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The Influence of Conjugated Linoleic Acid Enriched Tallow on Egg Hatchability and Quality in Chicken

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Abstract: Dietary conjugated linoleic acid (CLA) was shown to increase the level of saturated fatty acids in egg yolk, alter interior egg quality, and induce embryo mortality in fertile eggs. Since CLA may soon be used as an animal feed supplement, the objective of this study was to determine if feeding chickens tallow from beef cattle fed a CLA-supplemented diet would induce embryo mortality and cause changes in egg quality. Single Comb White Leghorn laying hens (10 per treatment) were fed a diet containing 0.5% canola oil (CO), 0.5% CLA (CLA), 10% regular tallow (RT), 9.5% regular tallow plus 0.5% CLA (RT+CLA), 10% tallow from beef cattle fed 1% CLA (TCLA1), or 10% tallow from beef cattle fed 2.5% CLA (TCLA2.5) for 18 d. For hatchability studies, hens were artificially inseminated weekly. Eggs were collected daily, stored at 15°C for 24 h, and then incubated. Twenty eggs per treatment were stored at 4°C for 30 d and analyzed for pH. After 18 d of feeding, 3 hens per group were euthanized with carbon dioxide exposure and liver and adipose tissue samples were obtained. Fat from yolk, liver and adipose tissue were extracted for fatty acid analysis. After the 8th d of feeding, embryo mortality was 100% in CLA group versus 7, 9, 5, 6, and 7% in CO, RT, RT+CLA, TCLA1, and TCLA2.5, respectively. Relative CLA levels (% of fatty acids) of yolk from CO, CLA, RT, RT+CLA, TCLA1, and TCLA2.5 were 0, 2.31, 0.99, 1.67, 1.18, and 1.35%, respectively. Diets containing CLA and RT+CLA resulted in increased C16:0 and C18:0 and decreased C16:1(n-7) and C18:1(n-9) in the egg yolk. The CLA but not TCLA1 or TCLA2.5 diet resulted in decreased levels of C20:4(n-6) in yolk and liver compared to CO. Yolk pH increased and albumen pH decreased in the eggs from the CLA relative to CO (8.09 and 8.63 versus 6.13 and 9.04, respectively). Abnormal pH changes did not develop in the eggs from CO, RT, RT+CLA, TCLA1 and TCLA2.5. These results showed that tallow from beef cattle fed CLA or tallow supplemented with CLA had no adverse effects on hatchability and egg quality.

Key words: Tallow, conjugated linoleic acid, CLA, fatty acid content, embryo mortality, egg quality

Introduction

Conjugated linoleic acid (CLA) is the term for a group of positional and geometrical isomers of linoleic acid, an essential fatty acid for human and animal nutrition. CLA was shown to have anticarcinogenic (Ha *et al*., 1990; Ip *et al*., 1994), antiatherogenic (Lee *et al*., 1994; Nicolosi *et al*., 1997) and immune modulating activity (Cook *et al*., 1993) in animal models. CLA also was shown to reduce body fat content and increase lean body mass in animal models (Dugan *et al*., 1997; Park *et al*., 1997). Dietary sources of CLA are mainly meat and dairy products of ruminant animals. Rumen bacteria (i.e. *Butyrovibrio fibrosolvens*) produce CLA as a stable first intermediate (Parodi *et al*., 1997).

Research has demonstrated means of enriching the CLA content of animals and their products. Feeding a CLA mixture caused mainly c-9, t-11 CLA and t-10, c-12 CLA isomers to be incorporated into liver, heart, backfat, and omental fat of pigs (Kramer *et al*., 1998). When dairy cattle were fed a diet supplemented with oils high in polyunsaturated fatty acids, the level of milk CLA was increased significantly (Kelly *et al*., 1998). Compared to meat and dairy products from ruminant animals, eggs and meat from poultry contain far less CLA. Laying hens fed a diet supplemented with CLA had enriched levels of CLA in egg yolks (Chamruspollert and Sell, 1999). However, CLA in low-fat diets increased the level of saturated fatty acids (SFA) (mainly C16:0 and C18:0) and decreased the levels of monounsaturated fatty acids (MUFA) [mainly C16:1(n-7) and C18:1(n-9)] presumably in liver due to an inhibitory effect of the t-10, c-12 CLA isomer on stearoyl-CoA desaturase enzyme (Lee *et al*., 1998). Thus; the increase in SFA resulted in increased yolk firmness during cold storage and discoloration of egg yolk and albumen (Ahn *et al*., 1999; Aydin *et al*., 2001). In addition to adverse effects on the quality of eggs, dietary CLA was demonstrated to reduce hatchability in fertile eggs (Aydin, 2000; Aydin *et al*., 2001). Since rendered feed grade fat from animals enriched or fed CLA could enter feed grade fat stocks for breeding and laying chicken diets, our objective was to determine if feeding chickens CLA-enriched tallow

Table 1: Composition of experimental diets[1,2]

	Dietary Treatments (g/100g)					
Ingredients	CO	CLA	[3]RT	RT+CLA	[4]TCLA1	[5]TCLA2.5
Corn	68	68	24.3	24.3	24.3	24.3
Wheat middlings	0	0	42.3	42.3	42.3	42.3
Soybean meal (44%CP)	20.4	20.4	12.5	12.5	12.5	12.5
CO	0.5	0	0	0	0	0
CLA	0	0.5	0	0.5	0	0
Regular tallow	0	0	10	9.5	0	0
TCLA[1]	0	0	0	0	10	0
TCLA[2.5]	0	0	0	0	0	10
Calcium carbonate	8.3	8.3	8.7	8.7	8.7	8.7
Dicalcium phosphate	1.23	1.23	0.6	0.6	0.6	0.6
DL-Methionine	0.07	0.07	0.1	0.1	0.1	0.1
Sodium chloride	0.5	0.5	0.5	0.5	0.5	0.5
Premix[6]	1.0	1.0	1.0	1.0	1.0	1.0
Final Diet CLA						
c-9, t-11 CLA	0	0.18	0.36	0.52	0.34	0.78
t-10, c-12 CLA	0	0.18	0.15	0.33	0.20	0.24

[1]Diets were isonitrogenous and isocaloric and calculated to contain 15% CP and 2800 kcal/kg ME.
[2]Diets: CO (0.5% canola oil); CLA (0.5% CLA-80); RT (10% regular tallow); RT+CLA (0.5% CLA-80 plus 9.5% regular tallow); TCLA1 (10% tallow from beef cattle fed 1% CLA-60 salt); TCLA2.5 (10% tallow from beef cattle fed 2.5% CLA-60 salt).
[3]RT consisted of 3.09% C14:0; 25.63% C16:0; 2.34% C16:1(n-7); 20.52% C18:0; 35.37% C18:1(n-9); 2.96% C18:2(n-6); 0.26%α C18:3(n-3); 0.06% C20:4(n-6); 0.36% c-9, t-11 CLA; 0.15% t-10, c-12 CLA; 9.26% other fatty acids.
[4]TCLA1 consisted of 3.74% C14:0; 26.73% C16:0; 1.85% C16:1(n-7); 25.02% C18:0; 32.78% C18:1(n-9); 1.23% C18:2(n-6); 0.11% α C18:3(n-3); 0.34% c-9, t-11 CLA; 0.20% t-10, c-12 CLA; 8% other fatty acids.
[5]TCLA2.5 consisted of 2.74% C14:0; 23.80% C16:0; 0.97% C16:1(n-7); 26.07% C18:0; 25.33% C18:1(n-9); 2.42% C18:2(n-6); 0.18% α C18:3(n-3); 0.01% C20:4(n-6); 0.78% c-9, t-11 CLA; 0.24% t-10, c-12 CLA and 17.46% other fatty acids.
[6]Supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D_3, 9790 IU; vitamin E, 121 IU; B_{12}, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60mg.

caused adverse effects on egg quality and hatchability.

Materials and Methods

Animal care and dietary treatments: All procedures involving animals were approved by the University of Wisconsin Animal Care and Use Committee. Sixty 40-wk-old Single Comb White Leghorn (SCWL) laying hens (10 per treatment) were randomly assigned to six diets containing 0.5% canola oil (CO[1]), 0.5% CLA (CLA-80[2]), 10% regular tallow (RT), 0.5% CLA-80 plus 9.5% regular tallow (RT+CLA), 10% tallow from beef cattle fed 1% CLA-salt (TCLA1), or 10% tallow from beef cattle fed 2.5% CLA-salt (TCLA2.5). Table 1 shows the composition of the diets. Experimental diets were calculated to be isonitrogenous and isocaloric. To maintain isocaloric diets in the presence of high levels of tallow, wheat middlings was used as a diluent. Previous work has demonstrated that feeding hens up to 89% wheat middlings has no adverse effects on egg quality as has been shown by feeding CLA (Patterson *et al.*, 1988). The experimental diets were fed for 18 d. For the feeding study, tallow from beef cattle fed 1% CLA-60[3] (TCLA1) and 2.5% CLA-60 (TCLA-2.5) were donated by Allen Trenkle (Iowa State University, USA).

The laying hens had free access to feed and water and were exposed to a daily light period of 16 h. All laying hens were artificially inseminated weekly with 0.05 ml of pooled semen collected from New Hampshire roosters immediately prior to insemination. The number of eggs and egg weights were recorded daily. Eggs were held at 15°C for 24 h, and then incubated. After 18 d of incubation, all eggs were transferred into hatching trays. Eggs were candled weekly to detect fertility and embryo death. Hatchability was computed as a percentage of total fertile eggs on each d.

Analysis of fatty acid composition and ph measurements: On the 8th d of treatment feeding, three fresh eggs from each group were separated into egg yolk and albumen and egg yolks were stored at -20°C until fatty acid analysis. After 18 d of feeding, 3 laying hens per treatment were euthanized by carbon dioxide exposure. Liver and abdominal adipose tissue samples were obtained post mortem and stored at -20°C until fatty acid analysis.

Fat from egg yolk, liver, and abdominal adipose tissue was extracted with chloroform:methanol (2:1 v/v) (Folch *et al.*, 1957). Fatty acid methyl esters (FAME) were prepared by reaction with 4% HCl in methanol for 20 min at 60°C and the composition of the FAME was

Table 2: Effect of experimental diets[1] on fatty acid composition of liver lipids[2]

	Dietary Treatments (%)						
Fatty Acid	CO	CLA	RT	RT+CLA	TCLA[1]	TCLA[2.5]	SEM
C14:0	0.80^{b}	1.21^{a}	0.59^{c}	0.74^{bc}	0.78^{b}	0.72^{bc}	0.06
C16:0	29.08^{b}	36.78^{a}	26.20^{cd}	28.13^{bc}	26.48^{cd}	25.84^{d}	0.80
C16:1(n-7)	3.75^{a}	2.40b^{c}	2.55^{bc}	1.68^{c}	3.13^{ab}	3.06^{ab}	0.36
C18:0	11.64^{d}	17.23^{a}	13.26^{cd}	15.34^{b}	13.15^{cd}	14.44^{bc}	0.64
C18:1(n-9)	42.55^{a}	28.27^{e}	37.51^{bc}	35.32^{cd}	38.74^{b}	34.07^{d}	0.85
C18:2(n-6)	10.14^{c}	11.29^{bc}	13.91^{ab}	14.36^{a}	13.10^{ab}	15.03^{a}	0.73
aC18:3(n-3)	0.20^{c}	0.22^{bc}	0.26^{b}	0.30^{a}	0.32^{a}	0.32^{a}	0.01
C20:4(n-6)	1.68^{cd}	0.73^{d}	4.86^{a}	2.95^{bc}	3.31^{b}	5.30^{a}	0.42
c-9, t-11 CLA	0.16^{d}	1.15^{a}	0.53^{c}	0.74^{b}	0.59^{bc}	0.77^{b}	0.04
t-10, c-12 CLA	nd	0.37	0.14	0.24	0.19	0.23	0.02
OtherCLA isomers	nd	0.35	0.19	0.20	0.21	0.22	0.04
SCLA	0.16^{c}	1.87^{a}	0.86^{b}	1.18^{ab}	0.99^{ab}	1.22^{ab}	0.31
SMUFA	46.3^{a}	30.67^{d}	40.06^{bc}	37.00^{c}	41.87^{b}	37.13^{c}	1.01
SPUFA	12.02^{c}	12.24^{c}	19.03^{ab}	17.61^{ab}	16.73^{b}	20.65^{a}	1.16
SSFA	41.52^{bc}	55.22^{a}	40.05^{d}	44.21^{b}	40.41^{cd}	41.00^{c}	0.89

[1]Diets: CO = 0.5% canola oil; CLA = 0.5% conjugated linoleic acid; RT = 10% regular tallow; CLA+RT = 0.5% CLA plus 9.5% regular tallow, TCLA1 =10% tallow from beef cattle fed 1% CLA60, TCLA2.5 = 10% tallow from beef cattle fed 2.5% CLA-60.

[2]Liver samples of 3 laying hens from each group were used for fatty acid analysis.

a, b, c Means within a row lacking a common superscript differ ($P < 0.05$)

Abbreviations: ΣCLA= total conjugated linoleic acid; ΣSFA = total saturated fatty acids; ΣMUFA = total monounsaturated fatty acids; ΣPUFA = total polyunsaturated fatty acids and do not include CLA-isomers; nd= not detectable.

determined by gas chromatography (GC). Briefly, a Hewlett-Packard 5890 series II GC was fitted with a flame-ionization detector and 3396A integrator (Hewlett-Packard, Andover, MA). A supelcovax-10 fused silica capillary column (60mX0.32mm i.d., 0.25µm film thickness) was used (Sigma-Aldrich, St. Louis, MO). Oven temperature was programmed from 50 to 200°C, increased 20°C per min., held for 50 min, increased 10°C per min to 230°C, and held for 20 min. Heptadecanoic acid (Sigma Chemical Co., St Louis, MO) was used as an internal standard. The FAME were identified by comparison of retention times with methylated fatty acid standards (Sigma Chemical Co., St Louis, MO and Nu-Chek Prep., Elysian, MN) and expressed as percentage of total FAME (Chin *et al.*, 1992).

Twenty eggs from each treatment on the 16th, 17th and 18th d of feeding were obtained and stored at 4°C for 30 d, and then yolk and albumen of the eggs were separated. After noting any observed discoloration in those eggs (data not shown), pH of the egg yolks and albumen was measured. For yolk and albumen pH measurements, the albumen and yolk samples were stirred with a glass rod during pH measurements [Fisher Scientific Accumet pH meter (910), Pittsburgh, PA].

Statistical analysis: For statistical analysis of hatchability data, time in the study was divided into two periods (First: d 1-8; Second: d 9-15) and two parameters (slope and intercept) were examined by t-test in any two regression lines. Different slope or intercept indicated that two treatments were different. A statistical analysis of fatty acid content and pH of yolk and albumen was performed by one-way ANOVA using the General Linear Models procedure of SAS (SAS Institute, 1994). In cases in which the overall effect was significant ($P<0.05$), multiple and pair-wise comparisons were made through Fisher's least significant difference (LSD) procedure.

Results and Discussion

Fig. 1 represents the effects of dietary treatments on hatchability (%) of fertile eggs. Similar to previous studies (Aydin *et al.*, 2001), embryo mortality reached 100% within 8 d of feeding CLA to laying hens. Average hatchability (%) of eggs over the 16 d of feeding period for the treatments CO, RT, RT+CLA, TCLA1, and TCLA2.5 was not statistically different at $P < 0.05$ (93, 91, 95, 94 and 93%, respectively). In the present study, when CLA was added to a diet containing 9.5% tallow, CLA-related embryo mortality was not observed. Tallow from beef cattle fed CLA (TCLA1 or TCLA2.5) had no adverse effect on egg hatchability (Fig. 1). Previously, it was shown that CLA added to diets with 10% olive oil did not cause any adverse effects on egg hatchability (Aydin *et al.*, 2001). A fertile egg could be enriched to 5.3% CLA (as % of fatty acids) by using a combination of CLA and vegetable oil (2% CLA plus 4% canola oil in the diet) without negatively influencing hatchability of fertile eggs (Aydin, 2000). When chickens were fed a diet containing high levels of oil, *de novo* fatty acid synthesis was

Table 3: Effect of experimental diets[1] on fatty acid composition of egg yolk lipid[2]

	Dietary Treatments (%)						
Fatty Acid	CO	CLA	RT	RT+CLA	TCLA[1]	TCLA[2.5]	SEM
C14:0	0.54^{c}	0.94^{a}	0.70^{bc}	0.67^{c}	0.83^{ab}	0.61^{c}	0.05
C16:0	28.43^{b}	36.74^{a}	24.49^{c}	27.92^{b}	25.60^{c}	25.98^{c}	0.46
C16:1(n-7)	3.13^{a}	1.72^{cd}	2.19^{bc}	1.38^{d}	2.40^{b}	1.73^{cd}	0.16
C18:0	10.56^{cd}	17.63^{a}	9.53^{d}	13.80^{b}	9.43^{d}	11.43^{c}	0.41
C18:1(n-9)	41.86^{bc}	25.96^{d}	46.42^{a}	38.88^{c}	44.90^{ab}	41.33^{c}	1.05
C18:2(n-6)	13.30	13.22	13.6	13.77	13.59	14.90	0.55
aC18:3(n-3)	0.36^{ab}	0.39^{a}	0.30^{c}	0.34^{abc}	0.35^{abc}	0.34^{bc}	0.02
C20:4(n-6)	1.82^{ab}	1.09^{c}	1.78^{b}	1.57^{b}	1.72^{b}	2.33^{a}	0.13
c-9, t-11 CLA	nd	1.35^{a}	0.49^{c}	0.98^{b}	0.46^{c}	0.48^{c}	0.04
t-10, c-12 CLA	nd	0.54^{a}	0.13^{d}	0.26^{bc}	0.19^{cd}	0.35^{b}	0.03
OtherCLA isomers	nd	0.42	0.37	0.43	0.53	0.52	0.1
SCLA	nd	2.31^{a}	0.99^{d}	1.67^{b}	1.18^{cd}	1.35^{c}	0.08
SMUFA	44.99^{a}	27.68^{c}	48.61^{a}	40.26^{b}	47.30^{b}	43.06^{a}	0.96
SPUFA	15.48^{c}	14.70^{ab}	15.68^{c}	15.68^{bc}	15.66^{c}	17.57^{a}	0.61
SSFA	39.53^{c}	55.31^{a}	34.72^{e}	42.39^{b}	35.86^{de}	38.02^{d}	0.60

[1]Diets: CO = 0.5% canola oil; CLA = 0.5% conjugated linoleic acid; RT = 10% regular tallow; CLA+RT = 0.5% CLA plus 9.5% regular tallow, TCLA1=10% tallow from beef cattle fed 1% CLA-60, TCLA2.5 =10% tallow from beef cattle fed 2.5% CLA-60.

[2]Yolk samples of 3 eggs from each group were used for fatty acid analysis.

a, b, c Means with different superscripts within the same row are significantly different ($P < 0.05$).

Abbreviations: ΣCLA = total conjugated linoleic acid; ΣSFA = total saturated fatty acids; ΣMUFA = total monounsaturated fatty acids; ΣPUFA = total polyunsaturated fatty acids and do not include CLA-isomers; nd = not detectable.

reduced (Naber and Biggert, 1989). When the fats are provided as a dietary constituent, they are directly deposited in the egg yolk and resulting fatty acid composition of the egg that resembles the fatty acid composition of the dietary fatty acids. Hence, it is possible that the reason CLA did not influence hatchability when added to tallow or when originating from tallow of CLA-fed animals could be due to the influences of other dietary fatty acids. Tallow from beef cattle fed CLA is unlikely to have adverse effects on egg hatchability within the constraints of this experiment. Since the CLA content of tallow is not high enough, we do not expect any adverse effects on embryo mortality in the fertile eggs. While this might be true, the tallow from beef cattle fed CLA did not appear to affect hatchability even when CLA was added (Fig. 1).

The effect of dietary treatments on the fatty acid composition of liver is shown in Table 2. Dietary CLA resulted in an increased level of C14:0, C16:0, and C18:0 and a decreased level of C16:1(n-7) and C18:1(n-9) in the liver. Feeding the RT diet caused significant decreases in the levels of C14:0, C16:0, and C18:1(n-9) and caused increases in C18:2(n-6) and C20:4(n-6) levels of liver compared to CO. However, the RT diet had no effect on the level of C18:0 in liver. Feeding tallow with 0.5% CLA (RT+CLA) had no effect on C14:0 and C16:0, but significantly increased C18:0 and C18:2(n-6) while it decreased the levels of C16:1(n-7) and C18:1(n-9) of liver. Livers from laying hens fed TCLA1 had similar levels of C14:0, C16:1(n-7) and C18:0, but lower levels of C16:0 and C18:1(n-9) compared to CO. Although tallow (including RT) used in the present study contained little or no C20:4(n-6), the tallow treatments (RT, TCLA1, and TCLA2.5) significantly had higher level of C20:4(n-6) in the liver compared to the CO ($P<0.05$). Liver can synthesize C20:4(n-6) from C18:2(n-6), which is the precursor of C20:4(n-6), by desaturation and elongation reactions. However, the CLA group actually decreased the level of C20:4(n-6) compared to CO ($P<0.05$). This may be due to the inhibitory effects of the CLA on the activity of Δ-6 desaturase enzyme in the liver. Similarly, dietary CLA also was shown to decrease the level of C20:4(n-6) in mice liver (Belury and Kempa-Steczko, 1997). In the present study, total CLA levels (as % fatty acids) of liver from CLA, RT, RT+CLA, RTCLA1, and TCLA2.5 increased 11.7, 5.4, 7.4, 6.2, and 7.6-fold, respectively, compared to the CO.

Table 3 represents the effects of dietary treatments on the fatty acid composition of egg yolk. In parallel to the fatty acid composition of liver lipids, the levels of C16:0 and C18:0 were significantly increased and C16:1(n-7) and C18:1(n-9) were decreased in the egg yolks from the CLA group compared to CO. Lee *et al.* (1998) showed that the t-10, c-12 CLA isomer (but not c-9, t-11 CLA isomer) inhibited stearoyl-CoA desaturase. Therefore, these results might be due to the inhibitory effect of the t-10, c-12 CLA isomer on the stearoyl-CoA desaturase activity. In the present study, adding tallow to the diet (RT+CLA) completely prevented CLA-related increase in C16:0 and decrease in C18:1(n-9) levels of egg yolk. The increase in total SFA and the decrease in total MUFA were much higher in the CLA group than the

Table 4: Effect of experimental diets[1] on fatty acid composition of abdominal adipose tissue lipids[2]

	Dietary Treatments (%)						
Fatty Acid	CO	CLA	RT	RT+CLA	TCLA1	TCLA2.5	SEM
C14:0	1.03^{d}	1.18^{cd}	1.56^{b}	1.42^{bc}	2.01^{a}	1.64^{b}	0.07
C16:0	21.80^{ab}	22.68^{a}	21.03^{b}	21.01^{b}	20.98^{b}	20.45^{b}	0.52
C16:1(n-7)	3.06^{a}	2.28^{c}	2.37^{c}	2.69^{b}	2.55^{bc}	1.66^{d}	0.10
C18:0	7.26^{c}	11.03^{b}	11.73^{ab}	9.73^{b}	11.43^{b}	14.09^{a}	0.71
C18:1(n-9)	43.04^{ab}	38.78^{c}	44.45^{a}	44.26^{a}	42.89^{ab}	41.74^{bc}	0.67
C18:2(n-6)	22.90^{a}	21.63^{a}	17.61^{b}	19.14^{b}	18.21^{b}	18.70^{b}	0.72
aC18:3(n-3)	0.86^{a}	0.62^{b}	0.64^{b}	0.64^{b}	0.69^{b}	0.61^{b}	0.03
C20:4(n-6)	0.05	0.06	0.04	0.05	0.05	0.08	0.02
c-9, t-11 CLA	nd	1.18^{a}	0.37^{c}	0.70^{b}	0.79^{b}	0.62^{bc}	0.1
t-10, c-12 CLA	nd	0.33^{a}	0.13^{c}	0.19^{b}	0.20^{b}	0.24^{b}	0.03
OtherCLA isomers	nd	0.23^{a}	0.07^{c}	0.17^{b}	0.20^{ab}	0.17^{b}	0.05
SCLA	nd	1.74^{a}	0.57^{b}	1.06^{c}	1.19^{c}	1.03^{c}	0.06
SMUFA	46.10^{a}	41.06^{b}	46.82^{a}	46.95^{a}	45.44^{a}	43.40^{b}	0.69
SPUFA	23.81^{a}	22.31^{a}	18.29^{b}	19.83^{b}	18.95^{b}	19.39^{b}	0.74
SSFA	30.09^{a}	34.89^{b}	34.32^{b}	32.16^{b}	34.42^{b}	36.18^{b}	1.05

[1]Diets: CO = 0.5% canola oil; CLA = 0.5% conjugated linoleic acid; RT = 10% regular tallow; CLA+RT = 0.5% CLA plus 9.5% regular tallow, TCLA1=10% tallow from beef cattle fed 1% CLA-60 salt, TCLA2.5 =10% tallow from beef cattle fed 2.5% CLA-60 salt.[2]Samples of 3 adipose tissues from each group were used for fatty acid analysis.

a, b, c Means with different superscripts within the same row are significantly different ($P < 0.05$).

Abbreviations: ΣCLA = total conjugated linoleic acid; ΣSFA = total saturated fatty acids; ΣMUFA = total monounsaturated fatty acids; SPUFA = total polyunsaturated fatty acids and do not include CLA-isomers; nd = not detectable.

RT+CLA group ($P < 0.05$). The level of C16:1(n-7) was significantly lower in all groups compared to CO. The levels of C18:0 and C18:1(n-9) in eggs from hens fed TCLA1 and TCLA2.5 did not differ from CO. In addition, egg yolk from laying hens fed TCLA1 and TCLA2.5 had less C16:0 level compared to CO ($P<0.05$). Dietary CLA alone significantly decreased the level of C20:4(n-6) in egg yolk relative to the group CO ($P<0.05$). The level of C20:4 (n-6) was decreased significantly in the egg yolks from CLA-fed laying hens with an increase in dietary levels of CLA (Chamruspollert and Sell, 1999). The decrease in the level of C20:4(n-6) in the egg yolk was probably due to decrease in the level of C20:4(n-6) in the liver. In the present study, total CLA (% of fatty acids) levels of egg yolk from CO, CLA, RT, RT+CLA, TCLA1, and TCLA2.5 were 0, 2.31, 0.99, 1.67, 1.18, and 1.35%, respectively. The apparent deposition rate (%) is clearly higher for the c-9, t-11 CLA isomer than for the t-10, c-12 CLA isomers in the treatment.

Table 4 shows the influence of dietary treatments on fatty acid composition of adipose tissue. In contrast to fatty acid composition of both liver and egg yolk lipid, dietary CLA had no effect on the levels of C14:0, C16:0, and C20:4(n-6) of adipose tissue. In parallel to fatty acid composition of liver and egg yolk, CLA decreased the levels of C16:1(n-7) and C18:1(n-9) and increased the level of C18:0 of adipose tissue. Although the level of C18:1(n-9) of adipose tissue was significantly decreased by the CLA group, the relative level (%) of C18:1(n-9) was greater in adipose tissue than in egg yolk and liver. Interestingly, the level of C20:4(n-6) in adipose tissue was barely detectable and far less than that in liver and egg yolk. These findings were also reported by Chamruspollert and Sell (1999). The presence of CLA in a low-fat diet significantly influenced the fatty acid composition of liver, egg yolk, and adipose tissue, but these changes were more pronounced in egg yolk and liver than in adipose tissue.

Table 5 shows the effects of experimental diets on pH values of yolk and albumen of eggs stored at 4°C for 30 d. The CLA treatment caused an increased yolk pH and a decreased albumen pH relative to CO (8.09 and 8.63 versus 6.13 and 9.04, respectively). Feeding RT+CLA, TCLA1 and TCLA2.5 had no negative effect on the pH of yolk and albumen of eggs stored at 4°C for 30 d compared to CO. Under normal conditions, when eggs are stored at 4°C, the content of yolk water increases as a consequence of water migration from albumen (Ahn *et al.*, 1999) resulting in a 1.9% increase in yolk weight. Ahn *et al.* (1999) showed that yolks of eggs from CLA group had higher water content than those from CO. This may partly explain why dietary CLA causes negative effects on the quality (i.e. color defects in yolk and albumen of eggs) of the eggs stored at 4°C (data not shown). Ahn *et al.* (1999) reported that the amount of water in yolk of eggs from CLA-fed hens increased depending on the level of CLA in the diet and storage time. Previously, it was reported that 5.4% increase was observed, due to CLA feeding, in yolk weight (% of egg) compared to the control (Aydin, 2000).

The actual reason for abnormal pH changes of yolk and albumen of the eggs from the CLA group has yet to be

Table 5: Influence of dietary treatments[1] on pH of albumen and yolks of eggs2 stored at 4°C for 30 d

	pH Values	
Dietary Treatments	Albumen	Yolk
CO	9.04[a]	6.13[b]
CLA	8.63[b]	8.09[a]
RT	9.11[a]	6.14[b]
RT+CLA	9.09[a]	6.13[b]
TCLA1	9.12[a]	6.11[b]
TCLA2.5	9.10[a]	6.12[b]
SEM	0.04	0.05

[1]Diets: CO= 0.5% canola oil; CLA = 0.5% conjugated linoleic acid; RT= 10% regular tallow; CLA+RT= 0.5% CLA plus 9.5% regular tallow, TCLA1=10% tallow from beef cattle fed 1% CLA-60, TCLA2.5=10% tallow from beef cattle fed 2.5% CLA-60. [2]Values are means of 20 yolk or albumen samples per treatment.

a, b Means with different superscripts within the same column are significantly different (P < 0.05).

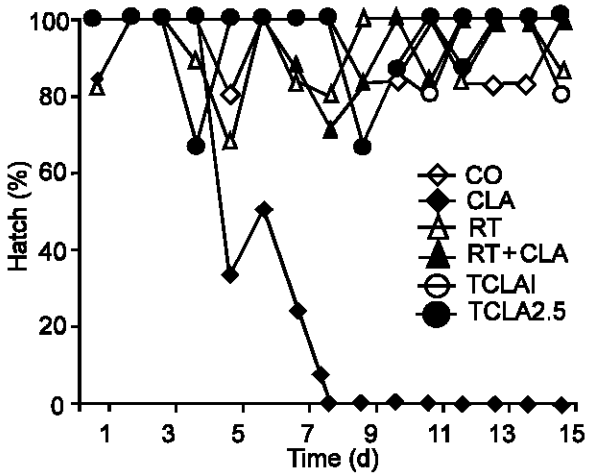

Fig. 1: Hatchability[1] (%) of fertile eggs from laying hens fed dietary treatments[2,3]

[1]Eggs were collected daily, held at 15°C for 24 h and then incubated.

Hatchability (%) was computed as a percentage of total number of fertile eggs that hatched in the treatments on each day.

[2]Diets CO = 0.5% canola oil; CLA = 0.5% conjugated linoleic acid; RT = 10% regular tallow; CLA+RT = 0.5% CLA plus 9.5% regular tallow, TCLA1 = 10% tallow from beef cattle fed 1% CLA-60 salt, TCLA2.5 = 10% tallow from beef cattle fed 2.5% CLA-60 salt.

[3]Only slope of the CLA group was significantly different from other treatments (P < 0.05). CO, RT, RT+CLA, RTCLA1 and RTCLA2.5 were not significantly different from one another.

shown. However, there is evidence that mineral exchange and water movement from albumen to yolk and vice versa play an important role in the abnormal pH changes in the eggs (stored at 4°C) from CLA-fed laying hens (Aydin *et al.*, 2001). In the present study, the increase in yolk pH and the decrease in albumen pH may be related to the migration of minerals and water (Aydin, 2000; Aydin *et al.*, 2001). It is speculated that CLA increases the level of SFA content of the vitelline membrane so that during cold storage temperature the vitelline membrane is disrupted, causing leakage of minerals and water from the albumen into the egg yolk. When hens were fed TCLA2.5, there was also a slight, but significant increase in percent yolk and decrease in percent albumen (Aydin, 2000). While not quality defects were visually seen in eggs from hens fed this dietary treatment (data not shown), these results suggested that these subtle changes might represent a slight adverse effect of feeding tallow from CLA-fed cattle. However, it is unlikely that commercial laying hens will ever be fed tallow at the levels used in the present study. The present study confirmed that CLA in low-fat diets caused significant alteration in the yolk fatty acid composition, increased embryo mortality, and changed the quality of eggs stored at cold temperatures. The present study also showed that CLA incorporated into tallow or tallow from beef cattle fed CLA (TCLA1 and TCLA2.5) had no adverse effects on egg hatchability and quality when fed to laying or breeding hens. This study also suggests that CLA-related color changes observed in yolk and albumen of eggs stored at cold temperature depend on the increase in the ratio of SFA to unsaturated fatty acids (UFA).

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[1]CO was purchased from Sysco Baraboo, Baraboo, WI, USA.

[2]CLA-80 was a gift from Natural Lipids Ltd. AS., Hovdebygda, Norway and consisted of 0.12% C14:0; 6.37% C16:0; 0.11% C16:1(n-7); 2.19% C18:0; 14.88% C18:1(n-9); 1.11% C18:2(n-6); 34.2% c-9, t-11 CLA; 34.21% t-10, c-12 CLA; 6.81% other fatty acids.

[3]CLA-60 was analyzed by Natural Lipids Ltd. AS., Hovdebygda, Norway and used in the feeding of beef cattle as a calcium salt. CLA-60 consisted of 0.16% C14:0; 5.38% C16:0; 0.06% C16:1(n-7); 2.91% C18:0; 20.50% C18:1(n-9); 5.46% C18:2(n-9); 24.14% c-9, t-11 CLA; 19.11% t-10, c-12 CLA and 22.28% others.

Abbreviation Key: CLA= conjugated linoleic acid; MUFA= monounsaturated fatty acids; PUFA= polyunsaturated fatty acids; UFA= unsaturated fatty acids; SFA= saturated fatty acids