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Effects of dietary conjugated linoleic acid isomers and vitamin E on fatty acid composition and cholesterol content of hen egg yolks*

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ABSTRACT

The objective of this study was to develop functional eggs, by feeding hens with conjugated linoleic acid (CLA) isomers (0.0 vs 1.5%) and α-tocopherol (0 vs 120 mg/kg of diet) as an antioxidant. Thirty-six 25-week-old laying hens were randomly distributed into four groups of 9 hens and maintained in individual laying cages throughout 12 weeks of experiment. They were assigned to the four commercial layer diets (2770 kcal ME/kg; 16.7% CP): 0.0% CLA, 0.0% CLA+120 mg vit. E/kg of diet, 1.5% CLA and 1.5% CLA + 120 mg vit. E/kg of diet. The laying performance, fatty acid composition (relative %) and cholesterol content of egg yolk lipids were determined. No apparent effects of dietary CLA on laying performance were observed. The rate of laying and egg production per hen were increased ($P<0.01$) by vitamin E. When compared to the 0.0% CLA diets, vitamin E added to the 1.5% CLA diets was more effective in increasing the rate of laying ($P<0.01$). Feeding the 1.5% CLA diets resulted in substantial deposition of CLA isomers (6.7%) in egg yolk lipids. Compared to the 0.0% CLA diets, the 1.5% CLA diets increased SFA (31.7 vs 51.9%; $P<0.01$), decreased MUFA (45.4 vs 27.3%; $P<0.01$) and less apparently PUFA content (21.7 vs 19.6%; $P<0.01$). Vitamin E had no effect on SFA, it decreased MUFA and slightly increased PUFA ($P<0.01$). When fed to hens in the 0.0% CLA diets, it decreased MUFA (wk 4, 8 and 12; $P<0.01$) and increased PUFA (wk 4; $P<0.01$) whereas in the 1.5% CLA diets, it decreased SFA (wk 4; $P<0.05$) and increased MUFA (wk 4 and 8; $P<0.05$). Egg cholesterol (mg per egg), was reduced ($P<0.01$) by both dietary supplements and the effect of vitamin E was more pronounced in the 0.0% CLA diets ($P<0.01$). In conclusion, vitamin E may exert alleviating effects on fatty acid composition of CLA-enriched eggs. However, the extent of these effects is negligible. Therefore, the composition of CLA-enriched eggs must be improved to consider them functional food products.

KEY WORDS: CLA, vitamin E, egg yolk lipids, fatty acid profile, cholesterol

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INTRODUCTION

A new class of functional (i.e. health-promoting) components of animal origin comprises conjugated linoleic acid (CLA) isomers. CLA are the positional and geometric (*cis, trans*) conjugated dienoic isomers of linoleic acid (18:2*n*-6), present mainly in ruminant milk and meat (Lawson et al., 2001). The double bonds in CLA are usually either in C positions 9 and 11 or 10 and 12. Moreover, each of the double bonds can be in the *cis* or *trans* configuration. In a number of reliable studies, pure CLA were shown to decrease the risk of cancer and atherosclerosis, to enhance immune responses and reduce body fat accumulation in experimental animals (Azain, 2003). In humans, evident cardioprotective effects of CLA were demonstrated by Noone et al. (2002). Interestingly, CLA-enriched milk fat has been demonstrated to reduce the number of chemically-induced mammary tumors in female rats (Ip et al., 1999) and to inhibit the growth of human breast and colon cancer cell lines (Miller et al., 2003). Consequently, animal nutrition strategies have been developed to enrich foods of animal origin, including eggs (Jones et al., 2000; Schäffer et al., 2001; Cherian et al., 2002; Szymczyk and Pisulewski, 2003) with CLA isomers. The mechanisms of the above health-related effects of CLA isomers, such as anticarcinogenic and antiatheroslerotic action, have been attributed to their alleged antioxidant properties (Ha et al., 1990).

The above studies on 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. Also, in more recent studies, CLA isomers, as the free fatty acid and methyl ester forms, were reported to have extremely low oxidative stability. They were easily degraded in the presence of oxygen and acted finally as prooxidants (Yang et al., 2000). Indeed, when exposed to oxygen, CLA isomers were oxidatively degraded to yield furanoid fatty acids (Yurawecz et al., 1995). Therefore, efforts should be made to ensure lipid stability in animal feeds and food products enriched with CLA isomers.

In light of the evident functional properties of CLA isomers and their prooxidant potential, the objective of this study was to evaluate the effects of supplementing the diets of laying hens with CLA isomers and vitamin E, on their laying performance and the lipid composition of eggs. The use of vitamin E (α-tocopherol) as an effective natural antioxidant in laying hen diets (Qi and Sim, 1998) seems to be an obvious choice. Moreover, vitamin E was reported to stimulate Δ9-desaturase activity in rat liver (Okayasu et al., 1997), an enzyme that catalyses desaturation of C16:0 and C18:0 to C16:1 and C18:1 (Lee et al., 1998). Also, the *in vivo* antioxidant activity of vitamin E on *n*-6 fatty acids in fat from pigs (Lauridsen et al., 1999) and *n*-3 fatty acids in egg yolk lipids (Cherian et al., 1996) was demonstrated.

MATERIAL AND METHODS

Animals and diets

CLA-enriched eggs were obtained by feeding hens with CLA (Natural Lipids Ltd., Norway) isomers (0.0 vs 1.5%) and vitamin E (0 vs 120 mg/kg of diet). Thirty-six 25-week-old laying hens were randomly distributed into four groups of 9 hens each and maintained in individual laying cages, throughout 12 weeks of experiment. They were assigned to the four commercial layer diets (2770 kcal ME/kg, 16.7% CP) containing the following combinations of treatments: I - 0.0% CLA, II - 0.0% CLA+120 mg vitamin E/kg of diet, III-1.5% CLA

TABLE 1

The ingredient composition and nutrient content of CLA-enriched experimental diets (0.0 and 1.5 %) fed to laying hens

Indices	Percentage
Ingredient	
ground maize	8.0
ground wheat	25.0
ground barley	28.0
soyabean meal	14.6
rapeseed meal	4.0
meat-and-bone meal	4.0
lucerne meal	2.0
sunflower oil[1]	5.0
limestone	8.3
dicalcium phosphate	0.3
NaCl	0.3
mineral and vitamin premix[2]	0.5
Calculated nutrient content	
metabolizable energy, kcal/kg	2770
crude protein	16.70
ether extract	7.20
total sulphur amino acids	0.62
methionine	0.35
cystine	0.27
lysine	0.75
Ca	3.53
P (available)	0.34
crude fibre	4.21

[1] the CLA source (60% CLA) was substituted for sunflower oil at 0.00 or 2.50% of the diet to obtain 0.0 or 1.5% CLA-enriched diets, respectively

[2] mineral and vitamin premix (*Lutamix DJ*) provided per kg of diet: IU: vit. A, 12000; vit. D_3, 2000; mg: vit. E, 15; vit. K_3, 2; vit. B_1, 1; vit. B_2, 4; vit. B_6, 1.5; biotin, 1; vit. B_{12}, 0.01; D-calcium panthotenate, 8; nicotinamide, 25; choline chloride, 250; Mn, 100; I, 0.8; Zn, 5; Co, 0.2; Se, 0.2; DL-methionine, 500

and IV-1.5%CLA + 120 mg vitamin E/kg of diet. The CLA source, obtained from Natural Lipids Ltd. (Norway), contained 60% CLA. Appropriate amounts of sunflower oil were included in the diets to equalize the total fat added to all diets at 5% (Table 1). Fatty acid composition of the sunflower oil and CLA supplement used in this study is given in Table 2. Feed and water were available *ad libitum*. All procedures involving animals were approved by the Animal Ethics Committee at the National Institute of Animal Production in Poland.

TABLE 2

The fatty acid composition (relative %) of sunflower oil and conjugated linoleic acid (CLA) source fed to laying hens

Fatty acid	Sunflower oil	CLA
12:0	-	0.1
14:0	0.1	0.1
16:0	6.2	4.9
16:1	-	0.1
18:0	3.6	2.0
18:1	24.4	29.9
18:1	-	0.7
18:2	62.9	0.5
CLA isomers (total)[1]	-	58.7
cis-9, *trans*-11 CLA	-	9.5
trans-8, *cis*-10 CLA	-	8.6
cis-11, *trans*-13 CLA	-	9.8
trans-10, cis-12 CLA	-	11.2
other CLA	-	19.6
18:3	1.0	-
20:0	0.3	0.9
22:0	0.6	0.3
24:0	0.2	0.1
Total fatty acids	99.3	98.3

[1] CLA isomers were identified by double bond positions from the carboxyl end and bond configurations (*cis*; *trans*)

Sampling procedures

Individual feed intake was determined weekly. Eggs were collected daily, counted and weighed individually to obtain egg production and egg mass for the entire study. The rate of laying (egg production per 100 hens, %) and feed conversion efficiency per one egg (feed intake per number of eggs, g) and per

1 kg of eggs (feed intake per egg mass, kg) were calculated. In addition, eggs from each hen, collected every four weeks were broken, yolks were separated from albumen, weighed, and then frozen at -20°C for further analyses.

Chemical analyses

On the day of analyses, the frozen yolks were thawed, mixed and then analysed for fatty acid composition and cholesterol content. Total egg yolk lipids were extracted according to the method of Folch et al. (1957). They were saponified (10 min, 75°C) in 0.5M KOH/Me-OH and then methylated (10 min, 75°C) in 14% BF_3/Me-OH (Morrison and Smith, 1964). Finally, fatty acid methyl esters were extracted with hexane and analysed on a Hewlett-Packard (model 5890) gas chromatograph, equipped with a BPX 70 fused silica capillary column (length 50 m × 0.22 mm i.d. × 0.25 μm film thickness; SGE International, Australia), and a flame ionization detector. Helium was the carrier gas used at a split ratio of 50:1. The operating conditions were as follows: the temperature of injector was 210°C, and that of detector was 240°C. The initial oven temperature was 160°C for 35 min, increasing progressively by 3°C/min to 210°C, and held constant at 210°C for 10 min. The fatty acid percentage was integrated and calculated using the HP ChemStation Computer Programme. Fatty acid methyl esters were identified by comparison of their retention times with authentic standards purchased from Sigma-Aldrich (Poland) and the CLA reference standards (9*cis,* 11*trans*, and 10*trans,*12*cis* isomers) were obtained from Larodan Fine Chemicals AB (Malmö, Sweden). The above analyses were performed at the Meat and Fat Research Institute (Warszawa, Poland). The total egg yolk cholesterol was extracted according to the method of Folch et al. (1957) and determined enzymatically (Allain et al., 1974) using commercial kits (Sigma-Aldrich, Poland).

Statistical analyses

The data were analysed as a factorial arrangement of two dietary CLA levels (0.0 vs 1.5%) and two levels of vitamin E (0 vs 200 mg/kg) with an interaction, using two-way ANOVA generated by the STATISTICA v. 5.1 package. Data were presented as the means of each combination of treatments and the pooled standard errors (SE) together with the significance levels ($P<0.05$ and $P<0.01$) of the main effects and interactions.

RESULTS

Generally, no effects of dietary CLA on laying performance were observed among hens fed the diets corresponding to four combinations of experimental treatments: 0.0% CLA, 0.0% CLA+120 mg vitamin E/kg of diet, 1.5% CLA and 1.5% CLA + 120 mg vitamin E/kg of diet (Table 3). The only exception was higher feed ($P<0.01$) conversion expressed in kg of feed per one kg of eggs. On the other hand,

TABLE 3

Effect of dietary conjugated linoleic acid (CLA: 0.0% vs 1.5%) and vitamin. E (E: 0 mg vs 120 mg/kg feed) on laying performance of hens during 12 weeks of experiment

	CLA				SE	Significance of effects, P[1]		
	0.0%		1.5%					
	E, mg					CLA	E	CLA × E
	0	120	0	120				
Rate of laying, %	95.2	96.5	93.8	97.0	0.46	NS	**	**
Number of eggs per layer	116.0	119.0	115.0	119.0	0.49	NS	**	NS
Egg mass, g	63.7	61.0	61.2	61.4	0.01	NS	NS	*
Feed intake, g per hen per day	123.7	123.0	126.0	123.3	0.59	NS	*	*
Feed conversion								
per one egg, g	131.4	128.0	130.5	128.0	0.66	NS	NS	NS
per one kg of eggs, kg	2.07	2.1	2.12	2.06	0.31	**	NS	**

[1] significance of effects: ** $P<0.01$; * $P<0.05$; NS $P\geq0.05$

the rate of laying and egg production per hen were increased ($P<0.01$) and feed intake was slightly decreased ($P<0.05$) by vitamin E. Several interactions between dietary CLA and vitamin E were also observed. Namely, when compared to the 0.0% CLA diets, vitamin E added to the 1.5% CLA diets was more effective in increasing the rate of laying ($P<0.01$) and decreasing the feed intake of hens ($P<0.05$). At the same time, the opposite effects of vitamin E added to the 0.0 and the 1.5% CLA diets were observed for egg mass ($P<0.05$) and feed conversion expressed in terms of kg feed per one kg of eggs ($P<0.01$).

The fatty acid composition of egg yolk lipids, expressed as a percentage of total methyl esters of fatty acids, was significantly altered by dietary CLA (Table 4). No CLA was detected in the egg yolk lipids of hens fed the 0.0% CLA diets, whereas feeding the 1.5% CLA diets resulted in substantial deposition of CLA isomers (6.4 and 6.9% of total FA, respectively). Moreover, *cis*-9, *trans*-11 and *cis*-11, *trans*-13 isomers were incorporated into egg yolk lipids more efficiently than the remaining CLA. The concentrations of individual SFA (C14:0, C16:0 and C18:0) were significantly ($P<0.01$) increased, whereas those of individual MUFA (C16:1 and C18:1, except C20:1) and PUFA (in all cases), were significantly decreased ($P<0.01$). The effects of

vitamin E were less consistent. Among MUFA, C18:1 was decreased and C20:1 was increased, and among PUFA C18:2 and C22:4 were increased (P<0.01 and P<0.05, respectively) whereas concentration of C18:3 was decreased (P<0.01). No consistent interactions (CLA × E; Table 4) on concentrations of individual fatty acids were observed. Overall, compared to the 0.0% CLA diets, the 1.5% CLA diets increased total SFA (30.8 and 32.5% vs 53.3 and 50.4%; P<0.01) and decreased total MUFA (48.6 and 42.1 vs 25.9 and 28.7%; P<0.01). Total PUFA content (19.4 and 23.9% vs 19.5 and 19.7%; P<0.01) was less affected and that of total non-CLA PUFA was significantly decreased (P<0.01). Vitamin E had no effect on SFA, it decreased MUFA and slightly increased PUFA and non-CLA PUFA content in egg yolk lipids (P<0.01). As indicated by significant interactions (P<0.01), effects of vitamin E added to either the 0.0 or the 1.5% CLA diets

TABLE 4

Effect of dietary conjugated linoleic acid (CLA: 0.0 vs 1.5%) and vitamin. E (E: 0 vs 120 mg/kg feed) on fatty acid composition (relative %) of egg yolk lipids at week 4 of the experiment

Fatty acids	CLA				SE	Significance of effects, P[1]		
	0.0%		1.5%			CLA	E	CLA × E
	E, mg							
	0	120	0	120				
14:0	0.3	0.3	0.7	0.7	0.04	**	NS	**
16:0	22.7	23.6	33.1	31.5	1.13	**	NS	**
16:1	3.1	2.7	1.1	1.1	0.01	**	NS	NS
18:0	7.8	8.6	19.5	18.8	1.31	**	NS	NS
18:1	45.2	39.2	24.4	26.8	2.33	**	**	**
18:2 *n*-6	14.9	20.4	11.1	10.3	0.72	**	**	**
18:3 *n*-3	1.3	0.7	0.4	0.7	0.07	**	**	**
20:1	0.3	0.2	0.4	0.8	0.02	**	**	**
20:3 *n*-6	0.1	0.2	0.1	0.2	0.00	**	**	**
20:4 *n*-6	1.6	1.9	1.1	1.0	0.10	**	NS	NS
22:4 *n*-6	0.1	0.2	0.1	0.1	0.11	**	*	**
22:5 *n*-3	0.1	0.1	0.1	0.2	0.00	**	NS	**
22:6 *n*-3	1.4	0.6	0.3	0.4	0.11	**	NS	NS
CLA isomers								
18:2 *cis*9 *trans*11	0.0	0.0	1.9	2.1	0.24	**	NS	NS
18:2 *trans*8 *cis*10	0.0	0.0	0.6	0.6	0.07	**	NS	NS
18:2 *cis*11 *trans*13	0.0	0.0	1.4	1.4	0.18	**	NS	NS
18:2 *trans*10 *cis*12	0.0	0.0	0.9	1.1	0.11	**	NS	NS
other CLA isomers	0.0	0.0	1.6	1.7	0.20	**	NS	NS
Total SFA[a]	30.8	32.5	53.3	50.4	2.60	**	NS	**
total MUFA[b]	48.6	42.1	25.9	28.7	2.64	**	**	**
total PUFA[c]	19.5	24.1	19.6	19.8	0.98	**	**	**
total *n*-6	16.7	22.7	18.8	18.5	0.65	**	**	**
total *n*-3	2.8	1.4	0.8	1.3	0.12	**	**	**

[a] SFA : saturated fatty acids; [b] MUFA: monounsaturated fatty acids; [c] PUFA: polyunsaturated fatty acids[1] significance of effects: ** P<0.01; * P<0.05; NS P≥0.05

were different. In more detail, vitamin E, when fed to hens in the 0% CLA diets (Figure 1), decreased MUFA (wk 4, 8 and 12; P<0.01), tended to increase SFA and increased PUFA (wk 4; P<0.01) content in egg yolk lipids. In contrast, vitamin E fed in the 1.5% CLA diets (Figure 2), tended to decrease SFA (wk 4; P<0.05) and to increase MUFA (wk 4 and 8; P<0.05), without affecting PUFA content in these lipids.

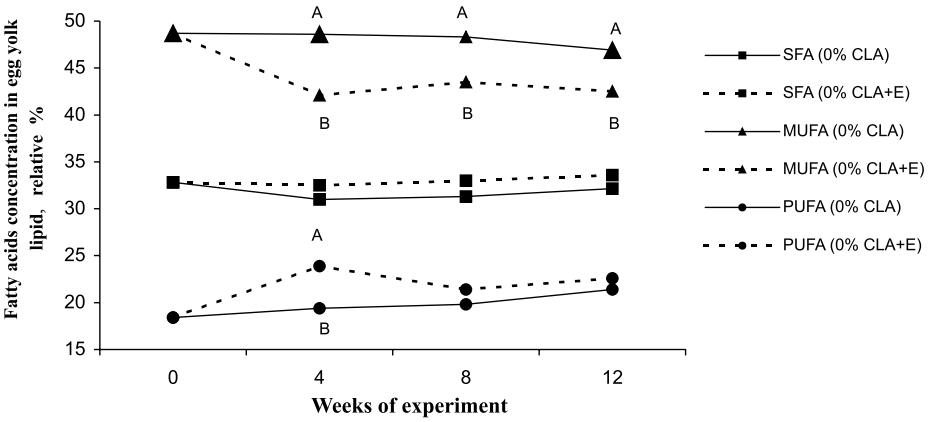

Figure 1. Effect of vitamin E (E: 0 mg vs 120 mg/kg feed) on relative proportions of SFA, MUFA and PUFA in egg yolk lipids from hens fed 0.0% CLA diets during 12 weeks of experiment. Means for a week bearing different letters (A,B) differ significantly (P<0.01)

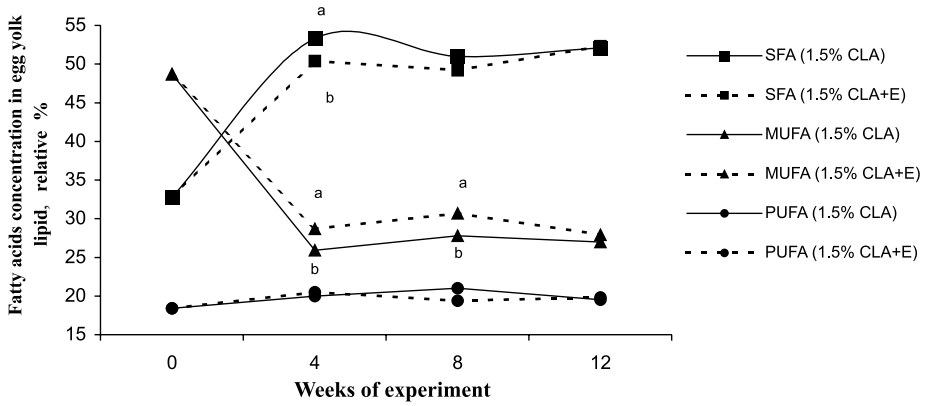

Figure 2. Effect of vitamin E (E: 0 mg vs 120 mg/kg feed) on relative proportions of SFA, MUFA and PUFA in egg yolk lipids from hens fed 1.5% CLA diets during 12 weeks of experiment. Means for a week bearing different letters (a,b) differ significantly (P<0.05)

Weight of yolk (Table 5) was significantly increased (P<0.01) by both dietary CLA and vitamin E. The interaction was also observed, in which the effect of vitamin E added to 1.5% CLA diet, on the yolk weight was more pronounced (P<0.01). At the same time, cholesterol content of eggs, expressed in mg per g of yolk and mg per egg, was significantly (P<0.01) reduced by both dietary supplements. As indicated by significant (P<0.01) interaction, a lowering effect of vitamin E on egg cholesterol (mg per egg) was more marked in the 0.0% CLA diet.

TABLE 5

Effect of dietary conjugated linoleic acid (CLA: 0.0 vs 1.5%) and vitamin E (E: 0 vs 120 mg/kg feed) on egg cholesterol content during 12 weeks of the experiment

Specification	CLA				SE	Significance of effects, P[1]		
	0.0%		1.5%			CLA	E	CLA × E
	E, mg							
	0	120	0	120				
Weight of yolk, g	17.3	17.4	16.9	18.3	0.82	**	**	**
Cholesterol content								
mg per g of yolk	16.3	15.4	14.9	13.8	0.59	**	**	NS
mg per egg	281.1	268.3	253.3	251.7	3.69	**	**	**

[1]significance of effects: ** P<0.01; NS P≥0.05

DISCUSSION

Animal performance

Overall, the laying performance of hens (Table 3) was little affected by the experimental treatments. First of all, there were no significant differences in the performance measurements among hens fed either the 0.0% CLA or the 1.5% CLA diets. In contrast, there were several apparent relationships concerning effects of vitamin E supplement. Our results, at least partly, correspond to those derived from similar studies using laying hens fed CLA-supplemented diets. For example, there were no changes in feed intake nor in the rate of laying and only egg mass was decreased in laying hens fed the diet containing as much as 5% CLA (Chamruspollert and Sell, 1999; Experiment 1). In similar studies of Schäfer et al. (2001) no adverse effects of dietary CLA (1.8%) on the rate of laying nor egg mass were found whereas feed intake was slightly decreased. Likewise, feeding laying hens with relatively low concentrations of dietary CLA (1%) had no adverse effects on the rate of laying, feed intake, nor egg mass (Du et al., 2000). In our recent experiment (Szymczyk and Pisulewski, 2003), using hens fed with increasing amounts of dietary CLA (0, 0.5, 1.0, and 1.5%), the rate of laying and feed intake were unaffected whereas egg mass was decreased

by the dietary CLA. At the same time, as reported by Jones et al. (2000), the rate of laying was significantly decreased ($P<0.05$) in hens fed diets enriched with very low concentrations of CLA (0.0, 0.01, 0.5, and 1.0%). In addition, the birds of the CLA-fed groups (0.01 and 1.0% CLA) consumed slightly less feed, relative to body mass, than birds of the control and 0.5% CLA group, over the entire study. In addition, no significant differences between treatments were observed for egg mass. It seems probable that the data mentioned above indicate that the concentration of dietary CLA is not the only factor affecting production performance of laying hens. The effects of dietary vitamin E (0 vs 120 mg/kg) on laying performance of hens (Table 3), contradict the results of similar studies in which no apparent effects of dietary vitamin E on laying performance of hens were noted (Jiang et al., 1994; Qi and Sim, 1998). Moreover, in the experiment of Qi and Sim (1998), feed intake was decreased only in hens fed the highest level (400 mg/kg) of this vitamin. We have no explanation for the above disagreement. In the above context, there is at present no apparent explanation for the significant interactions between the experimental treatments (CLA × E; Table 3) on laying performance of hens.

Fatty acid in egg yolk lipids

The finding that dietary CLA isomers were incorporated into egg yolk lipids (6.4-6.9% of total fatty acid methyl esters) was the expected feature of this study (Table 4). The preferential incorporation of *cis*-9, *trans*-11 and *cis*-11, *trans*-13 isomers into egg yolk lipids was observed in several previous experiments (Jones et al., 2000; Szymczyk and Pisulewski, 2003).The effects of dietary CLA isomers on concentrations of individual fatty acids can be related to inhibitory effects of these compounds on fatty acid desaturation in avian tissues such as liver, heart and muscles (Simon et al., 2000). The increase in the relative percentage of C14:0, C16:0 and C18:0 at the expense of C16:1 and C18:1 could result from inhibition of Δ9-desaturase. In this respect, CLA isomers were demonstrated to reduce the activity of this enzyme in the liver of mice fed CLA-supplemented diets (Lee et al., 1998). More recently, CLA was shown to depress Δ9-desaturase activity in porcine adipose tissue (Smith et al., 2002). Significant decreases in relative concentrations of all PUFA in egg yolk lipids could be related to substituting the CLA oil for sunflower oil (a source of 18:2 and 18:3; Table 2). Similar effects of replacing soyabean oil with a CLA source in diets fed to hens were reported by Ahn et al. (1999), Chamruspollert and Sell (1999) and Du et al. (2000). More probably, a decrease in concentrations of *n-6* (20:4 and 22:4) and *n-3* fatty acids (22:6) in egg yolk lipids, could result from inhibitory effects of CLA isomers on the metabolism of 18:2 and 18:3 series of fatty acids in laying hens. Indeed, there

is evidence that CLA isomers suppress the action Δ6- and Δ 5-desaturases, thus resulting in impaired desaturation of linoleic acid (18:2) to arachidonic acid (20:4) and α-linolenic acid (C18:3) to docosahexaenoic acid (C22:6). An inhibition of the desaturation of linoleic (C18:2) and α-linolenic (C18:3) acid by CLA is physiologically important because the more unsaturated long-chain detrivatives of these acids are cell membrane constituents which determine important membrane functions, e.g., synthesis of eicosanoids. In line with the above findings, an increase in total SFA ($P<0.01$) and a decrease in total MUFA ($P<0.01$) content were observed. Total PUFA content was not markedly altered in egg yolk lipids whereas that of non-CLA PUFA was substantially ($P<0.01$) decreased (Table 4). Similar, changes in fatty acid composition of eggs were observed in earlier studies (Schäfer et al., 2001; Cherian et al., 2002; Szymczyk and Pisulewski, 2003). The observation that the proportions of SFA in egg yolk lipids increased, whereas those of MUFA and non-CLA decreased, with CLA feeding, is of special interest. Namely, the above changes are associated with the development of hypercholestorolaemia and atherosclerosis in humans, as indicated by relevant studies (Schaefer, 2002). Although there were several significant effects of vitamin E on fatty acid composition of egg yolk lipids, i.e. decreased C18:1 and increased C20:1, increased C18:2, 20:4 and C22:4, decreased C18:3 and C22:6, the reasons for these effects are not clear. In contrast to our findings, the proportion of *n*-9 fatty acids in polar lipids of brain and liver of growing chickens was increased when 125 mg/kg of α-tocopheryl acetate was added to their diets (Fuhrmann and Sallman, 1996). On the other hand, in line with the findings of Lauridsen et al. (1999), the increased concentrations of *n*-6 fatty acids, i.e. C18:2, C20:4, and C22:4, can be attributed to the protective, antioxidant activity of this vitamin on the linoleic acid (18:2), derived from sunflower oil. In view of the findings in which dietary tocopherols either significantly increased the content of *n*-3 fatty acids (C20:5 and C22:6) in egg yolk lipids (Cherian et al., 1996) or have no effect on their composition (Qi and Sim, 1998), we have no explanation for our opposite results. Overall, vitamin E unexpectedly decreased total MUFA content and increased that of total PUFA and non-CLA PUFA in egg yolk lipids (Table 4). However, of more interest are significant interactions between dietary CLA and vitamin E, on concentrations of total SFA, MUFA, PUFA, and non-CLA PUFA in egg yolk lipids. We have no explanation for the unexpected and adverse effects of vitamin E on SFA and MUFA concentrations in egg yolk lipids from hens fed the 0% CLA diets (Figure 1). On the same diets, the increased concentrations of PUFA and non-CLA PUFA could be attributed to antioxidant effects this vitamin on *n*-6 fatty acids. The opposite interactions were noted for egg yolk lipids from hens fed the 1.5% CLA diet, supplemented with 120 mg vitamin E/kg feed (Figure 2). On these diets, the increase in the relative percentage of MUFA at the expense of SFA, could result

from a stimulatory effect of vitamin E on Δ9-desaturase activity (Smith et al., 2002). However, although the above effects of vitamin E, associated only with the 1.5% CLA diet, can be considered beneficial, their extent was too small to prevent the negative effects of dietary CLA on the composition of egg yolk lipids.

Cholesterol in egg yolk lipids

The cholesterol content of eggs was decreased by both dietary supplements (Table 5). The reduction of cholesterol in eggs from hens fed the 1.5% CLA diets can be attributed to inhibitory effects of CLA isomers on hepatic synthesis of apolipoprotein B (apo-B). The resulting impaired secretion of cholesterol-rich VLDL could finally decrease cholesterol deposition in egg yolks. Indeed, it was recently found that that *trans*-10, *cis*-12 isomer significantly reduced secretion of apo-B in murine hepatocytes (Yotsumoto et al., 1999). On the other hand we have no explanation for an unexpected inhibitory effect of vitamin E on egg cholesterol concentration. In fact, vitamin E when fed to laying hens, seems to stabilize egg cholesterol by preventing its oxidation and formation of harmful cholesterol oxides in egg yolks (Sim, 2000).

CONCLUSIONS

From a nutritional point of view, the egg is considered an ideal target for dietary modification leading to development of a functional food. The benefits of improving the quality of eggs by enhancing the concentrations of *n*-3 fatty acids, vitamin E, carotenoids, and selenium and folic acid are known. The same may be true for CLA isomers as novel functional components with evident health-protective properties in animal models and human subjects, as reported recently by Noone et al. (2002). However, adverse effects of CLA feeding to laying hens on fatty acid profile of egg yolks, i.e. an increase in SFA and a decrease in MUFA, are commonly observed (e.g., Szymczyk and Pisulewski, 2003). As evidenced in this experiment, vitamin E may exert protecting effects on fatty acid composition of CLA-enriched eggs. However, at the present concentrations of dietary CLA (1.5%) and vitamin E (120 mg/kg), the practical importance of these effects is questionable. Therefore, the composition of CLA-enriched eggs must be further improved to consider them functional food products.

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STRESZCZENIE

Sprzężony kwas linolowy (CLA) oraz witamina E w żywieniu kur niosek jako czynniki modyfikujące skład kwasów tłuszczowych i zawartość cholesterolu w żółtku jaja

Celem badań było nadanie jajom cech funkcjonalnych, poprzez żywienie kur niosek izomerami sprzężonego kwasu linolowego (0,0 vs 1,5% mieszanki) i witaminą E (0 vs 120 mg/kg mieszanki), jako przeciwutleniaczem. Doświadczenie przeprowadzono na 36 kurach nioskach, w wieku 25 tygodni, rozdzielonych losowo do 4 równoliczebnych grup. Ptaki żywiono w indywidualnych klatkach, przez 12 tygodni doświadczenia, następującymi czterema mieszankami (2770 kcal EM/kg; 16,7% BO): 0,0% CLA; 0,0% CLA+120 mg vitaminy E/kg; 1,5% CLA i 1,5% CLA+120 mg vitaminy E/kg. Oceniano wskaźniki nieśności, skład kwasów tłuszczowych (%) lipidów żółtek i zawartość cholesterolu w żółtkach jaj. Nie stwierdzono wyraźnego wpływu SKL na wskaźniki nieśności. Dodatek witaminy E zwiększał natomiast nieśność (%) i liczbę znoszonych jaj ($P<0,01$). Dla wskaźnika nieśności stwierdzono istotną interakcję 1,5% SKL × 120 mg E/kg ($P<0,01$). Wprowadzenie SKL (1,5%) do mieszanek dla niosek spowodowało wzrost udziału SKL (0.0 vs 6,7%; $P<0,01$), a także wzrost udziału SFA (31.7 vs 51,9%; $P<0,01$), spadek udziału MUFA (45,4 vs 27,3; $P<0,01$) i mniej wyraźny spadek udziału PUFA (21,7 vs 19,6; $P<0,01$) w lipidach żółtek jaj. Witamina E nie wpływała na udział SFA, obniżała udział MUFA i zwiększała udział PUFA ($P<0,01$) w lipidach żółtek jaj. Jednocześnie, zgodnie z interakcją 0,0% SKL × 120 mg E/kg, witamina E obniżała udział MUFA (4, 8 i 12 tydzień; $P<0,01$) i zwiększała udział PUFA (4 tydzień; $P<0,01$). Natomiast zgodnie z interakcją 1,5% SKL × 120 mg E/kg, witamina E obniżała udział SFA (tydzień 4; $P<0,05$) i zwiększała udział MUFA (tydzień 4 i 8; $P<0,05$) w lipidach żółtka jaja. SKL i witamina E obniżały ($P<0,01$) zawartość cholesterolu w jajach (mg/jajo). Dla efektu witaminy E wystąpiła istotna interakcja 0,0% CLA × 120 mg E/kg ($P<0,01$). W podsumowaniu, witamina E może zapobiegać niepożądanym zmianom w składzie lipidów jaja kurzego wzbogacanego w SKL, efekt ten jest jednak znikomy. Niezbędne są zatem dalsze prace nad składem jaja kurzego wzbogacanego w SKL, jako potencjalnego produktu funkcjonalnego.