

Effects of Conjugated Linoleic Acid Supplementation in Layer Diet on Fatty Acid Compositions of Egg Yolk and Layer Performances

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ABSTRACT Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds. Conjugated linoleic acids have been reported to have a wide range of health-beneficial effects, including anticarcinogenic, antiatherogenic, anti-diabetic, and immune stimulatory effects. The objective of this study was to investigate the incorporation of CLA into eggs. Three hundred 27-wk-old layers were allocated to 5 dietary treatments (0, 1, 2, 3, and 4% CLA) with 5 replicates. The results of the study showed that average daily feed intakes were similar in all treatment groups, although hens fed with 4% CLA tended to consume less feed than other hens. Body weight gain and mortality rate were not significantly different ($P > 0.05$). Hens fed 4% dietary CLA had reduced egg, yolk, and albumen

weights ($P < 0.05$). Yolk color significantly decreased as dietary CLA increased ($P < 0.01$). Shell thickness and Haugh units were not influenced by the dietary CLA. Concentrations of CLA and saturated fatty acids in egg yolk lipids increased as dietary CLA increased ($P < 0.01$), whereas concentrations of monounsaturated fatty acids and polyunsaturated fatty acids decreased as dietary CLA increased ($P < 0.01$). It can be concluded from the present experiments that increasing the amount of CLA fed to hens will increase the amount of CLA in egg yolk and that this increase is accompanied by a reduction in the amount of yolk polyunsaturated fatty acids but an increase in yolk saturated fatty acids. Egg size, yolk weight, and Roche-fan determined yolk color significantly decreased at the highest level of CLA supplementation.

Key words: conjugated linoleic acid, egg yolk, fatty acid, layer, egg quality

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INTRODUCTION

The roles of dietary cholesterol and fatty acid composition in the etiology of cardiovascular disease and other ailments remain controversial. Many attempts have been made to reduce egg cholesterol contents; however, these attempts have met little practical application (Hargis, 1988). An alternative way to reduce cholesterol in eggs is by altering the yolk fatty acid composition. The cholesterol-lowering effects of polyunsaturated fatty acids (PUFA) have been recognized for some years (Kinsella et al., 1990; Hargis and Van Elswyk, 1993; Demeyer and Doreau, 1999). Feeding layers a diet rich in PUFA resulted in a large increase in the relative and absolute concentrations of PUFA in yolk total lipid (Caston and Leeson, 1990; Farrell and Gibson, 1991; Hargis et al., 1991).

Recently, conjugated linoleic acid (CLA), a group of isomers of octadecadienes, has received much attention. Several health-beneficial effects are attributed to these

isomers (e.g., anticarcinogenic and antiatherosclerotic effects), influencing both fat metabolism and protein deposition (Ha et al., 1987; Ip et al., 1991; Pariza et al., 2000). However, if the antiatherogenic activity of CLA found in rabbits (Lee et al., 1994), hamsters (Nicolosi et al., 1997), and mice (Munday et al., 1999) could be measured in humans, the benefit of CLA to human health might be of interest. Consumption of CLA by humans has been shown to elicit many favorable health benefits, such as the modulation of immune function, weight reduction, and protection against diseases such as cancer and arteriosclerosis (Chin et al., 1992; Lee et al., 1994; Nicolosi et al., 1997). Conjugated linoleic acid is naturally present in products originating from ruminants as a result of the specific metabolism of the rumen producing, in particular, *c9, t11* CLA as the predominant isomer. However, recent studies have suggested that the endogenous synthesis of CLA by the action of $\Delta 9$ -desaturase on trans 18:1 fatty acids is probably more important than that of the ruminal production (Griinari et al., 2000; Santora et al., 2000). Eggs and other products from monogastric animals contain negligible amounts of CLA (Chin et al., 1992). It is worthwhile to consider the enrichment of food with CLA, because CLA is readily incorporated into the fat fraction of animal foods. The egg yolk can

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Table 1. Ingredients (kg) and nutrient composition of the experimental diets fed to laying hens (as-fed basis)

Feed ingredients	Diets (% CLA)				
	0	1	2	3	4
Ground corn	48.00	48.00	48.00	48.00	48.00
Soybean meal	24.80	24.80	24.80	24.80	24.80
Fish meal	2.80	2.80	2.80	2.80	2.80
Extracted rice bran	0.80	0.80	0.80	0.80	0.80
Dl-Met	0.10	0.10	0.10	0.10	0.10
Oyster shell	8.50	8.50	8.50	8.50	8.50
Dicalcium phosphate ¹	0.96	0.96	0.96	0.96	0.96
NaCl	0.20	0.20	0.20	0.20	0.20
CLA (30%) ²	0.00	3.34	6.67	10.00	13.34
Soybean oil	6.67	5.00	3.34	1.67	0.00
Silica	6.67	5.00	3.34	1.67	0.00
Premix ³	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00
Moisture content (%)	6.25	6.27	6.30	6.20	6.08
CP (%)	16.78	16.73	16.68	16.62	16.60
Ether extract (%)	9.01	9.20	9.22	9.00	9.00
Crude fiber (%)	4.40	4.25	4.30	4.20	4.26
Ash (%)	18.16	18.25	18.34	18.74	18.80
Ca (%)	3.75	3.78	3.75	3.77	3.79
P (%)	0.43	0.44	0.40	0.41	0.42
Calculated composition ⁴					
Lys (%)	0.91	0.91	0.91	0.91	0.91
Met + Cys (%)	0.65	0.65	0.65	0.65	0.65
Tryptophan (%)	0.21	0.21	0.21	0.21	0.21
Thr (%)	0.64	0.64	0.64	0.64	0.64
ME (kcal/kg)	2,862	2,862	2,862	2,862	2,862

¹22% Ca and 18% P.

²The conjugated linoleic acid (CLA) contained 15% of the *c9,t11* isomer and 15% of the *t10,c12* isomer.

³Provided the following per kilogram of diet fed: vitamin A, 8,800 IU; vitamin D₃, 2,500 IU; vitamin E, 5 IU; vitamin K, 0.5 mg; thiamin, 8 mg; riboflavin, 2.2 mg; vitamin B₆, 3 mg; vitamin B₁₂, 0.03 mg; niacin, 10 mg; folic acid, 0.25 mg; Zn, 53 mg; Mn, 70 mg; Fe, 8 mg; Cu, 5 mg; Co, 0.25 mg; Mo, 0.1 mg; and Se, 0.1 mg.

⁴Based on NRC (1994) ingredient composition.

be considered to be a good carrier, because it contains 30 to 35% fat. Many studies have suggested that eggs from CLA-fed hens are a good source of CLA in the human diet (Chamruspollert and Sell, 1999; Du et al., 1999; Raes et al., 2002; Sun et al., 2003). One way to increase the CLA content in eggs is through supplementation of the layer diet with CLA. A combined incorporation of CLA into egg yolk, together with that of PUFA, would lead to even greater health benefits. The aim of this experiment was to study the effects of various CLA levels upon laying performance, egg yolk fatty acid compositions, and egg quality.

MATERIALS AND METHODS

Experimental Birds

All experiments were conducted in accordance with the principles and guidelines approved by the Suranaree University of Technology Animal Care and Use Committee, which followed Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Federation of Animal Science Societies, 1999). Three hundred laying hens (Hisex Brown; 27 wk old) were randomly divided into 5 groups. Each group (60 hens) was further randomly divided into 5 replicates of 12 hens. In each replicate, hens were then randomly allocated to 4 metal cages of 3 laying hens, under condi-

tions of an evaporative cooling system and lighting program (16L:8D) throughout the entire experiment. Hens were fed diets and water ad libitum during the entire experimental period.

Experimental Diets

Each group of hens was randomly fed an experimental diet containing 0, 1, 2, 3, and 4% CLA. All experimental diets were isonitrogenous and isocaloric and formulated to meet the NRC (1994) requirements. Hens were fed experimental diets containing 2,862 kcal of ME/kg, 16.5% CP, 0.91% Lys, 0.65% Met + Cys, 3.78% Ca, and 0.41% P. Feed ingredients and chemical compositions of the experimental diets are presented in Table 1. Commercial liquid CLA [BASF (Thai) Ltd., Bangkok, Thailand], containing 60% CLA (30% *c9, t11*; 30% *t10, c12*), 22% oleic acid, 6% palmitic acid, 4% stearic acid, 2% linoleic acid, and 6% of other isomers of fatty acids, was premixed with silica to obtain a CLA concentration of 30% (15% *c9, t11*; 15% *t10, c12*). To equalize the concentration of total fat, the CLA source was substituted for soybean oil. Silica was also thoroughly mixed into the diets to maintain equivalent silica content in the diets. Each of the 5 experimental diets was fed to each group of laying hens for five 28-d periods, giving a total of 140 d for the entire experimental periods. Chemical analysis of the

Table 2. Analyses of fatty acid composition in the experimental diets

Fatty acid profile	Diets (% CLA ¹)				
	0	1	2	3	4
	(%) of diet				
Myristic acid (C14:0)	0.01	0.01	0.01	0.01	0.01
Palmitic acid (C16:0)	1.20	1.11	1.03	0.85	0.76
Palmitoleic acid (C16:1)	0.01	0.02	0.02	0.01	0.01
Stearic acid (C18:0)	0.46	0.44	0.41	0.36	0.34
Oleic acid (C18:1)	2.42	2.54	2.62	2.57	2.56
Linoleic acid (C18:2)	4.39	3.83	2.94	2.00	1.17
Linolenic acid (C18:3)	0.45	0.37	0.28	0.16	0.07
Arachidonic acid (C20:4)	0.05	0.05	0.05	0.04	0.03
Docosahexaenoic acid (C22:6)	0.02	0.02	0.02	0.04	0.02
CLA	0.00	0.82	1.85	2.98	4.03
	(%) of total fatty acid				
Myristic acid (C14:0)	0.13	0.13	0.14	0.11	0.12
Palmitic acid (C16:0)	13.35	12.06	11.16	9.45	8.41
Palmitoleic acid (C16:1)	0.14	0.16	0.16	0.15	0.15
Stearic acid (C18:0)	5.12	4.79	4.45	4.02	3.76
Oleic acid (C18:1)	26.86	27.56	28.40	28.53	28.55
Linoleic acid (C18:2)	48.69	41.60	31.86	22.19	12.98
Linolenic acid (C18:3)	4.96	4.00	3.05	1.83	0.75
Arachidonic acid (C20:4)	0.55	0.58	0.51	0.39	0.32
Docosahexaenoic acid (C22:6)	0.20	0.19	0.19	0.18	0.20
CLA	0.00	8.93	20.08	33.14	44.77

¹CLA = conjugated linoleic acid.

diets was made for CP, crude fiber, ether extract, and ash (Association of Official Analytical Chemists, 1998).

Layer Performances and Egg Quality Characteristics

Egg production and egg weight were recorded daily, whereas feed consumed was recorded weekly. Every 14 d throughout the experimental period, uniform samples of 4 eggs from each replication from each treatment were used to determine the mass of the main egg components (shell, yolk, and albumen). Yolk color was determined by comparing yolk color to the Roche color fan.

Fatty Acid Analysis

Fatty acid analysis was determined as previously described (Raes et al., 2000, 2001). In brief, lipids were extracted from fresh yolk using chloroform, methanol, or both (2:1, vol/vol; Folch et al., 1957). Nonadecanoic acid (19:0) was added as an internal standard. The fatty acid methyl esters (FAME) were analyzed by gas chromatography (GC; model 6890, Hewlett-Packard Co., Palo Alto, CA) using a CP-Sil88 column (Chrompack, Middelburg, The Netherlands) for FAME (100 m × 0.25 mm). The GC conditions were as follows: injected temperature, 240°C; detector temperature, 260°C; carrier gas, He; split ratio, 1:30; temperature program, 70°C for 4 min, followed by an increase of 13°C/min to 175°C then 4°C/min to 215°C. Peaks were identified by comparison of retention times with those of the corresponding standards (Supelco 37 component FAME Mix, Sigma-Aldrich Co., St. Louis, MO). Identification of the peak included fatty acids from 14:0 to 22:6 and the following CLA isomers: *c*9, *t*11; *t*10, *c*12; *c*9, *c*11; *t*9, *t*11.

Statistical Analysis

Because the CLA treatment caused similar responses in each of the five 28-d periods, the 5 observations from each replicate were averaged. The averaged observed effects between treatment groups were then statistically analyzed by ANOVA in a completely randomized designed (Steel and Torrie, 1986), and significant differences among means were compared by Duncan's new multiple range test, according to the methods previously described by the SAS institute (1994). The effects of increasing CLA were partitioned into linear and quadratic components using orthogonal polynomial contrasts (SAS Institute, 1994).

RESULTS AND DISCUSSION

Layer Performance

The fatty acid profiles of the diets, as analyzed by GC, are given in Table 2. Inclusion of CLA, together with replacing CLA with soybean oil, decreased the level (g/100g of total fatty acids, Table 2) of both linoleic and linolenic acids. This can be attributed to the high concentration of linoleic and linolenic acids in soybean oil when soybean oil was replaced by CLA; thus, the level of linoleic and linolenic acids decreased.

Average daily feed intakes were similar in all treatment groups, although hens fed with 4% CLA tended to consume less feed than other hens (Table 3). Body weight changes and mortality rates across the entire experimental period were also similar ($P > 0.05$). However, hens fed 4, 2, and 1% CLA diets produced fewer eggs than that of hens fed unsupplemented control diet ($P < 0.01$; Table 3). All hens showed similar albumen height,

Table 3. Effects of conjugated linoleic acid (CLA) on performance and egg quality of laying hens

Performances	Diets (% CLA)					SEM	P-value	P-value	
	0	1	2	3	4			Linear	Quadratic
ADFI ¹ (g/d)	107.4	103.3	103.0	106.8	99.9	1.82	0.0519	0.0784	0.9269
BWC ² (g)	52.8	44.2	54.0	51.0	37.2	10.49	0.7699	0.4707	0.5612
Mortality (%)	0.00	0.00	0.07	0.00	0.20	0.06	0.1894	0.0723	0.2980
Egg production (%)	86.73 ^d	79.32 ^{ef}	79.08 ^{ef}	83.70 ^{de}	72.89 ^f	2.09	0.0019	0.0022	0.8046
Egg quality									
Egg weight (g)	60.88 ^a	60.35 ^a	59.99 ^a	60.10 ^a	57.48 ^b	0.76	0.0426	0.0083	0.2040
Albumen height (mm)	8.07	8.17	7.78	7.96	7.98	0.15	0.4616	0.4076	0.4760
Haugh unit	89.29	90.10	87.95	89.09	89.77	0.83	0.4142	0.9422	0.3471
Yolk color	5.86 ^d	4.70 ^c	4.41 ^{ef}	4.33 ^f	3.80 ^g	0.10	0.0001	0.0001	0.0015
Shell thickness (mm)	0.38	0.38	0.38	0.39	0.38	0.004	0.9085	0.7861	0.4219
Yolk weight (g)	13.87 ^{ab}	13.79 ^{abc}	14.13 ^a	13.52 ^{bc}	13.39 ^c	0.14	0.0136	0.0135	0.0679
Albumen weight (g)	38.43 ^a	37.71 ^a	37.36 ^{ab}	38.09 ^a	36.04 ^b	0.47	0.0194	0.0085	0.3908

^{a-g}Values with no common superscript differ significantly ($P < 0.05$) and ($P < 0.01$) respectively when tested with Duncan's multiple range test.

¹ADFI = average daily feed intake.

²BWC =body weight change across five 28-d experimental periods.

Haugh units, and eggshell thickness, whereas hens fed a 4% CLA diet had lower egg weight ($P < 0.05$), yolk color ($P < 0.01$), yolk weight, and albumen weight ($P < 0.05$) than that of other hens.

It has been reported that the reduction in feed intake, BW gain, rate of egg production, egg weight, and feed efficiency are found with increasing CLA concentration in diets (Ahn et al., 1999; Szymczyk and Pisulewski, 2003; Watkins et al., 2003; Shang et al., 2004) Although, the results of this present study found no significant differences in average daily feed intakes and BW gain, they tended to decrease as the level of CLA increased. As feed consumption is positively correlated to egg weight, BW gain, and yolk weight, its reduction may account for the decrease of egg weight, BW gain, and yolk weight in this experiment. Jones et al. (2000) reported that the rate of egg production was significantly decreased, even at levels as low as 0.5 and 1.0% CLA in the diet.

Egg Yolk Fatty Acid Content

Conjugated linoleic acid supplementation in the diets significantly increased ($P < 0.001$) saturated fatty acids (SFA) and CLA content of egg yolk but significantly reduced monounsaturated and polyunsaturated fatty acid content of egg yolk. Linoleic acid content of egg yolk markedly decreased ($P < 0.001$) with increasing level of CLA addition in the diets. The dietary fatty acid composition affected the yolk fatty acid profile. Raes et al. (2002) found that when diets containing only plant oil without CLA addition were considered, the yolk fat was relatively unsaturated. The amount of SFA did not vary greatly among these diets, yet major differences were present in the 18 unsaturates, particularly 18:2. In contrast to the above research, the present study found significant increases in C14:0, C16:0, and C18:0 but found decreases in C18:2.

Because of the putative benefits of CLA to human health, food products rich in CLA have been extensively investigated in recent years. It has been found that egg yolk is a good carrier of fatty acids (Chamruspollert and

Sell, 1999). Chamruspollert and Sell (1999) found that the CLA concentration of egg yolk lipids increased linearly with increasing dietary CLA. Other studies have achieved similar results (Ahn et al., 1999; Cherian et al., 2002; Szymczyk and Pisulewski, 2003; Shang et al., 2004). It has been found that the greater the supplementation of dietary CLA, the more CLA is deposited in yolk lipids. In this study, the total CLA and its isomers in yolk lipids were increased linearly and quadratically.

The findings of the present study are comparable to the previous study, which found that the *c9*, *t11* CLA isomer accounted for 50 to 65% of the total CLA in yolk lipids (Chamruspollert and Sell, 1999). The concentration of individual CLA isomers in egg yolk lipids does not completely reflect those in diet. As shown in Table 3, the percentages of *c9*, *t11* and *t10*, *c12* in the CLA source were similar at 15 and 15%, respectively, however, their percentages in the yolk lipids differed appreciably at 75 and 25%, respectively. Szymczyk and Pisulewski (2003) reported that the relative proportions of the *c9*, *t11* CLA isomer in egg yolk lipids exceeded those found in the CLA product fed. However, the reason for the *c9*, *t11* isomer being deposited at higher levels in yolk lipids is poorly understood, although it may be connected with the fact that some isomers are more effectively metabolized compared with others (Park et al., 1999). Raes et al. (2002) reported that the deposition rate of the *c9*, *t11* isomer in yolk lipids was higher than that of the *t10*, *c12* isomer.

The results also demonstrated that all fatty acids in yolk lipids were significantly altered by dietary CLA supplementation (Table 4). The concentrations of C14:0, C16:0, and C18:0 in the egg yolk lipids were increased linearly and quadratically, whereas other fatty acids were decreased linearly and quadratically, except for C20:3, C20:4, and PUFA, which were reduced linearly. The results of this study, which combined increased dietary CLA and decreased dietary soybean oil, were significantly enhanced SFA concentration and significantly decreased monounsaturated fatty acid (MUFA) and PUFA concentration of the yolk.

Table 4. Effects of conjugated linoleic acid (CLA) on fatty acid composition of egg yolk

Analyzed composition	Diets (% CLA)					SEM	P-value	
	0	1	2	3	4		Linear	Quadratic
	(% of total fatty acids)							
Myristic acid (C14:0)	0.26 ^e	0.37 ^d	0.43 ^c	0.49 ^b	0.53 ^a	0.0075	0.0001	0.0001
Palmitic acid (C16:0)	22.72 ^e	26.97 ^d	28.24 ^c	29.04 ^b	29.68 ^a	0.2038	0.0001	0.0001
Palmitoleic acid (C16:1)	1.39 ^a	0.80 ^b	0.50 ^c	0.48 ^c	0.44 ^c	0.0374	0.0001	0.0001
Stearic acid (C18:0)	8.83 ^d	12.70 ^c	14.98 ^b	15.31 ^{ab}	15.46 ^a	0.1472	0.0001	0.0001
Oleic acid (C18:1)	34.71 ^a	28.27 ^b	25.79 ^c	25.33 ^c	26.02 ^c	0.2377	0.0001	0.0001
Linoleic acid (C18:2)	26.31 ^a	23.91 ^b	20.26 ^c	16.25 ^d	11.37 ^e	0.1784	0.0001	0.0001
Linolenic acid (C18:3)	1.29 ^a	1.12 ^b	0.93 ^c	0.71 ^d	0.33 ^e	0.0222	0.0001	0.0001
Eicosatrienoic acid (C20:3)	0.19 ^a	0.15 ^b	0.09 ^c	0.03 ^d	0.00 ^e	0.0071	0.0001	0.7432
Arachidonic acid (C20:4)	2.04 ^a	1.82 ^b	1.44 ^c	1.11 ^d	0.90 ^e	0.0353	0.0001	0.5445
Docosahexaenoic acid (C22:6)	1.98 ^a	1.32 ^b	0.92 ^c	0.70 ^d	0.58 ^d	0.0463	0.0001	0.0001
CLA <i>cis</i> -9, <i>trans</i> 11	0.00 ^e	1.67 ^d	4.33 ^c	6.98 ^b	9.63 ^a	0.0523	0.0001	0.0001
CLA <i>trans</i> 10, <i>cis</i> -12	0.00 ^e	0.40 ^d	1.50 ^c	2.73 ^b	4.02 ^a	0.0263	0.0001	0.0001
CLA <i>cis</i> -9, <i>cis</i> -11	0.00 ^c	0.00 ^c	0.00 ^c	0.14 ^b	0.22 ^a	0.0096	0.0001	0.0001
CLA <i>trans</i> 9, <i>trans</i> 11	0.01 ^d	0.02 ^d	0.15 ^c	0.21 ^b	0.28 ^a	0.0092	0.0001	0.1106
SFA ¹	31.81 ^d	40.03 ^c	43.66 ^b	44.84 ^a	45.68 ^a	0.3049	0.0001	0.0001
MUFA ¹	36.10 ^a	29.07 ^b	26.46 ^c	26.49 ^c	25.81 ^c	0.2333	0.0001	0.0001
PUFA ¹	31.82 ^a	30.40 ^b	29.63 ^c	28.86 ^d	27.34 ^e	0.2261	0.0001	0.8200
Total CLA ¹	0.01 ^e	2.08 ^d	5.98 ^c	10.05 ^b	14.15 ^a	0.0747	0.0001	0.0001

^{a-e}Values with no common superscript differ significantly ($P < 0.01$) when tested with Duncan's multiple range test.

¹Saturated fatty acids (SFA) = C14:0, C16:0, and C18:0; monounsaturated fatty acids (MUFA) = C16:1 and C18:1; polyunsaturated fatty acids (PUFA) = C18:2, C18:3, C20:4, C22:6; and CLA = 50% *c9,t11*, 50% *t10,c12*.

Fatty Acid Metabolism

The yolk fatty acid profile clearly reflected the dietary fatty acid composition. However, the unsaturated fatty acids:SFA ratio was more resistant to dietary manipulations than individual fatty acids. Analogous findings were reported by Huyghebaert et al. (1991) and Vahl et al. (1991). The effect of dietary CLA on the fatty acid composition of egg yolks has been extensively studied. Ahn et al. (1999) found that the concentration of SFA increased, whereas MUFA tended to decrease in yolk lipids of hens fed CLA compared with yolks from hens fed a control diet. Similar findings were also reported (Schafer et al., 2001; Cherian et al., 2002; Yang et al., 2002; Szymczyk and Pisulewski, 2003). Changes in SFA and MUFA may be due to the inhibition of the Δ^9 -desaturase (stearoyl-coenzyme A desaturase) enzyme system in the liver caused by CLA. Park et al. (1999) observed that hepatic stearoyl-coenzyme A desaturase was inhibited by *t10, c12* CLA and its derivatives in mice. Supportive evidence for this concept can be found in the study of Choi et al. (2000), which found the *t10, c12* isomers of CLA downregulated the activity of stearoyl-coenzyme A desaturase enzyme of mice. Furthermore, Shang et al. (2004) observed that supplementation of CLA in diets resulted in a dose-dependent decrease in the mRNA transcription and activity of the stearoyl-coenzyme A desaturase in the livers of laying hens. The Δ^9 -desaturase enzyme catalyzes the addition of a double bond at the ninth position of a SFA. If this enzyme is inhibited by CLA, MUFA would be decreased, and SFA would be increased.

The increase of SFA concentration in egg yolk is a potential human health concern, because it may increase blood cholesterol content and increase the incidence of

atherosclerosis. Humans eating CLA-supplemented eggs would consume increased amounts of SFA. Moreover, increased SFA intake increases cholesterol synthesis and plasma cholesterol concentrations, whereas the effect of dietary cholesterol is equivocal and dependent on dietary SFA intake. Reductions in dietary cholesterol intake do not remove health risks due to increased dietary SFA intake. However, if the antiatherogenic activity of CLA found in rabbits (Lee et al., 1994), hamsters (Nicolosi et al., 1997), and mice (Munday et al., 1999) could be measured in humans, the adverse consequences of increased SFA level could be counteracted by CLA. In addition, it has been reported that the cholesterol content in egg yolk was significantly reduced by dietary CLA (Sun et al., 2003). Therefore, the concerns about the increase of SFA appear to be unfounded.

Raes et al. (2002) found that the effects of CLA supplementation on the PUFA contents varied for the different fatty acids and was also diet-dependent for some fatty acids. They also found that no effect of CLA was observed on the amount of linoleic acid, whereas it clearly decreased the arachidonic acid (C20:4) level in the egg yolk. The other fatty acids showed a tendency to decrease by supplementing CLA. However, the present study found reduction in all PUFA present in egg yolk. A reduction in PUFA could be due to inhibition of Δ^6 -desaturase, the rate-limiting step in the conversion of linoleic and linolenic into C20:4. However, if CLA is a competitive inhibitor of Δ^6 -desaturase, an accumulation of linoleic and linolenic acids should be observed. A decrease in docosahexaenoic acid (C22:6) concentration in the yolk lipids due to feeding CLA was also found by Chamruspollert and Sell (1999), although in combination with a much wider change in dietary linolenic acid levels. This suggests that the CLA isomers may also inhibit

the enzyme responsible for this conversion (i.e., $\Delta 4$ -desaturase). The possible mechanism behind this effect was not clear.

In the present study, replacing soybean oil in the basal diet with the CLA source linearly decreased the concentration of linoleic acid in egg yolk lipids. This result was expected, because fatty acid composition of yolk lipids generally reflected the different combinations of CLA and soybean oil concentrations included in the diet (Ahn et al., 1999; Chamruspollert and Sell, 1999; Sun et al., 2003). In summary, dietary CLA supplementation was shown to increase the relative amount of CLA present in egg yolk fatty acids. This incorporation of CLA isomers into egg yolk lipids implies that CLA supplementation of layer diet could provide value-added healthful animal products for human consumption.

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