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EFFECTS OF DIETARY CONJUGATED LINOLEIC ACID AND SELECTED VEGETABLE OILS OR VITAMIN E ON FATTY ACID COMPOSITION OF HEN EGG YOLKS*

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Abstract

The objective of this study was to produce eggs enriched with conjugated linoleic acid (CLA) and ameliorate their fatty acid profile using the appropriate combination of dietary CLA with or without vegetable oils (olive oil or rapeseed oil) and vitamin E. In Experiment 1, 25-week-old laying hens were randomly distributed into eight groups of nine. Birds were fed with a standard diet with four different levels of CLA (0.0, 0.5, 0.75, 1.0%) and vegetable oils (olive oil or rapeseed oil, both in the amount of 1.46%). In Experiment 2, hens were randomly distributed into 12 groups of nine. The same four levels of CLA with three doses of vitamin E (0, 150, 300 mg/kg of diet) were applied. In both experiments, eggs were collected twice (at 4 and 8 weeks) for fatty acid profiling using GCMS. The differences between treatment means were considered significant at P<0.05. CLA treatments significantly increased the content of CLA, saturated fatty acids (SFA), and significantly decreased the content of monounsaturated fatty acids (MUFA) in the egg yolk, whereas levels of polyunsaturated fatty acids (PUFA) were unaffected. The vegetable oils used did not prevent the negative effects of CLA effectively. Only after eight weeks of experiment 1 SFA levels were significantly lower, but MUFA levels were significantly higher in groups fed with rapeseed oil compared to groups fed with olive oil. In experiment 2, the addition of vitamin E to the hen diet did not have an essential influence on the lipid profile of egg yolks.

Key words: CLA-enriched eggs, olive oil, rapeseed oil, vitamin E, fatty acid profile

Hen eggs contain many essential nutrients for chick embryo development and are considered valuable food for humans. Several epidemiological studies have demonstrated the lack of a relationship between egg intake and the risk of cardiovascular

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diseases (Herron and Fernandez, 2004; Katz et al., 2005; Rueda and Khosla, 2013; Fuller et al., 2015). According to Dietary Guidelines for Americans (2015), if the intake of dairy fat and meat is controlled, there is no need to restrict egg yolk intake strictly, although some limitation remains prudent. Whereas amino acids and total protein are hardly affected by dietary treatments, minerals, fat-soluble vitamins and fatty acids are easily modified through feed manipulation (Oliveira et al., 2010). The use of nutritional strategies to improve the composition and quality of food products of animal origin (dairy products, meat and eggs) has recently been well known in animal science, food science and human nutrition (Pisulewski, 2005; Kouba and Mourot, 2011; Sahoo and Jena, 2014). From a nutritional point of view an egg is considered an ideal target for dietary modification leading to the development of a functional food. Several oils have been fed to laying hens to produce designer eggs, including linseed (flax), soybean, sunflower, and fish oils (Oliveira et al., 2010). The benefits of improving the quality of eggs by enhancing the concentration of *n*-3 fatty acids, vitamin E, carotenoids, and Se (Surai and Sparks, 2001), as well as CLA isomers (Jones et al., 2000; Schäfer et al., 2001; Cherian et al., 2002; Raes et al., 2002; Szymczyk and Pisulewski, 2005; Suksombat et al., 2006; Franczyk-Żarów et al., 2008) are known.

Conjugated linoleic acid (CLA) is a term for a group of positional and geometric (*cis, trans*) conjugated dienoic isomers of linoleic acid (18:2, *cis*-9, *trans*-12, *n*-6), present mainly in ruminant milk and meat. A number of reliable studies show that pure CLA decreases the risk of cancer and atherosclerosis, enhances immune responses and reduces body fat accumulation in experimental animals (Crumb, 2011; Lehnen et al., 2015; Benjamin et al., 2015; Kim et al., 2016).

Eggs and other products from monogastric animals contain negligible amounts of CLA. As CLA is readily incorporated into the fat fraction of animal foods, an egg yolk can be a good carrier, because it contains 30–35% of fat. One way to increase the CLA content in eggs is through supplementation of the laying hen's diet with CLA. A combined incorporation of CLA into the egg yolk, together with that of (*n*-3) fatty acids, would lead to even greater health benefits for human consumers (Suksombat et al., 2006).

Eggs from CLA-fed laying hens are good sources of CLA in the human diet (Chamruspollert and Sell, 1999; Raes et al., 2002; Shang et al., 2004). However, unless the several adverse effects of feeding CLA to laying hens on the fatty acid profile of egg yolks are eliminated, CLA-enriched eggs cannot be considered as a functional food product in human nutrition. Apart from adequate nutritional effects, functional food should have beneficial effects on body functions that are relevant to an improved state of health and well-being and/or reduction of a risk of a disease (Hasler, 2002). Moreover, the functional food product should have unchanged organoleptic properties. Dietary CLA increases the concentration of SFA and decreases MUFA in egg yolks (Chamruspollert and Sell, 1999; Shang et al., 2004; Szymczyk and Pisulewski, 2005; Suksombat et al., 2006; Franczyk-Żarów et al., 2008), induces chick embryonic mortality (Aydin et al., 2001; Aydin and Cook, 2004), and alters egg quality, e.g. by increasing hardness (Shang et al., 2004; Franczyk-Żarów et al., 2008; Yari et al., 2015; Liu et al., 2017).

As free fatty acid and methyl ester forms, CLA isomers were reported to have extremely low oxidative stability (Moon et al., 2008). Supplementing hen's diets with antioxidants like vitamin E ensured lipid stability in animal feed and food products enriched with CLA. The use of vitamin E (α-tocopherol) as a natural antioxidant in diets of laying hens was shown in previous papers (Cherian et al., 1996; Qi and Sim, 1998; Szymczyk and Pisulewski, 2005; Rebolé et al., 2006; Shahriar et al., 2008; Hayat et al., 2010; Karsten et al., 2010). However, the extent of these effects was negligible. In addition, incorporating tocopherols into eggs might also provide a source of tocopherols for the human diet. The consumption of two eggs from hens fed with the 10% flaxseed oil + 150 IU of α-tocopherols diet could contribute to more than 500 mg of *n-3* fatty acids and 11 mg of vitamin E to the human diet (Hayat et al., 2010).

Botsoglou et al. (1998) and Al-Daraji et al. (2010) point out that feeding hens with flaxseed oil affects the linear increase of ALA, as well as EPA and DHA in egg yolks. The use of oils (olive oil or rapeseed oil) was shown to improve the omega-6 to omega-3 ratio and to have a beneficial effect on SFA. However, Aydin et al. (2001) show that olive oil inhibits the incorporation of CLA in egg yolk lipids.

This study was conducted to optimize the most efficient selection of vegetable oils (olive or rapeseed oil) (**Experiment 1**) or vitamin E (**Experiment 2**), which would alleviate the adverse effects of increasing CLA in the CLA-enriched eggs.

Material and methods

Animals and diets

All procedures involving animals were approved by the Animal Ethics Committee (No. 424/2006) at the National Research Institute of Animal Production in Poland. The feeding conditions to obtain CLA-enriched eggs were described previously (Szymczyk and Pisulewski, 2005). Hens were fed with diets supplemented with CLA isomers (0.0, 0.5, 0.75, 1.0%). The CLA oil received from Natural Lipids Ltd. (Norway) contained 65% CLA (*cis*-9, *trans*-11: 27.6 g/100 g total fatty acids and *trans*-10, *cis*-12: 27.6 g/100 g total fatty acids, Table 1). Differential amounts of CLA in diets were obtained by substituting appropriate amounts of CLA with sunflower oil. Feed and water were available *ad libitum.* The fatty acid profile of used oils and diet composition are shown in Tables 1 and 2, respectively. Two independent experiments were conducted. **Experiment 1** was conducted on seventy-two 25-week-old laying hens (Hy-Line Brown) kept in individual laying cages for eight weeks. Birds were randomly distributed into eight groups of nine hens. They were assigned to the commercial layer diets with the following content of CLA (0.0, 0.5, 0.75, 1.0%) and different vegetable oils (olive oil or rapeseed oil; 14.6 g/kg of diet). Similarly, **Experiment 2** was conducted on 108 birds randomly distributed into twelve groups of nine hens. They were fed with standard diets with the following content of CLA (0.0, 0.5, 0.75, 1.0%) and different levels of vitamin E (0, 150, 300 mg/kg of the diet).

Table 1. Fatty acid profile (% of total fatty acids) of oils used in experimental diets

Fatty acid	CLA oil	Olive oil	Rapeseed oil	Flaxseed oil	Sunflower oil
12:0	0.1	–	–	–	–
14:0	5.4	–	0.2	–	–
16:0	0.1	13.0	4.4	6.5	6.4
18:0	4.0	2.5	2.0	3.5	5.7
18:1*n-9*	32.5	74.0	55.2	18.1	23.2
18:2*n-6*	0.6	9.0	22.2	14.0	64.6
18:3*n-3*	–	0.5	11.8	58.9	0.1
18:2*c9t11*	27.6	–	–	–	–
18:2*t10c12*	27.6	–	–	–	–
Total SFA*	9.7	15.5	8.6	10.0	12.1
Total MUFA*	32.6	75.0	56.2	18.1	23.2
Total PUFA*	57.7	9.5	35.2	71.9	64.7

*SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids.

Table 2. Ingredients and chemical composition of a diet for laying hens (%)

Ingredients	Diet (%)
Ground wheat	26.00
Ground maize	35.00
Soybean meal	21.37
Grass droughts	3.00
Oils[1]	4.00
Limestone	8.10
Dicalcium phosphate	1.70
Sodium chloride	0.30
Mineral and vitamin premix[2]	0.50
DL-methionine	0.01
L-Lysine	0.02
In 1 kg of diet[3]:	
Metabolizable energy (MJ)	11.45
Crude protein (%)	16.95
Met (%)	0.37
Lys (%)	0.76
Ca (%)	3.65
P (%)	0.68

[1]For the total amount of 4% of oil in mixtures consisting of flaxseed oil FO – 1.0% (in all groups) + olive oil OO or rapeseed oil RO – 1.46% + sunflower oil and CLA – 1.54% in following manner:

– mixtures without CLA addition – 1.54% sunflower oil.

– mixtures with 0.5% pure CLA – 0.77% CLA oil and 0.77% sunflower oil.

– mixtures with 0.75% pure CLA – 1.15% CLA oil and 0.39% sunflower oil.

– mixtures with 1.0% pure CLA – 1.54% CLA oil.

[2]Vitamin-mineral premix (Lutamix DJ) supplies: vit. A, 12,000 IU; vit. D_3, 2,000 IU; vit. E, 15 mg; vit. K_3, 2 mg; vit. B_1, 1 mg; vit. B_2, 4 mg; vit. B_6, 1.5 mg; biotin, 1 mg; vit. B_{12}, 0.01 mg; D-calcium pantothenate, 8 mg; nicotinamide, 25 mg; choline, 250 mg; Mn, 100 mg; I, 0.8 mg; Zn, 5 mg; Co, 0.2 mg; Se, 0.2 mg; DL-methionine, 500 mg.

– Diet without vitamin E supplementation (0.0 mg vitamin E).

– Diet with 150 mg vitamin E supplementation (150 mg vitamin E).

– Diet with 300 mg vitamin E supplementation (300 mg vitamin E).

[3]Calculated nutrient content.

Sampling procedures

Eggs from each hen, collected in the 4th and 8th weeks of experiments, were broken, yolks were separated from albumen, weighed, frozen at –20°C and freeze-dried for further analyses.

Fatty acid analyses

Total egg yolk lipids were extracted using a Leco TFE 2000 fat analyzer (Leco, St. Joseph, USA) with liquid carbon dioxide as a solvent. Extracts from egg yolks were analyzed for the amount of total fat (Leco TFE 2000 fat analyzer, Leco, St. Joseph, USA). Lipids of each sample (1 g) were extracted using the modified Folch procedure (Folch et al., 1957), fatty acid methyl esters (FAME) were extracted with hexane (Franczyk-Żarów et al., 2017). Fatty acid composition (relative %), including CLA isomers, was analyzed using a Shimadzu GC-MS model (Model QP 5050A; Shimadzu Corporation, Kyoto, Japan). FAME were identified by comparing their retention times with authentic standards purchased from Sigma-Aldrich (Poland) and the CLA reference standards (*cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers) were obtained from Larodan Fine Chemicals AB (Malmö, Sweden). The following fatty acids were analyzed: c9,t11 and t10,c12 CLA isomers, C14:0, C15:0, C16:0, C16:1, C17:0, C18:0, C18:1*n-9*, C18:2*n-6*, C18:3*n-3*, C20:0, C20:2*n-6*, C20:3*n-6*, C20:4*n-6*. All chemical analyses were performed in triplicates (3 eggs per cage).

Statistical analyses

The results are presented as mean ± SD. The data were subjected to a two-way analysis of variance generated by STATISTICA 12.0 package (StatSoft Inc., Tulsa, OK, USA, 2015), followed by a *post-hoc* Duncan's multiple range test. The differences between treatment means were considered significant at $P<0.05$.

Results

The fatty acid compositions of egg yolk lipids after four and eight weeks of **Experiment 1**, expressed as a percentage of total methyl esters of fatty acids, are shown in Tables 3a and 3b, respectively; whereas those obtained after four and eight weeks of **Experiment 2** are demonstrated in Tables 4a and 4b, respectively.

In both studies no CLA was detected in the egg yolk lipids of hens fed with the 0.0% CLA diets, whereas the diets with increasing amount of CLA (0.5, 0.75 and 1.0%) resulted in a substantial deposition of CLA isomers. After eight weeks of both experiments the amount of CLA in egg yolk lipids was higher, when compared to that obtained after four weeks. After eight weeks of **Experiment 1**, rapeseed oil significantly enhanced the total CLA incorporation in egg yolks. Similarly, after eight weeks of **Experiment 2** vitamin E significantly enhanced the total CLA incorporation in egg yolks. The greatest amount of CLA (4.08%) in egg yolk lipids was observed in the group fed with 1% CLA and 300 mg of vitamin E after eight weeks of **Experiment 2.**

Table 3a. Effect of addition levels of dietary conjugated linoleic acid (CLA) and different vegetable oils on the fatty acid composition of egg yolk lipid at week 4 of the experiment

Fatty acids (%)	Dietary level of CLA (%)							
	0.00		0.50		0.75		1.00	
	Oils**							
	olive oil	rapeseed oil	olive oil	rapeseed oil	olive oil	rapeseed oil	olive oil	rapeseed oil
C14:0	0.28±0.03 b	0.23±0.07 ab	0.20±0.01 a	0.44±0.04 cd	0.45±0.05 cd	0.43±0.06 c	0.51±0.03 d	0.40±0.04 c
C15:0	0.08±0.02 b	0.09±0.03 b	0.04±0.04 a	0.10±0.01 b	0.11±0.02 b	0.04±0.01 a	0.04±0.01 a	0.04±0.01 a
C16:0	23.88±0.70 a	18.68±1.01 d	21.92±0.84 e	25.14±0.02 b	24.11±0.34 ab	27.01±0.15 f	30.06±0.66 c	29.19±0.57 c
C16:1	3.04±0.13 e	1.58±0.14 bc	1.07±0.02 d	1.63±0.06 c	1.46±0.06 b	0.92±0.03 a	0.82±0.06 a	0.86±0.06 a
C17:0	0.17±0.06 b	0.27±0.09 cd	0.22±0.02 bc	0.34±0.01 ad	0.52±0.07 e	0.43±0.03 ae	0.42±0.08 a	0.40±0.01 a
C18:0	4.68±0.49 b	6.11±0.32 c	9.51±0.18 d	12.09±0.22 a	13.50±0.27 f	12.10±0.19 a	11.27±0.14 e	12.48±0.38 a
C18:1	46.91±0.23 b	46.67±1.34 b	44.61±0.57 e	38.28±0.95 d	34.26±0.06 a	34.70±0.10 a	33.39±0.93 a	30.16±0.47 c
C18:2*n-6*	20.29±0.37 ab	24.37±1.27 e	21.43±0.72 bc	20.08±0.58 a	22.49±0.38 cd	21.79±0.56 cd	20.49±0.31 ab	22.85±0.24 d
C18:2*c9t11*	0.00	0.00	0.14±0.13 a	0.62±0.04 b	1.41±0.03 d	0.97±0.04 c	1.52±0.03 e	1.52±0.03 e
C18:2*t10c12*	0.00	0.00	0.04±0.05 a	0.10±0.01 b	0.33±0.06 d	0.22±0.02 c	0.44±0.05 e	0.53±0.05 f
C18:3*n-3*	0.35±0.05 b	0.82±0.11 a	0.42±0.07 b	0.53±0.02 c	0.84±0.07 a	0.90±0.04 a	0.59±0.05 c	1.12±0.07 d
C20:0	0.08±0.02 b	0.27±0.06 a	0.17±0.02 ab	0.28±0.17 a	0.22±0.03 a	0.16±0.03 ab	0.17±0.02 ab	0.17±0.02 ab
C20:2*n-6*	0.09±0.01 bc	0.29±0.05 e	0.06±0.03 b	0.13±0.03 a	0.12±0.03 ac	0.18±0.01 d	0.14±0.01 a	0.14±0.01 a
C20:3*n-6*	0.00	0.13±0.03 b	0.00	0.00	0.03±0.01 a	0.00	0.00	0.00
C20:4*n-6*	0.16±0.04 ab	0.49±0.07 c	0.18±0.02 ab	0.22±0.05 b	0.14±0.02 a	0.15±0.02 ab	0.16±0.01 ab	0.15±0.01 ab
Total CLA	0.00	0.00	0.17±0.17 a	0.72±0.04 b	1.74±0.07 d	1.19±0.06 c	1.96±0.07 e	2.05±0.08 e
Total SFA*	29.17±0.57 b	25.65±0.82 a	32.05±0.97 c	38.41±0.40 e	38.92±0.30 e	40.18±0.34 d	42.47±0.69 f	42.68±0.47 f
Total MUFA*	49.95±0.17 g	48.25±1.37 f	45.68±0.56 e	39.90±0.91 d	35.72±0.07 a	35.62±0.13 a	34.21±0.88 c	31.02±0.53 b
Total PUFA*	20.89±0.43 b	26.10±1.37 a	22.27±0.63 cd	21.69±0.52 bc	25.37±0.32 a	24.21±0.45 e	23.32±0.23 de	26.31±0.28 a

* SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; **olive oil or rapeseed oil were provided in the amount of 1.46%; a–g – means with different letters within the same row are significantly different at $P<0.05$; results are presented as mean±standard deviation (SD).

Table 3b. Effect of addition levels of dietary conjugated linoleic acid (CLA) and different vegetable oils on the fatty acid composition of egg yolk lipid at week 8 of the experiment

Fatty acids (%)	Dietary level of CLA (%)							
	0.00		0.50		0.75		1.00	
	Oils**							
	olive oil	rapeseed oil	olive oil	rapeseed oil	olive oil	rapeseed oil	olive oil	rapeseed oil
C14:0	0.32±0.04 ab	0.25±0.04 a	0.37±0.06 b	0.34±0.10 ab	0.69±0.03 d	0.55±0.06 c	1.20±0.03 e	0.60±0.01 cd
C15:0	0.06±0.01 a	0.07±0.02 a	0.07±0.02 a	0.11±0.03 b	0.07±0.02 a	0.14±0.02 b	0.08±0.02 a	0.07±0.01 a
C16:0	24.37±0.19 c	18.39±0.03 b	30.69±0.29 f	23.73±0.57 a	28.26±0.20 d	23.47±0.50 a	31.28±0.19 g	30.11±0.22 e
C16:1	3.03±0.13 d	1.49±0.03 a	1.38±0.19 ac	0.95±0.15 b	1.55±0.22 a	1.21±0.04 c	1.59±0.05 a	0.83±0.13 b
C17:0	0.16±0.02 a	0.21±0.02 a	0.18±0.03 a	0.36±0.05 b	0.31±0.03 bc	0.46±0.13 d	0.25±0.04 ac	0.36±0.03 b
C18:0	4.61±0.05 a	6.17±0.09 b	15.43±0.28 c	11.98±0.48 e	16.34±0.17 f	12.54±0.46 e	20.53±0.07d	16.45±0.62 f
C18:1	47.04±0.33 g	46.37±0.18 g	36.25 ±1.12 e	40.39±0.60 f	30.95±0.23 c	32.30±0.20 d	24.54±0.30a	26.35±0.82 b
C18:2*n-6*	19.83±0.12 a	25.14±0.22 e	14.24±0.42 b	20.15±1.27 a	18.60±0.55 d	26.12±0.20 f	16.38±0.23c	19.91±0.37 a
C18:2*c9t11*	0.00	0.00	0.50±0.04 a	0.65±0.05 b	1.41±0.04 c	1.70±0.06 d	2.07±0.05e	2.49±0.04 f
C18:2*t10c12*	0.00	0.00	0.11±0.01 a	0.12±0.03 a	0.45 ±0.06 c	0.30±0.03 b	1.02±0.03 e	0.92±0.03 d
C18:3*n-3*	0.29±0.04 a	0.81±0.06 c	0.32±0.03 a	0.59±0.08 b	0.79±0.07 c	0.32±0.03 a	0.59±0.07 b	1.32±0.06 d
C20:0	0.06±0.00 b	0.26±0.04 cde	0.15±0.03 a	0.19±0.05 ac	0.27±0.05 def	0.34±0.06 f	0.23±0.03 cd	0.31±0.03 ef
C20:2*n-6*	0.08±0.01 b	0.32±0.03 c	0.04±0.02 b	0.14±0.03 a	0.14±0.02 a	0.30±0.07 c	0.14±0.01 a	0.19±0.03 a
C20:3*n-6*	0.00	0.12±0.01 c	0.00	0.05±0.02 b	0.03±0.02 a	0.00	0.00	0.00
C20:4*n-6*	0.15±0.01 a	0.41±0.02 c	0.26±0.03 b	0.24±0.04 b	0.12±0.02 a	0.26±0.04 b	0.12±0.01 a	0.11±0.01 a
Total CLA	0.00	0.00	0.61±0.04 a	0.77±0.07 b	1.86±0.09 c	2.00±0.09 d	3.09±0.08 e	3.41±0.06 f
Total SFA*	29.58±0.17 b	25.35±0.10 a	46.89±0.59 f	36.71±0.85 c	45.95±0.04 e	37.50±0.25 d	53.56±0.16 h	47.89±0.41 g
Total MUFA*	50.07±0.27 h	47.86±0.19 g	37.63±0.93 e	41.35±0.45 f	32.50±0.41 c	33.51±0.16 d	26.13±0.31 a	27.17±0.70 b
Total PUFA*	20.35±0.10 a	26.79±0.28 e	15.48±0.37 c	21.94±1.24 b	21.55±0.37 b	28.99±0.22 f	20.31±0.15 a	24.94±0.30 d

*SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; **olive oil or rapeseed oil were provided in the amount of 1.46%; a–h – means with different letters within the same row are significantly different at $P<0.05$; results are presented as mean ± standard deviation (SD).

Table 4a. Effect of addition levels of dietary conjugated linoleic acid (CLA) and vitamin E on the fatty acid composition of egg yolk lipid at week 4 of the experiment

Fatty acids (%)	Dietary level of CLA (%)											
	0.00			0.50			0.75			1.00		
	Vitamin E level (mg/kg diet)											
	0	150	300	0	150	300	0	150	300	0	150	300
1	2	3	4	5	6	7	8	9	10	11	12	13
C14:0	0.23±0.07 c	0.21±0.03 c	0.27±0.03 ce	0.44±0.04 ad	0.33±0.02 be	0.39±0.03 ab	0.43±0.06 ad	0.42±0.03 a	0.59±0.07 f	0.40±0.04 ab	0.38±0.03 ab	0.50±0.02 d
C15:0	0.09±0.03 ac	0.09±0.02 ac	0.07±0.02 abe	0.10±0.01 c	0.07±0.02 ab	0.00	0.04±0.01 de	0.08±0.01 abc	0.06±0.02 bde	0.04±0.01 d	0.00	0.07±0.02 ab
C16:0	18.68±1.01 f	17.32±0.46 e	23.76±0.54 d	25.14±0.02 ab	22.48±0.13 c	26.05±0.09 g	27.01±0.15 h	22.88±0.65 c	24.37±0.27 ad	29.19±0.57 i	24.87±0.25 ab	25.26±0.38 b
C16:1	1.58±0.14 bc	1.21±0.04 a	2.18±0.11 g	1.63±0.06 bc	2.35±0.23 h	1.24±0.08 a	0.92±0.03 de	1.15±0.03 af	1.70±0.05 c	0.86±0.06 d	1.04±0.05 ef	1.50±0.09 b
C17:0	0.27±0.09 e	0.32±0.07 be	0.16±0.03 a	0.34±0.01 be	0.36±0.05 bc	0.32±0.03 be	0.43±0.03 cd	0.46±0.05 d	0.44±0.05 cd	0.40±0.01 bcd	0.37±0.04 bc	0.40±0.02 bcd
C18:0	6.11±0.32 bd	5.61±0.19 b	6.47±0.33 d	12.09±0.22 ac	5.67±0.17 b	12.26±0.25 a	12.10±0.19 ac	12.47±0.46 a	14.36±0.43 e	12.48±0.38 a	11.56±0.28 c	14.44±0.40 e
C18:1	46.67±1.34 h	50.39±0.23 d	50.30±0.35 d	38.28±0.95 c	45.50±0.03 g	38.61±0.30 c	34.70±0.10 b	35.20±0.15 b	33.34 ±0.91 a	30.16±0.47 e	37.05±0.64 f	33.22±0.32 a
C18:2*n-6*	24.37±1.27 f	22.77±0.29 de	16.00±0.36 a	20.08±0.58 c	21.82±0.46 b	19.45±0.15 c	21.79±0.56 b	24.16±0.04 f	21.53±0.29 b	22.85±0.24 e	21.93±0.37 bd	21.55±0.46 b
C18:2*c9t11*	0.00	0.00	0.00	0.62±0.04 d	0.10±0.01 a	0.60±0.05 d	0.97±0.04 b	1.41±0.03 e	1.49±0.06 f	1.52±0.03 f	1.11±0.04 c	1.40±0.06 e
C18:2*t10c12*	0.00	0.00	0.00	0.10±0.01 b	0.05±0.01 a	0.08±0.02 ab	0.22±0.02 c	0.23±0.02 c	0.38 ±0.03 d	0.53±0.05 f	0.30±0.04 e	0.37±0.04 d
C18:3*n-3*	0.82±0.11 b	0.77±0.07 be	0.31±0.16 a	0.53±0.02 c	0.60±0.05 cd	0.56±0.05 cd	0.90±0.04 bf	1.00±0.05 fg	1.15 ±0.02 h	1.12±0.07 gh	0.67±0.05 de	0.86±0.04 b
C20:0	0.27±0.06 bcd	0.36±0.04 d	0.19±0.02 abc	0.28±0.17 cd	0.25±0.03 abcd	0.15±0.03 a	0.16±0.03 ab	0.25±0.05 abcd	0.28±0.04 cd	0.17±0.02 abc	0.31±0.05 d	0.16±0.02 ab

C20:2*n-6*	0.29±0.05 e	0.27±0.05 e	0.07±0.01 a	0.13±0.03 bc	0.17±0.02 bcd	0.17±0.02 bcd	0.18±0.01 cd	0.13±0.02 b	0.15±0.01 bc	0.14±0.01 bc	0.19±0.02 d	0.12±0.02 b
C20:3*n-6*	0.13±0.03 b	0.10±0.01 a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:4*n-6*	0.49±0.07 e	0.58±0.05 f	0.22±0.03 cd	0.22±0.05 cd	0.23±0.04 d	0.13±0.02 a	0.15±0.02 ab	0.17±0.02 abcd	0.16±0.01 abc	0.15±0.01 ab	0.21±0.01 bcd	0.15±0.03 ab
Total CLA	0.00	0.00	0.00	0.72±0.04 a	0.15±0.01 b	0.67±0.05 a	1.19±0.06 c	1.64±0.04 e	1.88±0.07 g	2.05±0.08 h	1.41±0.08 d	1.77±0.10 f
Total SFA*	25.65±0.82 b	23.90±0.21 a	30.92±0.22 d	38.40±0.40 i	29.17±0.26 c	39.17±0.36 i	40.18±0.34 h	36.56±0.27 e	40.09±0.71 h	42.68±0.47 g	37.49±0.36 f	40.83±0.78 h
Total MUFA*	48.25±1.37 f	51.61±0.21 g	52.47±0.29 g	39.90±0.91 e	47.85±0.20 f	39.84±0.24 e	35.62±0.13 cd	36.34±0.18 d	35.04±0.88 c	31.02±0.53 a	38.09±0.58 b	34.71±0.29 c
Total PUFA*	26.10±1.37 e	24.49±0.40 c	16.60±0.50 a	21.69±0.52 d	22.98±0.38 b	20.99±0.15 d	24.21±0.45 c	27.10±0.12 f	24.87±0.21 c	26.31±0.28 ef	24.41±0.41 c	24.46±0.50 c

*SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. a–i – means with different letters within the same row are significantly different at $P<0.05$; results are presented as mean ± standard deviation (SD).

Table 4b. Effect of addition levels of dietary conjugated linoleic acid (CLA) and vitamin E on the fatty acid composition of egg yolk lipid at week 8 of the experiment

Fatty acids (%)	Dietary level of CLA (%)											
	0.00			0.50			0.75			1.00		
	Vitamin E level (mg/kg diet)											
	0	150	300	0	150	300	0	150	300	0	150	300
1	2	3	4	5	6	7	8	9	10	11	12	13
C14:0	0.25±0.04 c	0.21±0.03 c	0.46±0.06 b	0.34±0.10 a	0.41±0.03 ab	0.41±0.06 ab	0.55±0.06 d	0.90±0.02 g	0.71±0.03 e	0.60±0.01 d	1.11±0.05 h	0.79±0.04 f
C15:0	0.07±0.02 a	0.09±0.02 ab	0.06±0.03 a	0.11±0.03 bc	0.09±0.01 ab	0.15±0.03 c	0.14±0.02 c	0.12±0.02 bc	0.09±0.01 ab	0.07±0.01 a	0.11±0.03 bc	0.09±0.01 ab
C16:0	18.39±0.03 b	17.32±0.46 a	20.72±0.68 c	23.73±0.57 e	26.31±0.76 f	22.07±0.50 d	23.47±0.50 e	27.79±0.11 g	27.15±0.10 g	30.11±0.22 h	26.26±0.19 f	30.19±0.19 h
C16:1	1.49±0.03 c	1.21±0.04 a	3.55±0.25 e	0.95±0.15 bd	1.46±0.09 c	1.06±0.12 ad	1.21±0.04 a	1.58±0.07 c	1.25±0.05 a	0.83±0.13 b	1.15±0.06 a	0.84±0.06 b
C17:0	0.21±0.02 c	0.32±0.07 a	0.17±0.01 c	0.36±0.05 ab	0.31±0.03 a	0.55±0.09 d	0.46±0.13 b	0.38±0.04 ab	0.39±0.03 ab	0.36±0.03 ab	0.41±0.04 ab	0.32±0.02 a
C18:0	6.17±0.09 c	5.61±0.19 c	8.57±0.35 f	11.98±0.48 a	13.17±0.50 b	12.52±0.49 ab	12.54±0.46 ab	16.08±0.08 d	18.70±0.03 e	16.45±0.62 d	20.42±0.03 g	18.94±0.58 e
C18:1	46.37±0.18 i	50.40±0.22 k	47.83±0.29 j	40.39±0.60 h	35.78±0.17 f	36.69±0.56 g	32.30±0.20 e	28.95±0.10 a	28.92±0.11 a	26.35±0.82 d	25.56±0.11 c	23.70±0.22 b
C18:2*n-6*	25.14±0.22 f	22.77±0.29 d	17.10±0.06 e	20.15±1.27 b	20.96±0.26 c	23.47±0.40 d	26.12±0.20 g	20.34±0.12 bc	19.21±0.21 a	19.91±0.37 ab	19.26±0.09 a	19.26±0.13 a
C18:2*c9t11*	0.00	0.00	0.00	0.65±0.05 a	0.63±0.02 a	0.98±0.04 b	1.70±0.06 d	1.61±0.04 c	1.88±0.04 e	2.49±0.04 f	2.76±0.09 g	2.92±0.03 h
C18:2*t10c12*	0.00	0.00	0.00	0.12±0.03 a	0.13±0.02 a	0.19±0.03 c	0.30±0.03 d	0.49±0.03 e	0.54±0.04 f	0.92±0.03 g	1.14±0.01 b	1.17±0.02 b
C18:3*n-3*	0.81±0.05 f	0.77±0.07 cf	0.50±0.07 ab	0.59±0.08 b	0.44±0.09 a	0.85±0.05 f	0.32±0.03 d	1.07±0.05 e	0.69±0.03 c	1.32±0.06 g	1.30±0.03 g	1.26±0.07 g
C20:0	0.26±0.04 abc	0.36±0.04 a	0.36±0.20 a	0.19±0.05 bcd	0.10±0.02 d	0.21±0.02 bcd	0.34±0.06 a	0.28±0.04 abc	0.17±0.02 bd	0.31±0.03 ac	0.29±0.04 abc	0.27±0.03 abc

C20:2*n-6*	0.32±0.03 e	0.27±0.05 ef	0.14±0.02 abc	0.14±0.03 abc	0.09±0.01 b	0.22±0.03 df	0.30±0.07 e	0.16±0.02 acd	0.12±0.01 ab	0.19±0.03 cd	0.16±0.02 acd	0.12±0.01 ab
C20:3*n-6*	0.12±0.01 d	0.10±0.01 c	0.08±0.01 b	0.05±0.02 a	0.00	0.05±0.03 a	0.00	0.06±0.01 a	0.00	0.00	0.00	0.00
C20:4*n-6*	0.41±0.02 d	0.58±0.05 e	0.44±0.05 d	0.24±0.04 c	0.10±0.01 ab	0.58±0.08 e	0.26±0.04 c	0.16±0.02 a	0.16±0.01 a	0.11±0.01 ab	0.07±0.01 b	0.13±0.01 ab
Total CLA	0.00	0.00	0.00	0.77±0.07 a	0.76±0.04 a	1.17±0.06 b	2.00±0.09 c	2.11±0.06 d	2.43±0.08 e	3.41±0.06 f	3.91±0.10 g	4.08±0.05 h
Total SFA*	25.35±0.10 b	23.90±0.21 a	30.35±0.26 c	36.71±0.85 e	40.40±0.29 g	35.91±0.18 d	37.50±0.25 f	45.56±0.27 h	47.21±0.13 i	47.89±0.41 j	48.59±0.11 k	50.60±0.41 l
Total MUFA*	47.86±0.19 d	51.61±0.21 h	51.38±0.15 h	41.35±0.45 c	37.24±0.15 g	37.75±0.52 g	33.51±0.16 b	30.53±0.06 f	30.18±0.14 f	27.17±0.70 e	26.71±0.07 e	24.55±0.26 a
Total PUFA*	26.79±0.28 d	24.49±0.40 ac	18.26±0.11 e	21.94±1.24 b	22.36±0.14 b	26.34±0.51 d	28.99±0.22 f	23.90±0.22 c	22.62±0.20 b	24.94±0.30 a	24.70±0.09 a	24.85±0.23 a

*SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. a–l – means with different letters within the same row are significantly different at $P<0.05$; results are presented as mean ± standard deviation (SD).

Considering the vegetable oils used in **Experiment 1**, it was observed that after four weeks of the study the CLA content was not affected, whereas after eight weeks of the study olive oil caused the inhibited incorporation of CLA in egg yolks, especially in the group fed with 1% of CLA as compared to rapeseed oil (3.09% vs 3.41%). In both studies the amount of individual isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12 linearly increased in egg yolk lipids with the growing amount of CLA (0.0, 0.5, 0.75 and 1.0%) in diets. Moreover, *cis*-9, *trans*-11 was incorporated into egg yolk lipids more efficiently than the *trans*-10*, cis*-12 CLA.

Dietary CLA in **Experiment 1** significantly increased the concentration of individual SFA (C16:0 and C18:0), whereas those of individual MUFA (C16:1 and C18:1) were significantly decreased after four weeks of the experiment. Similarly, after eight weeks of the experiment the content of SFA was considerably increased. Taking into account the vegetable oils used in the study, olive oil caused a lower increase (about 45%) of SFA as compared to rapeseed oil (66%), especially in the group fed with 1% of CLA after four weeks of the experiment. Moreover, after eight weeks of the experiment the increase of SFA was enhanced (81% in the olive oil and 89% in the rapeseed oil group). Dietary CLA decreased MUFA levels after four and eight weeks of the experiment. However, MUFA level was significantly elevated in olive oil groups without CLA after four and eight weeks of studies. PUFA levels were unaffected.

Taking into consideration levels of individual fatty acids obtained in **Experiment 1**, the greatest changes were in stearic acid (C18:0) in groups fed with olive oil. The 3-fold significant ($P<0.05$) increase of C18:0 after four weeks of the experiment and the 5-fold significant ($P<0.05$) increase after eight weeks of the experiment were observed in the group fed with 1% CLA. Oleic acid (C18:1, *n-9*) was significantly ($P<0.05$) decreased with an increased content of CLA in the diets after four and eight weeks (about 50%) of the experiment. Among PUFA, the highest amount was observed for linoleic acid (C18:2, *n-6*), but its level was unchanged. Only the amount of linolenic acid (C18:3, *n-3*) in the group fed with olive oil was significantly ($P<0.05$) increased to 0.75% CLA in the diet and afterwards decreased. After four weeks of the study in groups fed with rapeseed the significantly ($P<0.05$) increased level of C18:3 was observed, when compared to olive oil.

In **Experiment 2** (Tables 4a and 4b), the greatest concentration of SFA (50.6%) was obtained after eight weeks of the study in the eggs from the hens fed with 1% CLA and 300 mg/kg vitamin E. Regardless of the CLA content, vitamin E in a dose of 150 mg/kg significantly reduced SFA and increased MUFA after four weeks of the study. Dietary CLA decreased MUFA levels and the lowest concentration of MUFA (24.55%) was in the group fed with 1% CLA and 300 mg/kg vitamin E after eight weeks of study, whereas in the group without CLA vitamin E in a dose of 300 mg/kg significantly decreased PUFA after four as well as eight weeks of the study.

To describe **Experiment 2** by levels of individual fatty acids affected by vitamin E, the significant differences were obtained for the majority of FA. Similarly, as in **Experiment 1**, the C18:0 level was significantly increased and the C18:1 level was decreased. Moreover, both doses of vitamin E significantly decreased C18:1 in CLA-fed groups.

Discussion

The finding that the amounts of CLA isomers incorporated into egg yolk lipids were proportional to the levels of CLA in the diet confirmed the results indicated by the previous data (Shang et al., 2004, 2005; Suksombat et al., 2006). The increase of CLA was related to vegetable oils and different levels of vitamin E. Jones et al. (2000) also showed that the content of CLA isomers increased with time elongation (36 days) of the experiment. Raes et al. (2002) used different fat sources and levels in the diet of laying hens. Flaxseed oil increased CLA content in the egg yolk lipid to the higher level than soybean oil and animal fat, but to a lesser extent than in the diet with a low fat content. In contrast, olive oil inhibited the incorporation of CLA in egg yolk lipids (Aydin et al., 2001), which was in line with the results of the present study. In addition, we noted that the amount of *cis*-9, *trans*-11 was approximately 3-4-fold higher than the *trans*-10, *cis*-12. The preferential incorporation of *cis*-9, *trans*-11 isomer into egg yolk lipids was observed in several previous experiments (Jones et al., 2000; Szymczyk and Pisulewski, 2005; Shang et al., 2005; Suksombat et al., 2006).

In line with our previous experiments (Szymczyk and Pisulewski, 2002, 2005; Franczyk-Żarów et al., 2008) also in the present study we indicated that eggs enriched with CLA isomers had adverse changes in the composition of SFA and MUFA. These findings confirmed our previous observations that increased dietary CLA concentrations altered relative proportions of SFA and MUFA in egg yolk lipids, namely the proportions of SFA (e.g. 16:0 and 18:0) were increased, whereas those of MUFA (e.g. 18:1, *n-9*) were decreased. Considering the effect of vegetable oils on a yolk lipid profile, we found out that only rapeseed oil increased PUFA and decreased MUFA after four and eight weeks of the study.

The inclusion level of vitamin E (0, 150 and 300 mg/kg diet) affected the fatty acid profile of CLA-enriched egg yolks. Qi and Sim (1998) showed that the diet enriched with *n*-3 fatty acids supplemented with a natural tocopherol (0, 200, 400 and 800 mg/kg diet) caused an increased stability of egg yolk lipids, whereas it had no effect on the composition of fatty acids in egg yolks. On the contrary, Szymczyk and Pisulewski (2005) observed that the addition of vitamin E to the layer diet reduced the total amount of MUFA, whereas it raised the content of PUFA in egg yolk lipids. Nevertheless, Lauridsen et al. (1999) showed that the increase in the concentration of *n*-6 fatty acids, such as 18:2, 20:4, or 22:4, may be associated with protective antioxidant activity of vitamin E on linoleic acid derived from sunflower oil. Therefore, it was shown that the development of CLA-enriched eggs resulted not only in the incorporation of CLA isomers into egg yolk, but also in increased SFA and decreased MUFA proportions in egg yolk lipids. Indeed, many authors confirmed that the concentration of SFA increased at the expense of MUFA, which tended to decrease in yolk lipids of hens fed with CLA-supplemented diets (Schäfer et al., 2001; Cherian et al., 2002; Yang et al., 2002; Suksombat et al., 2006; Franczyk-Żarów et al., 2008).

There are several mechanisms which explain how dietary CLA affects the fatty acid content of egg yolk (Shang et al., 2005). Park et al. (2000) reported that hepatic stearoyl-coenzyme A desaturase-1 (SCD-1) activity in mice was significantly

inhibited by *trans*-10*, cis*-12 CLA and its derivatives. Similarly, Shang et al. (2005) showed that with increased concentration of dietary CLA the hepatic SCD-1 activity was significantly decreased, as SCD-1 catalyzes the insertion of a double bond between carbons 9 and 10 in both C16:0 and C18:0 fatty acid (Cook, 1991). It was shown that dietary CLA inhibited SCD-1 activity and the expression of the hepatic SCD-1 gene. Thus, decreased amount of SFA converted into MUFA, and changes of the relative concentrations of SFA and MUFA in the tissues of laying hens were observed (Shang et al., 2005). If this enzyme was inhibited by CLA, MUFA would be decreased and SFA would be increased (Suksombat et al., 2006). Moreover, vitamin E was reported to stimulate Δ9-desaturase activity in rat liver, the enzyme that catalyzes desaturation of C16:0 and C18:0 to C16:1 and C18:1 (Okayasu et al., 1977). However, in the present study this effect was not observed.

Unless adverse effects of feeding CLA to laying hens on the fatty acid profile of egg yolks are eliminated, the CLA-enriched eggs obtained by the nutritional treatments described in this study cannot be considered functional food products in human nutrition (Hasler, 2002). To the best of our knowledge the only effective way to obtain CLA-enriched eggs without negative changes in the fatty acid composition is to feed hens with a diet supplemented with pomegranate seed oil as a source of conjugated trienoic fatty acids (CLnA) (Kostogrys et al., 2017).

Conclusion

In conclusion, the co-supplementation of CLA, vitamin E or vegetable oils had no positive effects on fatty acid composition of CLA-enriched eggs.

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