	7.15
Crude fiber	3.70	3.60	3.50	3.60	3.50
Crude ash	12.38	12.38	12.38	12.38	12.38
Lysine	0.74	0.76	0.77	0.76	0.77
Methionine + Cysteine	0.60	0.60	0.59	0.60	0.59
Calcium	3.63	3.11	3.11	3.11	3.11
Available phosphorus	0.27	0.27	0.27	0.27	0.27
Linoleic acid	2.80	2.60	2.30	2.60	2.30

* Composition per kg of feed: Vitamin A, 3,333.3 IU; Vitamin D3, 833,3 IU; Vitamin E, 11.6 mg; Vitamin K3, 1.3 mg; Vitamin B1, 666.7 mg; Vitamin B2, 2.0 mg; Pantothenic acid, 2,666.7 mg; Vitamin B6, 1,333.3 mg; Vitamin B12, 5 mg; Folic acid, 250 mg; Biotin, 15 mg; Choline, 133,333.3 mg; Cu, 1,666.7 mg; Fe, 13,333.3 mg; I, 250 mg; Mn, 33,333.3 mg; Se, 83.3 mg; Zn, 20.000 mg.

[1] Diets with 5% of 17% saturated margarine

[2] Diets with 10% of 17% saturated margarine

[3] Diets with 5% of 35% saturated margarine

[4] Diets with 10% 35% saturated margarine

Table 3. Fatty acid composition of experimental diets.

Fatty acids, %	Control	M17-5[1]	M17-10[2]	M35-5[3]	M35-10[4]
C12:0 (Lauric acid)	0.06	4.53	7.07	5.33	7.01
C14:0 (Myristic acid)	0.11	1.75	2.60	1.75	0.83
C16:0 (Palmitic acid)	8.71	21.24	26.27	23.07	28.00
C16:1 (Palmitoleic acid)	0.14	0.25	0.09	0.12	0.17
C18:0 (Stearic Acid)	3.04	5.59	4.69	5.14	6.64
C18:1 n-9 (Oleic acid)	27.85	28.19	27.46	27.17	24.41
C18:2 n-6 (Linoleic Acid)	56.87	34.83	28.21	33.09	30.26
C18:3 n-3 (alpha-linolenic acid)	0.82	0.72	0.67	0.67	0.59
C20:4 n-6 (Arachidonic acid)	0.68	0.64	1.63	0.81	0.53
C20:1 (cis-11 eicosenoic acid)	0.26	0.41	0.22	0.26	0.30
C22:0 (Behenic acid)	0.34	0.19	0.09	0.19	0.11
∑MUFA	27.99	28.44	27.55	27.29	24.59
∑PUFA	58.38	36.19	30.51	34.57	31.38
∑SFA	12.53	33.69	40.92	35.74	42.89
∑UFA	86.36	64.63	58.06	61.86	55.96
PUFA/SFA	4.66	0.79	0.98	0.91	1.01
UFA/SFA	7.21	1.59	1.70	1.63	1.79

[1] Diets with 5% inclusion of 17% saturated margarine

[2] Diets with 10% inclusion of 17% saturated margarine

[3] Diets with 5% inclusion of 35% saturated margarine

[4] Diets with 10% inclusion of 35% saturated margarine

Table 4. Laying performance of hens fed different dietary inclusion levels of margarine with different degrees of saturation.

Item	Week	Control	M17-5[1]	M17-10[2]	M35-5[3]	M35-10[4]	P-value
Feed consumption, g/d	**1-4**	109±2.35	108±2.75	120.40±8.17	112±4.14	116±4.79	0.361
	5-8	115±5.15	114±2.02	115.55±5.01	114±3.72	118±1.57	0.962
	Mean	112±3.40	111±2.04	117.98±6.27	113±3.74	117±3.02	0.671
Weekly egg yield, %	**1-4**	79.76±3.28	83.33±2.28	85.41±1.55	82.14±2.48	85.11±1.76	0.441
	5-8	89.40±0.78[b]	88.95±0.85[b]	90.50±0.55[ab]	90.71±0.52[ab]	91.82±0.31[a]	0.031
	Mean	85.11±1.72	86.46±1.14	88.24±0.74	86.90±1.36	88.84±0.89	0.241
Feed conversion ratio (dozen eggs/kg feed)	**1-4**	2.01±0.03	1.96±0.04	2.25±0.13	2.04±0.08	2.11±0.08	0.152
	5-8	2.00±0.10	1.94±0.04	2.03±0.09	1.96±0.07	2.07±0.03	0.721
	Mean	2.00±0.06	1.95±0.04	2.14±0.11	2.00±0.07	2.10±1.96	0.323
Feed conversion ratio (kg egg/kg feed)	**1-4**	1.13±0.06	1.16±0.04	1.32±0.10	1.14±0.04	1.26±0.09	0.294
	5-8	1.36±0.05	1.41±0.04	1.37±0.07	1.36±0.05	1.43±0.03	0.774
	Mean	1.25±0.05	1.28±0.03	1.34±0.08	1.25±0.04	1.34±0.05	0.531

[a,b] Means with different superscripts within the same row differ significantly.
[1] Diets with 5% inclusion of 17% saturated margarine
[2] Diets with 10% inclusion of 17% saturated margarine
[3] Diets with 5% inclusion of 35% saturated margarine
[4] Diets with 10% inclusion of 35% saturated margarine

Table 5. Initial and final live weights of laying hens (g) fed different dietary inclusion levels of margarines with different degrees of saturation.

Item	Control	M17-5[1]	M17-10[2]	M35-5[3]	M35-10[4]	P-value
Initial body weight, g	1592±40.20	1594±45.02	1658±35.21	1650±44.22	1606±51.47	0.711
Final body weight, g	1929±9.07[b]	1956±10.79[b]	1919±15.12[b]	2029±10.56[a]	2025±16.20[a]	≤0.001

[a,b] Means with different superscripts within the same row differ significantly.
[1] Diets with 5% inclusion of 17% saturated margarine
[2] Diets with 10% inclusion of 17% saturated margarine
[3] Diets with 5% inclusion of 35% saturated margarine
[4] Diets with 10% inclusion of 35% saturated margarine

Table 6. Serum cholesterol and triglyceride levels (mg/dL) of laying hens fed different dietary inclusion levels of margarines with different degrees of saturation.

Item	Control	M17-5[1]	M17-10[2]	M35-5[3]	M35-10[4]	P-value
Cholesterol	102.53±2.41	136.97±14.80	106.38±13.64	107.68±13.31	134.82±20.07	0.258
Triglyceride	1429±140.45	1330±140.45	928±164.60	1155±282.11	873±75.91	0.098

[1] Diets with 5% inclusion of 17% saturated margarine
[2] Diets with 10% inclusion of 17% saturated margarine
[3] Diets with 5% inclusion of 35% saturated margarine
[4] Diets with 10% inclusion of 35% saturated margarine

Table 7. Egg quality of laying hens fed different dietary inclusion levels of margarines with different degrees of saturation.

Item	Week	Control	M17-5[1]	M17-10[2]	M35-5[3]	M35-10[4]	P-value
Shape index, %	**1-4**	80.16±0.46	77.87±0.95	78.14±0.58	79.80±1.41	77.54±0.60	0.151
	5-8	79.69±1.02	77.89±0.90	78.47±0.81	79.21±0.90	78.75±0.81	0.672
	Mean	79.93±0.50	77.88±0.75	78.30±0.60	79.51±1.07	78.14±0.62	0.233
Yolk index, %	**1-4**	47.44±0.33	48.46±0.93	47.04±0.47	47.85±0.54	46.63±2.07	0.781
	5-8	44.42±1.15	46.01±0.73	44.02±1.31	46.52±0.87	44.43±0.71	0.312
	Mean	45.93±0.69	47.24±0.38	45.53±0.85	47.18±0.59	45.53±1.21	0.342
Albumen index, %	**1-4**	10.35±0.71	11.72±1.05	10.09±0.67	12.16±0.58	11.16±0.34	0.254

	5-8	9.41±0.74	9.51±0.71	8.36±0.51	9.81±0.93	9.65±0.69	0.653
	Mean	9.88±0.69	10.61±0.76	9.22±0.58	10.98±0.73	10.40±0.52	0.391
Yolk color	**1-4**	12.17±0.17	12.33±0.25	12.33±0.31	12.33±0.17	12.67±0.17	0.601
	5-8	12.33±0.21	11.92±0.15	12.08±0.24	12.08±0.24	12.42±0.20	0.460
	Mean	12.25±0.45	12.13±0.17	12.21±0.18	12.21±0.16	12.54±0.12	0.451
Haugh unit	**1-4**	96.26±2.11	99.31±2.26	96.14±1.77	100.65±1.41	99.24±1.62	0.354
	5-8	95.53±1.95	96.95±1.60	93.23±1.40	97.61±2.74	97.70±1.89	0.482
	Mean	95.89±1.92	98.13±1.52	94.69±1.56	99.13±2.05	98.47±1.47	0.341
Egg weight, g	**1-4**	55.21±1.56	55.86±0.70	53.81±0.81	55.21±0.64	55.12±0.97	0.692
	5-8	57.77±1.39	58.61±0.51	57.27±0.75	57.84±0.44	57.57±0.74	0.841
	Mean	56.49±1.42	57.24±0.59	55.54±0.76	56.52±0.51	56.34±0.79	0.753

[1] Diets with 5% inclusion of 17% saturated margarine
[2] Diets with 10% inclusion of 17% saturated margarine
[3] Diets with 5% inclusion of 35% saturated margarine
[4] Diets with 10% inclusion of 35% saturated margarine

Table 8. Yolk fatty acid composition of laying hens fed different dietary inclusion levels of margarines with different degrees of saturation.

Fatty acids, %	Control	M17-5[1]	M17-10[2]	M35-5[3]	M35-10[4]	P-value
C6:00 (Caproic acid)	0.02±0.00^{bc}	0.05±0.01^{a}	0.03±0.01^{b}	0.01±0.00^{c}	0.01±0.00^{c}	≤0.001
C12:0 (Lauric acid)	0.04±0.00^{b}	0.09±0.00^{b}	0.05±0.01^{b}	0.03±0.00^{b}	0.14±0.07^{a}	≤0.001
C14:0 (Myristic acid)	0.49±0.02^{b}	0.49±0.05^{b}	0.46±0.03^{b}	0.50±0.01^{b}	0.60±0.01^{a}	0.023
C14:1 (Myristoleic acid)	0.11±0.01^{b}	0.10±0.01^{bc}	0.07±0.01^{d}	0.09±0.01^{c}	0.14±0.01^{a}	≤0.001
C15:0 (Pentadecanoic acid)	0.04±0.00^{c}	0.10±0.01^{a}	0.06±0.01^{b}	0.04±0,00^{c}	0.06±0.01^{b}	≤0.001
C16:0 (Palmitic acid)	28.32±0.72^{ab}	29.03±0.60^{a}	29.38±1.34^{a}	25.69±0,34^{b}	26.73±0.99^{ab}	0.028
C16:1 (Palmitoleic acid)	2.80±0.27	2.55±0.39	2.63±0.17	3.03±0.13	2.96±0.18	0.609
C17:0 (Heptadecanoic acid)	0.16±0.01^{a}	0.14±0.01^{ab}	0.14±0.01^{ab}	0.12±0.01^{b}	0.12±0.01^{b}	0.005
C18:0 (Stearic Acid)	7.82±0.19^{a}	8.53±0.55^{a}	7.91±0.11^{a}	5.95±0.17^{b}	5.84±0.16^{b}	≤0.001
C18:1 n-9 (Oleic acid)	44.85±0.74^{d}	44.96±0.26^{d}	46,67±1.18^{bc}	48.46±0.07^{b}	51.42±0.26^{a}	≤0.001
C18:2 n-6 (Linoleic Acid)	12.35±0.20^{ab}	11.87±0.44^{b}	11.38±0.11^{b}	13.35±0.39^{a}	9.94±0.18^{c}	≤0.001
C18:3 n-3 (Alpha-linolenic acid)	0.22±0.00^{ab}	0.23±0.00^{a}	0.10±0.01^{d}	0.22±0.00^{ab}	0.12±0.00^{c}	≤0.001
C18:3 n-6 (Gamma linolenic acid)	0.10±0.00^{c}	0.18±0.01^{b}	0.12±0.01^{c}	0.24±0.02^{a}	0.18±0.01^{b}	≤0.001
C20:0 (Arachidic Acid)	0.30±0.11^{a}	0.10±0.01^{bc}	0.15±0.03^{b}	0.09±0.02^{bc}	0.08±0.01^{c}	≤0.001
C20:1 (cis-11 eicosenoic acid)	0.14±0.01^{c}	0.24±0.01^{a}	0.11±0.01^{d}	0.13±0.00^{cd}	0.17±0.01^{b}	≤0.001
C20:4 n-6 (Arachidonic acid)	1.37±0.13^{a}	0.53±0.02^{b}	1.19±0.15^{a}	0.59±0.05^{b}	0.74±0.08^{b}	≤0.001
C22:0 (Behenic acid)	0.23±0.01^{a}	0.18±0.01^{c}	0.23±0.00^{ab}	0.22±0.00^{ab}	0.22±0.00^{b}	≤0.001
∑MUFA	47.90±0.51^{c}	47.85±0.59^{c}	49.49±1.09^{c}	51.71±0.09^{b}	54.69±0.08^{a}	≤0.001
∑PUFA	14.05±0.12^{a}	12.81±0.42^{b}	12.79±0.25^{b}	14.40±0.35^{a}	10.98±0.53^{c}	≤0.001
∑SFA	37.37±0.57^{a}	38.68±0.25^{a}	38.36±1.27^{a}	32.92±0.33^{b}	33.88±0.81^{b}	≤0.001
∑UFA	61.95±0.50^{b}	60.66±0.40^{b}	62.27±0.85^{b}	66.11±0.30^{a}	65.67±0.60^{a}	≤0.001
PUFA/SFA	0.38±0.00^{b}	0.33±0.01^{c}	0.34±0.01^{c}	0.44±0.01^{a}	0.33±0.02^{c}	≤0.001
UFA/SFA	1.66±0.04^{b}	1.57±0.02^{b}	1.64±0.08^{b}	2.01±0.03^{a}	1.95±0.06^{a}	≤0.001

[a.b.c,d] Means with different superscripts within the same row differ significantly.
[1] Diets with 5% inclusion of 17% saturated margarine
[2] Diets with 10% inclusion of 17% saturated margarine
[3] Diets with 5% inclusion of 35% saturated margarine
[4] Diets with 10% inclusion of 35% saturated margarine

DISCUSSION

Laying performance: The addition of fat to balance the energy level in the diet is a common practice in commercial animal feed industry. However, laying performance of hens relies on several factors such as breed, body weight, age, and energy level of the diet (Hoan and Khoa, 2016). In this study, it was expected that margarine with two different degrees of saturation may alter the nutritional composition of eggs and the performance of laying hens. This study showed that the addition of margarine with different saturation points at 5 or 10% dietary inclusion levels had no effect on feed consumption, egg yields, and feed conversion rates of laying hens. Similar findings were reported by Hosseini-Vashan and Afzali (2008) following the use of 0, 1.5, 3, and 4.5% palm oil in laying hen diets. Other studies

reported that the addition of different fat levels in laying hen diets was not effective in the manipulation of egg weight (Grobas *et al*., 2001) and feed conversation (Lelis *et al*., 2009). On the contrary, Fouladi *et al*. (2021) reported that adding different fat levels to the diets of laying hens reduces feed intake. These findings might be attributed to the levels of added fat that increased the energy level of the ration thereby reducing egg production, egg weight, and feed intake (Agah *et al*., 2010). Hoan and Khoa (2016) reported that laying performance of laying hens such as egg production, egg weight, and feed intake were decreased in response to the supplementation of different dietary sesame oil levels but not feed conversion ratio. Another study showed that feed intake, egg production, egg weight, egg yolk weight, liver mass, and cholesterol levels of liver and egg yolk were not affected in laying hens fed diets enriched with plant sterols (Liu *et al*., 2010).

Egg quality: Egg quality attributes like shape index, breaking strength, shell thickness, weight, yolk color, yolk index, flow index, Haugh unit, and egg yolk fatty acid composition depend significantly on the composition of diet rather than energy levels. Vegetable oils, widely used in animal diets, are rich in polyunsaturated fatty acids. Most of these fatty acids significantly relate to the growth and production performance of laying hens (Hoan *et al*., 2016). In the present study, shape index, yolk index, albumen index, yolk color, Haugh unit, and egg weight remained unaffected across the groups. Similarly, Kucukersan *et al*. (2010) reported that different oils added to laying hen diets affect the egg production and egg weight but not feed consumption and efficiency. Rowghani *et al*. (2017) reported that addition of 3% or 5% rapeseed oil to the basal diet of 24-week-old Hy-Line Brown chickens had no significant effect on egg weight. Hosseini-Vashan and Afzali (2008) reported that Haugh unit score, yolk color index, yolk index, egg shape index, shell weight, shell thickness, and egg density of laying hens were not affected by the addition of 0, 1.5, 3, and 4.5% dietary palm oil. Likewise, Hoan and Khoa (2016) described that increasing levels of sesame oil in laying hen diets did not affect the shape index, breaking strength, or shell thickness. Similarly, Liu *et al*. (2010) reported that laying hens receiving diets enriched with plant sterols had no effect on the Haugh unit of eggs.

In our study, serum cholesterol and triglyceride levels remained unaffected across the groups. Likewise, Sim and Bragg (1977) reported that the addition of cholesterol to the basal diet including 8% safflower oil caused a significant increase in serum cholesterol levels, while in combination with hydrogenated coconut oil, it did not change the serum cholesterol level.

In the present study, inclusion of margarines with different degrees of saturation significantly altered the fatty acids composition of egg yolk. In addition, total UFA levels were higher when laying hens were fed dietary margarine with 35% degree of saturation. Besides these, there was no defined relationship between MUFA composition of egg yolks, and the type of margarine added to the diet. Several studies have reported that egg quality and yolk fatty acid profile can be modified by the inclusion of lipid sources in the diets of laying hens (Wu *et al*., 2005; Ribeiro *et al*., 2007; Oliveira *et al*., 2010). These differences may be related to the content of saturated and unsaturated fatty acids in the diets and genetic differences in the metabolism of each chicken. Oliveira *et al*. (2010) reported that, regardless of the source of lipids and the age of the laying hens, the percentage of trans fat in the egg yolk is meagre. The main problem with trans-fat is that despite being plant-based and unsaturated, it is metabolized more like saturated fat and converted to cholesterol, which is one of the contributing causes of cardiovascular diseases (Oliveira *et al*., 2010). In our study, C18:2 and C18:3 concentrations were proportional to their levels in the diet. Because these fatty acids play an essential role in human health and are the precursors of longer-chain fatty acids such as the n-3 and n-6 series, it is desirable to have these fatty acids in balanced concentrations in the egg yolk. Also, mammals do not synthesize these fatty acids and are obtained only from the diet (Oliveira *et al*., 2010). For linoleic acid (C18:2 n-6), laying hens fed diets containing 5% of 35% saturated margarine and control diets had higher concentrations in the yolk, followed by those in M17-5, M17-10, and M35-10 groups. The highest concentration of alpha-linolenic acid (C18:3 n-3) was seen in M17-5 and control groups followed by M35-10 and M17-10 groups. Shafey *et al*. (1992) also confirmed that adding vegetable oils to the diets of laying hens increases the linoleic acid concentration in the egg yolk.

Conclusions: In conclusion, inclusion of different dietary levels of margarines with different degrees of saturation may not affect the laying performance, serum fat metabolites, and egg quality of laying hens. However, dietary margarines with different degrees of saturation at different inclusion levels may alter the fatty acid composition of egg yolk. Further studies elucidating the hepatic metabolites of fats and liver fatty acids may enlighten the possible net anabolism and net catabolism of fatty acids involved in the final fatty acid composition of eggs.

Acknowledgements: This study was financially supported under the 2209-A Research Project Support Programme for Undergraduate Students framework of The Scientific and Technological Research Council of Türkiye (TÜBİTAK).

REFERENCES

Agah, H. J., H. Nasriri-Moghaddam, A. M. Tahmasbi and H. Lotfollahian (2010). Performance and fatty acid compositions of yolk lipid from laying hens fed with locally produced Canola Seeds (*Brassica napus L.*). Res. J. Agric. Biol. Sci. 5: 228-232.

Bardakçı, B. and H. Seçilmiş (2006). Investigation of chemical composition of rose oil from Isparta Region by GC-MS and FTIR Spectroscopy Technique. SDU Faculty of Arts and Sciences J. Science. 1(1): 64-69.

Fouladi, P., N. R. Salamat Doust, A. Ahmadzade, H. Aghdam Shahriar and A. Noshadi (2021). Effects of canola and sesame oil on the internal organs and carcass weight of broiler chickens. J. Anim. Vet. Adv. 7: 1160-1163.

Gao, Z., J. Zhang, F. Li, J. Zheng and G. Xu (2021). Effect of oils in feed on the production performance and egg quality of laying hens. Animals 11(12): 3482. DOI: 10.3390/ani11123482

Gopi, M., K. Karthik, H. V. Manjunathachar, P. Tamilmahan, M. Kesavan, M. Dashprakash and M. R. Purushothaman (2014). Essential oils as a feed additive in poultry nutrition. Adv. Anim. Vet. Sci. 2(1): 1-7.

Grobas, S., J. Mendez, R. C. Lazaro and G. G. Mateos (2001). Influence of source and percentage of fat added to diet on performance and fatty acids composition of eggs yolks of two strains of laying hens. Poult. Sci. 80: 1171-1179. DOI: 1093/ps/80.8.1171

Hoan, N. D. and M. A. Khoa (2016). The effect of different levels of sesame oil on productive performance, egg yolk and blood serum lipid profile in laying hens. Open J. Anim. Sci. 6(1): 85-93. DOI: 10.4236/ojas.2016.61011

Hosseini-Vashan, S. J. and N. Afzali (2008). Effect of different levels of palm olein oil in laying hen`s performance and yolk cholesterol. Int. J. Poult. Sci. 7 (9): 908-912.

Hu, P., X. Xu and L. L. Yu (2017). Effect of fatty acid chain length on the crystallization behaviour of trans-free margarine base stocks during storage. J. Oleo Sci. 66(4): 353–362. DOI: 10.5650/jos.ess16210

Karadağoğlu, O., T. Şahin, M. Ölmez, U. Ahsan, B. Özsoy and K. Onk (2019). Fatty acid composition of liver and breast meat of quails fed diets containing black cumin (*Nigella sativa* L.) and/or coriander (*Coriandrum sativum* L.) seeds as unsaturated fatty acid sources. Livest. Sci. 223: 164-171. DOI: 10.1016/j.livsci.2019.03.015

Küçükersan, K., D. Yeşilbağ and S. Küçükersan (2010). Influence of different dietary oil sources on performance and cholesterol content of egg yolk in laying hens. Biol. Environ. 4(12): 117-122.

Lelis, G. R., M. D. Silva, F. C. Tevernari, L. F. Z. Albino and H. S. Rostagno (2009). Performance of layers fed diets containing different diets. Braz. J. Poult. Sci. 11: 235-240. DOI: 10.1590/S1516-635X2009000400004

Li, C., L. K. Cobb, H. W. Vesper and S. Asma (2019). Peer Reviewed: Global Surveillance of trans-Fatty Acids. Prev. Chronic Dis. 16. DOI: 10.5888/pcd16.190121

Liu, X., H. L. Zhao, S. Thiessen, J. D. House and P. J. H. Jones (2010). Effect of plant sterol-enriched diets on plasma and egg yolk cholesterol concentrations and cholesterol metabolism in laying hens. Poult. Sci. 89(2): 270-275. DOI: 10.3382/ps.2009-00249

NRC. (1994). Nutrient Requirements of Poultry, 9th rev. Natl. Acad. Press, Washington, DC, US.

Oliveira, D. D., N. C. Baião, S. V., Cançado, R. Grimaldi, M. R. Souza, L. J. C. Lara and A. M. Q. Lana (2010). Effects of lipid sources in the diet of laying hens on the fatty acid profiles of egg yolks. Poult. Sci. 89(11): 2484-2490. DOI: 10.3382/ps.2009-00522

Özdoğan, M. and M. Sarı (2001). Fat addition to poultry rations. Anim. Prod. 42(1): 28-34.

Palmquist, D. L. (2009). Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal product foods. Prof. Anim. Sci. 2009; 25(3): 207-249. DOI: 10.15232/S1080-7446(15)30713-0

Puprasit, K., D. Wongsawaeng, K. Ngaosuwan, W. Kiatkittipong and S. Assabumrungrat (2020). Non-thermal dielectric barrier discharge plasma hydrogenation for production of margarine with low trans-fatty acid formation. Innov. Food Sci. Emerg. Technol. 66: 102511. DOI: 10.1016/j.ifset.2020.102511

Ribeiro, B. J. C., N. C. Lara, C. A. A. Baião, M. Lopez, S. V. Fiuza, G. M. Cançado and M. Silva (2007). Efeito do nível de ácido linoléico na ração de matrizes pesadas sobre o peso, composição e eclosão dos ovos. Arq. Bras. Med. Vet. 59: 789-796. DOI: 10.1590/S0102-09352007000300034

Rowghani, E., M. Arab, S. Nazifi and Z. Bakhtiari (2017). Effect of canola oil on cholesterol and fatty acid composition of egg-yolk of laying hens. Int. J. Poult. Sci. 6: 111–114.

Shafey, T. M., J. G. Dingle and M. W. McDonald (1992). Comparison between wheat, triticale, rye, soyabean oil and strain of laying bird on the production, and cholesterol and fatty acid

contents of eggs. Br. Poult. Sci. 33(2): 339-346. DOI: 10.1080/00071669208417472

Sim, J. S. and D. B. Bragg (1977). Effect of dietary factors on serum and egg yolk cholesterol levels of laying hens. Poult. Sci. 56(5): 1616-1621. DOI: 10.3382/ps.0561616

Skrtic, Z., G. Kralik, Z. Gajcevic, D. Hanzek and I. Bogut (2008). Effect of different source of oils on fatty acid profile and organoleptic traits of eggs. Acta Agric. Slov. 2: 129-134.

Stevanović, Z. D., J. Bošnjak-Neumüller, I. Pajić-Lijaković, J. Raj and M. Vasiljević (2018). Essential oils as feed additives—Future perspectives. Mol. 23(7): 1717. DOI: 10.3390/molecules23071717

Virtanen, J. K. (2018). Randomized trials of replacing saturated fatty acids with n-6 polyunsaturated fatty acids in coronary heart disease prevention: Not the gold standard? Prostaglandins Leukot. Essent. Fatty Acids. 133: 8-15. DOI: 10.1016/j.plefa.2018.04.002

Wu, M. M., R. A. Bryant and D. A Voitle (2005). Roland Effect of dietary energy on performance and egg composition of Bovans White and Dekalb White hens during phase I. Poult. Sci. 84: 1610-1615. DOI: 10.1093/ps/84.10.1610