Effects of dietary conjugated linoleic acid on fatty acid composition and cholesterol content of hen egg yolks

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The main objectives of the present study were to determine the effect of dietary conjugated linoleic acid (CLA) isomers on the fatty acid composition and cholesterol content of egg-yolk lipids. Forty-five 25-week-old laying hens were randomly distributed into five groups of nine hens each and maintained in individual laying cages, throughout 12 weeks of the experiment. They were assigned to the five treatments that consisted of commercial layer diets containing 0, 5, 10, 15 or 20 g pure CLA/kg. Feed intake of hens varied little and insignificantly. Egg mass was uniformly lower ($P<0.05$) in the hens fed the CLA-enriched diets. Feed conversion efficiency, when expressed per kg eggs, was impaired ($P<0.05$), although without obvious relation to the dietary CLA concentration. Feeding the CLA-enriched diets resulted in gradually increasing deposition of CLA isomers ($P<0.01$) in egg-yolk lipids. Saturated fatty acids were increased ($P<0.01$) and monounsaturated fatty acids decreased ($P<0.01$). Polyunsaturated fatty acids (PUFA), when expressed as non-CLA PUFA, were also significantly decreased ($P<0.01$). The most striking effects ($P<0.01$) were observed for palmitic (16 : 0) and stearic (18 : 0) acids, which increased from 23·6 to 34 % and from 7·8 to 18 %, respectively. On the other hand, oleic acid (18 : 1n-9) decreased from 45·8 to 24·3 %. Among non-CLA PUFA, linoleic (18 : 2n-6) and α-linolenic (18 : 3n-3) acids were strongly ($P<0.01$) decreased, from 14·2 to 7·7 % and from 1·3 to 0·3 %, respectively. The same was true for arachidonic (20:4n-6) and docosahexaenoic (22 : 6n-3) acids. The cholesterol content of egg yolks, when expressed in mg/g yolk, was not affected by the dietary CLA concentrations. In conclusion, unless the adverse effects of CLA feeding to laying hens on the fatty acid profile of egg yolks are eliminated, the CLA-enriched eggs cannot be considered functional food products.

Conjugated linoleic acid: Egg production: Fatty acid composition

The use of nutritional strategies to improve the composition and quality of food products of animal origin has emerged recently at the interface of animal science, food science and human nutrition. This new approach has been effectively used to alter product composition to be more consistent with human dietary guidelines. For example, feeding dairy cows with oil seeds, plant and fish oils, as rich sources of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), resulted in their subsequent incorporation into milk lipids (Kowalski *et al.* 1999; Goodridge *et al.* 2001). Equally, the dietary pattern of fatty acids was reproduced in carcass fat of beef cattle (Scollan *et al.* 2001), pigs (Wiseman & Agunbiade, 1998; Matthews *et al.* 2000) and poultry (Leskanich & Noble, 1997). Also egg-yolk lipids were easily enriched in dietary MUFA and PUFA (van Elswyk, 1997; Botsoglou *et al.* 1998; Ayerza & Coates, 2001). Nutritionally modified butter (Noakes *et al.* 1996) or pork (Sandström *et al.* 2000; Stewart *et al.* 2001) or eggs (Lewis *et al.* 2000) decreased plasma cholesterol concentrations when consumed by human subjects.

Recently, there has been increased research on isomers of conjugated linoleic acid (CLA). CLA is a collective name referring to the positional and geometric (*cis*, *trans*) conjugated dienoic isomers of linoleic acid (18 : 2n-6), present mainly in ruminant milk and meat (Fritche & Steinhart, 1998; 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. The CLA isomers have been shown to have health-promoting properties as components of animal diets. Of the major effects, evident anticarcinogenic and anti-atherogenic properties of CLA, and its ability to enhance immune responses in experimental animals were recently described (Roche *et al.* 2001). This has led to efforts to obtain CLA-enriched milk (Kelly *et al.* 1998; Dhiman *et al.* 1999; Baumgard *et al.* 2001), meat (Simon *et al.* 2000; Szymczyk *et al.* 2001; Joo *et al.* 2002) and eggs (Du *et al.* 2000; Jones *et al.* 2000) by nutritional means. Interestingly, CLA-enriched butter has been demonstrated to reduce the number of chemically induced mammary tumours

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in female rats (Ip *et al.* 1999). Similarly, CLA-enriched egg yolks tended to induce hypocholesterolaemia in adult rats (Szymczyk & Pisulewski, 2002).

Although direct extrapolation of the results from animal studies to man may be premature, it seems desirable to obtain and to evaluate CLA-enriched products as functional foods for human consumption. Therefore, the main objectives of the present study were to determine the effect of dietary CLA isomers on the fatty acid composition and cholesterol content of egg yolks.

Material and methods

Forty-five 25-week-old laying hens (Hy-Line Brown) were randomly distributed into five groups of nine hens each and maintained in individual laying cages, throughout 12 weeks of the experiment. They were assigned to the five treatments that consisted of modified Polish commercial layer diets ('DJ') containing 0, 5, 10, 15 or 20 g pure CLA/kg. The CLA source (Natural Lipids Ltd, Hovdebygda, Norway) contained 600 g CLA/kg. Appropriate amounts of sunflower-seed oil were included in the diets to equalise the total fat added to all diets at 50 g/kg (Table 1). The fatty acid composition of the sunflower-seed oil and CLA supplement used in the present 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.

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 kg eggs (feed intake per egg mass; kg) were calculated. In addition, eggs from each hen, collected every 4 weeks were broken, yolks were separated from albumen, weighed, and then frozen at −20°C for further 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 0·5 M KOH/Me-OH and then methylated (10 min, 75°C) in 14 % (v/v) BF₃/Me-OH (Morrison & 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 internal diameter × 0·25 μm film thickness; SGE International, Ringwood, Australia), and a flame ionisation detector. The carrier gas was He used at a split ratio of 50:1. The operating conditions were as follows: the temperature of the injector was 210°C, and that of the 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 Hewlett Packard ChemStation Computer Program (Palo Alto, CA). Fatty acid methyl esters were identified by comparison of their retention

Table 1. The ingredient composition and nutrient content of conjugated linoleic acid (CLA)-enriched experimental diets (0, 5, 10, 15, and 20 g/kg) fed to laying hens

Ingredients (g/kg)	
Ground maize	80
Ground wheat	250
Ground barley	280
Soyabean meal	146
Rapeseed meal	40
Meat-and-bone meal	40
Lucerne meal	20
Sunflower-seed oil*	50
Limestone	83
Dicalcium phosphate	3·0
NaCl	3·0
Mineral and vitamin premix†	5·0
Calculated nutrient content (g/kg)	
Metabolisable energy (MJ/kg)	11·59
Crude protein (N × 6·25)	167·0
Total S amino acids	6·2
Methionine	3·5
Cystine	2·7
Lysine	7·5
Ca	35·3
P (available)	3·4
Crude fibre	42·1

* The CLA source (600 g CLA/kg) was substituted for sunflower-seed oil at 0·00, 8·40, 16·7, 25·0, or 33·3 g/kg diet to obtain 0, 5, 10, 15, or 20 g/kg CLA-enriched diets, respectively.
† Mineral and vitamin premix (Lutamix DJ; Natural Lipids Ltd, Hovdebygda, Norway) provided (per kg diet) vitamin A, 3600 μg; vitamin D₃, 50 mg; vitamin E, 15 mg; vitamin K₃, 2 mg; vitamin B₁, 1 mg; vitamin B₂, 4 mg; vitamin B₆, 1·5 mg; biotin, 1 mg; vitamin B₁₂, 0·01 mg; D-calcium panthotenate, 8 mg; nicotinamide, 25 mg; choline chloride, 250 mg; Mn, 100 mg; I, 0·8 mg; Zn, 5 mg; Co, 0·2 mg; Se, 0·2 mg; DL-methionine, 500 mg.

times with authentic standards purchased from Sigma-Aldrich (Poznan, 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 (Warsaw, 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).

The data were analysed using one-way ANOVA generated by the STATISTICA version 5.1 package (StatSoft, Tulsa, OK). Where appropriate, the Duncan's multiple range test (Duncan, 1955) was used to determine the significance of differences between treatment means at the $P<0.05$ and $P<0.01$ levels of significance.

Results

Feeding hens with graded amounts of dietary CLA (0, 5, 10, 15, and 20 g/kg diet) had no apparent effects on their production performance over the 12-week experimental period (Table 3). The overall means for feed intake

Table 2. Fatty acid composition (relative %) of sunflower-seed oil and conjugated linoleic acid (CLA) source fed to laying hens

Fatty acid	Sunflower-seed oil	CLA
12 : 0	–	0·1
14 : 0	0·01	0·1
16 : 0	6·2	4·9
16 : 1n-7	–	0·1
18 : 0	–	2·0
18 : 1n-9	24·4	29·9
18 : 1n-7	–	0·7
18 : 2n-6	62·9	0·5
CLA isomers*		
Total	–	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 : 3n-3	1·0	–
20 : 0	0·3	0·9
22 : 0	0·6	0·3
24 : 0	0·2	0·1
Total fatty acids	95·6	98·3

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

of hens varied little and insignificantly. The hens fed the CLA-enriched diets (5–20 g/kg) tended to have lower laying rates than those fed the control diet (0 g/kg). Similarly, egg mass was uniformly lower ($P < 0.05$) in the hens fed the CLA-enriched diets. Feed conversion efficiency, when expressed per one egg, tended to decrease with increasing concentrations of dietary CLA. Conversely, when expressed per kg eggs, it was slightly increased ($P < 0.05$), although without obvious relation to the dietary CLA concentration.

The fatty acid composition of egg-yolk lipids, expressed as a percentage of total methyl esters of fatty acids, was significantly altered by increasing concentrations of dietary CLA (Table 4). The total and individual concentration of CLA isomers reached a maximum on week 4 and decreased slightly thereafter (Fig. 1). No CLA was found in the egg lipids of hens fed the control diet. In contrast,

feeding the CLA-enriched diets resulted in increasing deposition of CLA isomers ($P < 0.01$). Interestingly, concentrations of individual CLA isomers in egg-yolk lipids did not entirely reflect those of the commercial CLA product, thus indicating preferential incorporation of these compounds. Generally, the relative proportions of cis-9, trans-11 and cis-11, trans-13 CLA isomers in egg-yolk lipids exceeded those found in the CLA product. On the other hand, trans-8, cis-10 and trans-10, cis-12 were incorporated into egg lipids less efficiently. The CLA-enriched diets significantly ($P < 0.01$) increased the egg-yolk saturated fatty acid (SFA) concentrations and significantly ($P < 0.01$) decreased those of MUFA and non-CLA PUFA. Of individual SFA, the most striking effects ($P < 0.01$) were observed for 16 : 0 and 18 : 0, which increased from 23·6 to 34 % and from 7·8 to 18 %, respectively. On the other hand, striking changes ($P < 0.01$) in concentrations of 18 : 1n-9, which decreased from 45·8 to 24·3 %, produced overall decreases in MUFA concentrations. Among non-CLA PUFA, 18 : 2n-6 and 18 : 3n-3 concentrations were strongly ($P < 0.01$) reduced, from 14·2 to 7·7 % and from 1·3 to 0·3 %, respectively. The same was true for 20 : 4n-6 and 22 : 6n-3. Concentrations of other non-CLA PUFA varied inconsistently.

The cholesterol content of egg yolks (Table 5), when expressed in mg per g yolk, was not affected by the increasing dietary CLA concentrations. Also, the reduction of egg cholesterol (mg per egg) became significant ($P < 0.01$) only as a result of reduction of egg yolk size.

Discussion

Overall, the production performance of laying hens (Table 3) was little affected by feeding CLA-enriched diets (0, 5, 10, 15, and 20 g/kg diet). However, there were several apparent relationships between performance characteristics. Since the rate of laying (%) was constant in hens fed the CLA-enriched diets and feed intake tended to be decreased by the 20 g/kg CLA-enriched diet, the hens fed this diet required slightly less feed ($P > 0.05$) to produce one egg, as compared with the birds fed the other dietary treatments. On average, the feed intake and mass of eggs produced by hens fed

Table 3. Effect of feeding hens with conjugated linoleic acid (CLA)-enriched diets (0, 5, 10, 15, and 20 g/kg) on their production characteristics during 12 weeks of experiment*

(Mean values and standard errors of the mean)

Dietary level of CLA (g/kg)	Rate of laying (%)		Egg mass (g)		Feed intake (g/hen per d)		Feed conversion			
							per egg (g)		per kg eggs (kg)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	95·2	1·03	63·7[a]	0·39	124·0	1·77	131·2	3·79	2·07[a]	0·05
5	93·1	0·87	59·7[b]	1·01	120·0	0·82	131·0	2·85	2·19[b]	0·02
10	93·7	1·39	61·2[b]	0·47	126·0	2·04	130·7	1·84	2·13[ab]	0·01
15	93·8	1·29	61·2[b]	0·22	123·0	1·03	130·5	1·55	2·14[ab]	0·02
20	93·0	0·67	60·6[b]	0·65	119·2	0·85	128·7	1·18	2·12[ab]	0·04

a,b Mean values within a column with unlike superscript letters were significantly different ($P \leq 0.05$; analysed by Duncan's multiple range test).
* For details of diets and procedures, see Tables 1 and 2 and p. 94.

Table 4. Effect of feeding hens with conjugated linoleic acid (CLA)-enriched diets (0, 5, 10, 15, and 20 g/kg) on fatty acid composition (relative %) of egg-yolk lipids after 4 weeks of experiment*

(Mean values and standard errors of the mean)

| | Pre-experimental | | Dietary level of CLA (g/kg) | | | | | | | | | |
| | | | 0 | | 5 | | 10 | | 15 | | 20 | |
Fatty acids	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14 : 0	0·3	0·01	0·3[a]	0·02	0·5[b]	0·02	0·6[bc]	0·04	0·7[c]	0·04	0·7[c]	0·02
16 : 0	24·4	0·12	23·6[a]	0·27	29·6[b]	0·78	30·3[b]	0·22	33·1[c]	0·93	34·0[c]	0·69
16 : 1n-7	2·6	0·08	2·2[a]	0·13	1·5[b]	0·07	1·1[c]	0·04	1·1[c]	0·1	1·2[bc]	0·02
17 : 0	0·2	0·00	0·2[a]	0·00	0·3[b]	0·04	0·3[b]	0·00	0·3[b]	0·02	0·3[b]	0·00
18 : 0	7·9	0·16	7·8[a]	0·23	14·6[b]	0·26	17·6[c]	0·50	18·7[c]	0·94	18·0[c]	0·33
18 : 1n-9	45·9	0·34	45·8[a]	0·42	30·3[b]	0·54	26·3[c]	0·20	23·2[cd]	0·75	24·3[cd]	0·50
18 : 2n-6	12·3	0·28	14·2[a]	0·34	13·8[b]	0·55	12·9[ab]	0·27	11·1[b]	0·55	7·7[c]	0·23
18 : 3n-3	1·5	0·01	1·3[a]	0·04	1·3[b]	0·05	0·9[b]	0·02	0·3[c]	0·02	0·3[c]	0·00
20 : 1n-9	0·2	0·00	0·2[a]	0·02	0·3[b]	0·00	0·3[b]	0·02	0·4[c]	0·00	0·5[c]	0·00
20 : 4n-6	1·9	0·04	1·6[a]	0·06	1·3[ab]	0·06	1·2[abc]	0·04	1·1[c]	0·04	0·8[d]	0·04
22 : 5n-3	0·1	0·00	0·1[a]	0·00	0·3[b]	0·00	0·3[b]	0·02	0·3[b]	0·00	0·1[a]	0·00
22 : 6n-3	0·7	0·02	1·4[a]	0·06	1·0[b]	0·02	0·6[c]	0·04	0·3[d]	0·00	0·2[d]	0·02
CLA isomers												
Cis-9, _trans_-11	0·0	0·00	0·0[a]	0·00	0·7[b]	0·02	1·4[c]	0·05	1·9[d]	0·02	2·5[e]	0·02
Trans-8, _cis_-10	0·0	0·00	0·0[a]	0·00	0·3[b]	0·02	0·5[c]	0·00	0·6[c]	0·00	0·8[d]	0·04
Cis-11, _trans_-13	0·0	0·00	0·0[a]	0·00	0·5[b]	0·02	1·0[c]	0·02	1·4[d]	0·04	1·8[e]	0·06
Trans-10, _cis_-12	0·0	0·00	0·0[a]	0·00	0·3[b]	0·02	0·6[c]	0·02	0·9[d]	0·02	1·3[e]	0·04
Other CLA isomers	0·0	0·00	0·0[a]	0·00	0·5[b]	0·00	1·0[b]	0·06	1·6[c]	0·07	2·0[d]	0·09
Total SFA	32·8	0·30	31·0[a]	0·35	45·2[b]	1·20	49·1[c]	0·45	53·1[d]	0·93	53·3[d]	1·50
Total MUFA	48·7	0·68	49·1[a]	0·87	33·6[b]	1·41	29·1[bc]	0·54	26·0[c]	0·81	28·1[bc]	0·54
Non-CLA PUFA	18·4	0·21	19·3[a]	0·36	18·6[b]	0·34	16·4[c]	0·38	13·9[d]	0·27	9·9[e]	0·43

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[a,b,c,d,e]Mean values for CLA dietary level effect within a row with unlike superscript letters were significantly different ($P \leq 0.01$; analysed by Duncan's multiple range test).
* For details of diets and procedures see Tables 1 and 2 and p. 94.

the CLA-enriched diets were lower ($P > 0.05$ and $P < 0.05$, respectively) than those recorded for the control group. Thus, since the mass of eggs was more affected by dietary CLA, the resulting average feed conversion efficiency, when expressed as kg of feed required per kg eggs produced, was higher ($P < 0.05$) in the hens fed the CLA-enriched diets than in those fed the control diet. Our results partly correspond to those associated with much higher
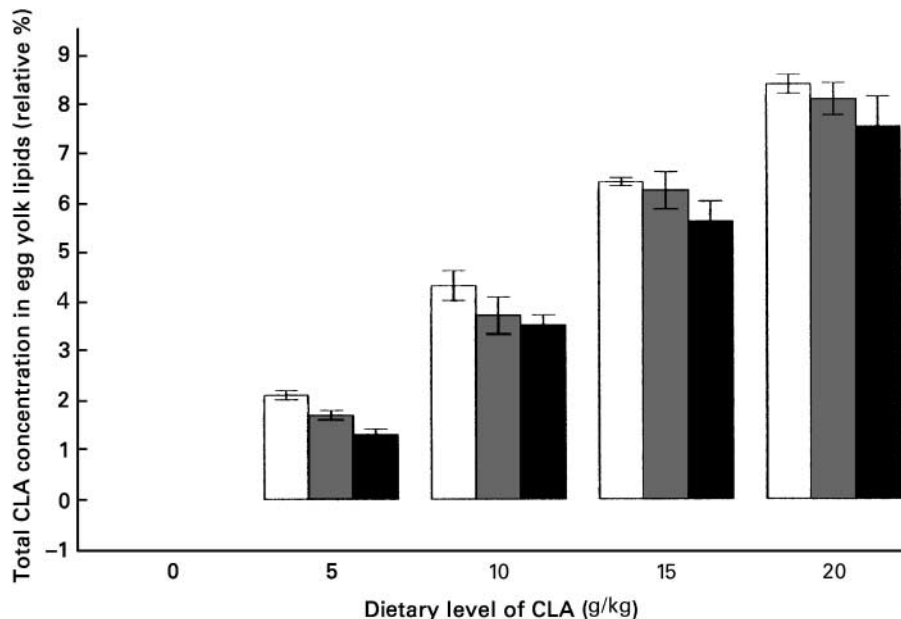

Fig. 1. Effect of feeding conjugated linoleic acid (CLA)-enriched diets to laying hens on the total concentration of CLA isomers in egg-yolk lipids at week 4 (□), week 8 (▦) and week 12 (■) of the experiment. Values are means, with standard errors of the mean represented by vertical bars.

Table 5. Effect of feeding laying hens with conjugated linoleic acid (CLA)-enriched diets (0, 5, 10, 15, and 20 g/kg) on egg cholesterol content (mg/g of yolk and mg/egg) during 12 weeks of experiment*

(Mean values and standard errors of the mean)

| | Weight of yolk (g) | | Cholesterol content | | | |
| | | | mg/g yolk | | mg/egg | |
Dietary level of CLA (g/kg)	Mean	SEM	Mean	SEM	Mean	SEM
0	17·22	0·31	15·24	0·62	262·43[a]	4·55
5	16·58	0·28	14·49	0·64	240·24[b]	3·82
10	16·73	0·23	14·28	0·70	238·90[b]	4·02
15	16·42	0·33	14·09	0·59	231·35[bc]	3·68
20	16·93	0·26	13·47	0·65	228·05[c]	3·80

[a,b,c]Mean values within a column with unlike superscript letters were significantly different ($P \leq 0.01$; analysed by Duncan's multiple range test).

* For details of diets and procedures see Tables 1 and 2 and p. 94.

concentrations of dietary CLA. For example, feeding laying hens with a diet containing 50 g CLA/kg (Ahn *et al.* 1999) adversely affected feed intake and the rate of laying, whereas no effect on egg mass was detected. The results of two separate experiments, using hens fed the 50 g/kg CLA-enriched diets (Chamruspollert & Sell, 1999), were contradictory. In the first experiment, feed intake and the rate of laying were not affected and egg mass was decreased, whereas in the second one, feed intake was decreased but neither the rate of laying nor egg mass were affected. Alternatively, feeding laying hens with relatively low concentrations of dietary CLA (10 g/kg) had no adverse effects on the rate of laying, feed intake, nor egg mass (Raes *et al.* 2002). On the other hand, as reported by Jones *et al.* (2000), the rate of laying was significantly decreased ($P < 0.05$) in hens fed diets enriched in even lower concentrations of CLA (0·0, 0·1, 5·0, and 10·0 g/kg). In addition, the birds of the CLA-fed groups (0·1 and 10·0 g CLA/kg) consumed slightly less feed, relative to body mass, than birds of the control and 5 g/kg CLA group, over the entire study. At the same time, no significant differences between treatments were observed for egg mass. The data reported earlier might indicate that the concentration of dietary CLA is not the only factor affecting production performance of laying hens.

The finding that the amounts of CLA isomers incorporated into egg-yolk lipids were proportional to the levels of CLA in the diet (Table 4) was the major feature of the present study. It was also found (Fig. 1) that the concentrations of total CLA isomers in egg-yolk lipids reached their maximum after the first 4-week period. From a physiological point of view, the period of 10–14 d seems to be sufficient to obtain maximum incorporation of CLA isomers into egg-yolk lipids (Chamruspollert & Sell, 1999; Du *et al.* 1999; Jones *et al.* 2000; Aydin *et al.* 2001; Raes *et al.* 2002). Moreover, we found that *cis*-9, *trans*-11 and *cis*-11, *trans*-13 were incorporated into egg-yolk lipids preferentially, when compared with *trans*-8, *cis*-10 and *trans*-10, *cis*-12 isomers. This also confirms earlier findings of Du *et al.* (1999), Jones *et al.* (2000) and, more recently, Raes *et al.* (2002). The preferential incorporation of CLA isomers into tissue lipids was also observed in our experiments with rats (Szymczyk *et al.*

2000) and broilers (Szymczyk *et al.* 2001) fed CLA-enriched diets. This may be because *trans*-10, *cis*-12 CLA appears to be metabolised more efficiently than *cis*-9, *trans*-11, at least in mice (Park *et al.* 1999).

As compared with the fatty acid composition of egg-yolk lipids produced by the control group, the proportions of SFA were increased ($P < 0.01$) with increasing dietary CLA concentrations, whereas those of MUFA decreased ($P < 0.01$). The earlier findings can be related to the inhibitory effect of CLA isomers on the liver Δ9-desaturase (stearoyl-CoA desaturase), the enzyme that catalyses the insertion of a double bond between C_9 and C_{10} atoms of either 16 : 0 or 18 : 0 (Cook, 1991). More recently it was shown that CLA inhibited stearoyl-CoA desaturase mRNA expression (Lee *et al.* 1998). Hence, it has been demonstrated (Table 4) that relative concentrations of 16 : 0 and 18 : 0 in egg-yolk lipids were increased, whereas those of 16 : 1*n*-7 and 18 : 1*n*-9 decreased significantly in hens fed CLA-enriched diets. These changes have been already reported for egg-yolk lipids of hens fed CLA-enriched diets. (Ahn *et al.* 1999; Chamruspollert *et al.* 1999; Du *et al.* 1999; Raes *et al.* 2002). The same was true for tissue lipids of rats (Szymczyk *et al.* 2000), broilers (Simon *et al.* 2000; Szymczyk *et al.* 2001), and pigs (Ramsay *et al.* 2001). These effects may be of concern since the increasing degree of saturation of egg-yolk lipids affects negatively the quality of eggs (Ahn *et al.* 1999; Aydin *et al.* 2001) and causes 100 % embryonic mortality in the fertile eggs (Aydin *et al.* 2001).

Significant decreases in the relative concentrations of non-CLA PUFA (Table 4), mainly 18 : 2*n*-6 and 18 : 3*n*-3 and their derivatives (20 : 4*n*-6 and 22 : 6*n*-3, respectively) in egg-yolk lipids, could be related to substituting the CLA oil for sunflower-seed oil (a source of 18 : 2*n*-6 and 18 : 3*n*-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 & Sell (1999), and Du *et al.* (1999, 2000). Also, the changes in concentrations of 20 : 4*n*-6 and 22 : 6*n*-3 in egg-yolk lipids could have resulted from inhibitory effects of CLA isomers on the metabolism of 18 : 2*n*-6 and 18 : 3*n*-3 series of fatty acids in laying hens. Indeed, it was found that that CLA isomers may compete with both 18 : 2*n*-6 and 18 : 3*n*-3 for Δ6-desaturase, the rate-limiting enzyme for the conversion of these

fatty acids to 20:4n-6 and 22:6n-3, at least in mouse liver microsomes (Belury & Kempa-Steczko, 1997). Moreover, it was reported that this effect was attributed to the cis-9, trans-11 CLA isomer (Bretillon et al. 1999).

The cholesterol content of eggs (Table 5), when expressed either in mg per g yolk or mg per egg, was not, in fact, affected by the increasing dietary CLA concentrations. The reduction of egg cholesterol, expressed in terms of mg per egg, was only apparent and became significant ($P<0.01$) as a result of reduction of egg yolk size. Therefore it can be clearly stated that for the 12-week period of our experiment, dietary CLA did not change cholesterol concentration in hen egg yolks.

The reported increases in concentrations of SFA and parallel decreases in concentrations of both MUFA and PUFA (n-6 and n-3) in egg-yolk lipids (Table 4) may be seen as undesirable for consumers. Namely, increased SFA consumption is associated with hypercholesterolaemia and the risk of CHD (Schaefer, 2002). Also, the overall data indicate that PUFA are hypocholesterolaemic and/or anti-atherogenic. Specifically, high intakes of n-3 PUFA are beneficial and associated with lower platelet aggregation, lower immune response, and lower systolic and diastolic blood pressure (Mantzioris et al. 2000; Schaefer, 2002). Interestingly, the commonly reported adverse effects of dietary CLA on fatty acid composition of egg-yolk lipids may be prevented by nutritional manipulation. Thus, olive oil (a rich source of 18:1n-9) fed to laying hens prevented CLA-induced increases in 16:0 and 18:0 and the decrease in 18:1n-9 in egg-yolk lipids (Aydin et al. 2001). However, feeding olive oil resulted also in considerably lower incorporation of CLA isomers into egg-yolk lipids (Aydin et al. 2001). In a similar approach, feeding laying hens with CLA-supplemented, α-linolenic acid-rich diets resulted in increased accumulation of n-3 PUFA, i.e. 18:3n-3, 20:5n-3 and 22:6n-3 (Du et al.2000). Less consistently, when an excess of dietary 18:3n-3 was fed, the amounts of 18:3n-3 and 20:5n-3 in egg-yolk lipids were decreased and those of 22:5n-3 and 22:6n-3 increased, as found by Raes et al. (2002). Thus, feeding plant oils rich in 18:3n-3 (for example, flaxseed oil) to laying hens seems to be a promising means to eliminate, at least partly, the adverse effects of CLA on fatty acid composition (n-3 series) of egg-yolk lipids.

From a nutritional point of view, the egg is considered an ideal target for dietary modification leading to the 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 Se were reported by Surai & Sparks (2001). The same may be true for CLA as a novel functional component with evident cardio-protective properties in human subjects, as reported recently by Noone et al. (2002). However, unless the several adverse effects of feeding CLA to laying hens on the fatty acid profile of egg yolks are eliminated, the CLA-enriched eggs cannot be considered functional food products in human nutrition. Further investigation is certainly needed to determine conclusively the efficient nutritional strategy for the production of CLA-enriched eggs with acceptable fatty acid composition and sensory properties.

Acknowledgements

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