Effect of storage temperature on the quality of eggs from conjugated linoleic acid-fed laying hens

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Abstract

This study was designed to investigate the effects of storage temperature on the quality of eggs from conjugated linoleic acid (CLA)-fed laying hens. For 40 days laying hens (20 per group) were fed a diet containing either 0.5% maize oil (Group A, Control) or 0.5% CLA (Group B). For fatty acid analysis, three eggs were collected after the eighth day of feeding. After two days of feeding, eggs were collected and stored for 28 days at 4 ºC, 15 ºC or 24 ºC for colour quality assessment. For pH measurements, 30 eggs from each group were stored at 4 ºC or room temperature (21-24 ºC) for one, two or three weeks. Dietary CLA caused higher levels of C16:0 and C18:0 and lower levels of C16:1(n-7) and C18:1(n-9) compared to the control group. Egg yolk from Group B had higher levels of c-9, t-11 and t-10, c-12 CLA than the control group. The ratio of total saturated to unsaturated fatty acids (SFA/UFA) increased 2.4-fold in the eggs from CLA-fed hens. CLA eggs stored at 4 ºC had higher pH values in yolk and lower pH values in albumen compared to those from the control group. However, pH values of yolk or albumen in eggs stored at 21-24 ºC were similar in the control and treatment groups. Dietary CLA caused significant colour changes in yolk and albumen of the eggs stored at 4 ºC and 15 ºC. No colour changes were observed in the yolk and albumen of CLA eggs at 21-24 ºC. Results indicated that dietary CLA influenced fatty acid composition of eggs and had negative effects on the quality of eggs stored at 4 °C or 15 °C, but not at room temperature (21-24 ºC). It is suggested that CLA probably changed the permeability of the vitelline membrane during cold storage.

Keywords: Conjugated linoleic acid, ratio of SFA/UFA, storage temperature, egg quality
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Introduction

Conjugated linoleic acid, briefly known as CLA**,** is a mixture of geometrical and positional isomers of linoleic acid (C18:2 c-9, c-12) and involves a double bond at positions 8 and 10, 9 and 11, 10 and 12 or 11 and 13 (Eulitz *et al.*, 1999). Each of these positional conjugated diene isomers can occur in cis-trans, trans-cis, cis-cis or trans-trans geometrical configurations. Originally isolated from grilled ground beef, CLA occurs naturally in meat and dairy products from ruminant animals (Ha *et al.*, 1990; Chin *et al*., 1992). The chicken egg contains little or no CLA (Chin *et al.*, 1992).

Since dietary CLA has some biological properties such as anticarcinogenic (Ip *et al.*, 1994), anti-atherogenic (Lee *et al*., 1994; Nicolosi *et al.*, 1997) and immune inducing activities (Cook *et al*., 1993), enhancing the CLA concentration of egg, milk and meat is of interest. The accumulation of CLA in these products is dose and time dependent. Chicken eggs were shown to be enriched with CLA (Chamruspollert & Sell, 1999; Du *et al.*, 1999; Raes *et al.*, 2002; Sun *et al.*, 2003). By feeding laying hens 5% CLA in the diet, an egg could be enriched in CLA as high as 11% (Chamruspollert & Sell, 1999). However, dietary CLA was reported to cause hardening of egg yolk and discoloration in the yolk and albumen of eggs stored at 4 ºC (Aydin *et al.*, 2001). Dietary CLA influences the fatty acid composition of egg yolk in a dose and time-dependent manner (Aydin & Cook, 2004). Dietary CLA was also reported to increase the firmness of hard-cooked egg yolk (Ahn *et al.*, 1999). In a previous study, it was shown that eggs from CLA-fed hens (stored at 4 °C for 10 weeks) had higher iron, calcium and zinc concentrations and lower magnesium, sodium and chloride concentrations in albumen relative to those from laying hens fed maize oil (Aydin *et al.*, 2001). A recent study showed that sodium, potassium and magnesium concentration of the egg yolk increased as the CLA levels increased in a diet (Shang *et al.*, 2004). The textural changes in the eggs from CLA-fed laying hens could be related to an increase of yolk water content, the movement of ions between yolk and albumen or change of egg yolk pH during cold storage (Ahn *et al.*, 1999). When CLA eggs were stored at 4 °C for 10 weeks, abnormal pH changes were observed in the egg yolk and albumen (Aydin *et al.*, 2001). It can be

speculated that CLA increases the saturated fatty acids (SFA) concentration of the vitelline membrane so that, during cold storage, the vitelline membrane is disrupted, causing leakage of minerals and water from the egg albumen into the yolk. Recently, Watkins *et al.* (2003) showed that the level of C18:0 in the vitelline membrane was increased by dietary CLA. A preliminary study showed that when eggs from CLA-fed laying hens were stored at a warmer room temperature, no abnormal colour and pH changes were observed in the yolk and albumen compared to the control group (R. Aydin, unpublished data). Therefore, the objective of this study was to evaluate the effects of storage temperature on the quality of the eggs from laying hens fed CLA.

Materials and Methods

Forty 28-week old Single Comb White Leghorn (SCWL) laying hens were assigned randomly into two groups of 20 hens per group. The hens were kept in individual cages and each hen was considered an experimental unit. They were fed for 40 days on a diet containing either 0.5% maize oil (Group A, control) or 0.5% CLA (Group B). Both diets contained 150 g crude protein and 11.7 MJ metabolisable energy/kg. The ingredient composition of the diets is presented in Table 1. The commercial source of CLA contained 80% CLA and consisted of 35.33% c-9, t-11 and t-9, c-11; 35; 72% t-10, c-12; 1.11% c-9, c-11; 1.57% c-10, c-12; 0.91% t-9, t-11 and t-10, t-12 CLA isomers. Other fatty acids in CLA were 6.64% palmitate, 2.39% stearate, 13.77% oleate, 0.81% linoleate and 1.75% unknown.

For fatty acid analysis, three eggs from each dietary group were collected on the 8th day of feeding. A previous study showed that dietary CLA inclusion of 0.5% could completely modify the fatty acid composition of eggs after 6-8 days of feeding laying hens (Aydin *et al*., 2001). Egg content was separated into yolk and albumen, and the yolk was stored at –20 °C pending fatty acid analysis. The fat from the egg yolk 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 FAME was determined by gas chromatography (Hewlett-Packard 5890). Heptadecanoic acid (C17:0) was used as an internal standard.

Table 1 Ingredients of experimental diets[1] supplemented with 0.5% maize oil or 0.5% CLA

Ingredients	Group A (Basal diet with maize oil)	Group B (Basal diet with CLA)
	g/100 g diet	
Maize grain	67.99	67.99
Soyabean meal (44% CP)	20.39	20.39
Calcium carbonate	8.34	8.34
Dicalcium phosphate	1.21	1.21
DL-Methionine	0.07	0.07
Maize oil	0.5	0
CLA	0	0.5
Salt	0.5	0.5
Vitamin/mineral premix[2]	1.0	1.0

[1] Diets were isonitrogenous and isoenergetic and calculated to contain 150 g crude protein (CP)/kg and 11.7 MJ metabolisable energy/kg

[2] Supplied/kg of diet: Vitamin A - 10,000 IU; vitamin D_3 - 9790 IU; vitamin E - 121 IU; vitamin B_{12} - 20 µg; riboflavin - 4.4 mg; calcium panthothenate - 40 mg; niacin - 22 mg; choline - 840 mg; biotin - 30µg; thiamine - 4 mg; zinc sulphate - 60 mg; manganese oxide - 60 mg

Eggs were examined for interior and exterior quality. Egg quality was assessed by measuring pH and yolk colour of eggs stored at cold or warm temperatures. For the measurements of egg yolk and albumen pH, 30 eggs per group were stored at 4 ºC or 24 ºC for one, two or three weeks (five eggs per each storage temperature and week duration). After noting any observable discoloration in the eggs, the albumen and yolk

samples were stirred separately with a glass rod during pH measurements. Tristimulus colour coordinates (L*, a*, b*) were recorded on the yolk of the eggs stored for 28 days at 4 °C, 15 °C or at room temperature (21-24 °C), using a chromameter (CR-300; Minolta Cameras, Osaka, Japan). The instrument was calibrated, using a white calibration plate (Calibration Plate CR-A43, Minolta Cameras) at the beginning of each session. Thirty eggs per group were collected during the last week of the study and were stored at 4 °C for 30 days. They were then weighed, broken open, and the albumen, yolk and shell were separated and weighed.

Data for fatty acid analysis, egg parameters, and yolk and albumen pH of eggs were analyzed using the general linear models procedure of SPSS, version 10.0 (SPSS Inc. Chicago, USA). The statistical models included the main effects of diet and temperature. Means were considered to be statistically significantly different at $P < 0.05$.

Results

The effect of diets supplemented with 0.5% maize oil or 0.5% CLA on the fatty acid composition of egg yolk is presented in Table 2. Dietary CLA increased the level of SFA (C16:0 and C18:0) and decreased the levels of monounsaturated fatty acids (MUFA; C16:1, n-7 and C18:1, n-9) in the egg yolk. The ratios of C16:0/C16:1(n-7) and C18:0/C18:1(n-9) increased approximately 2.8 and 3.8-fold, respectively, in eggs from the CLA-fed hens compared to the control group. The level of c-9, t-11 CLA isomer was increased 10-fold in eggs from the CLA-fed hens compared to the control group. The isomer, t-10, c-12 CLA, was not detectable in the fat of the control eggs, but fat in eggs from CLA-fed hens contained 0.52%. Dietary CLA decreased the ratio of unsaturated fatty acids/saturated fatty acids (UFA/SFA) significantly in the eggs compared to those from Group A.

Table 2 Fatty acid composition (mean ± s.e.) in egg yolk from laying hens fed diets containing 0.5% maize oil or 0.5% conjugated linoleic acid (CLA)[1]

Fatty Acid	% of Total fatty acids	
	Group A (Basal diet with maize oil)	Group B (Basal diet with CLA)
C10:0	0.06 ± 0.01	0.05 ± 0.01
C12:0	0.10 ± 0.02	0.10 ± 0.02
C14:0	0.61 [b] ± 0.01	1.02 [a] ± 0.01
C16:0	29.65 [b] ± 1.02	40.64 [a] ± 2.01
C16:1(n-7)	4.11 [a] ± 0.21	1.99 [b] ± 0.06
C18:0	8.50 [b] ± 0.25	17.61 [a] ± 1.02
C18:1(n-9)	40.24 [a] ± 0.62	22.23 [b] ± 0.15
C18:2(n-6)	14.62 ± 0.09	13.07 ± 1.06
αC18:3(n-3)	0.15 ± 0.01	0.14 ± 0.01
γC18:3(n-6)	0.54 ± 0.01	0.38 ± 0.00
C20:4(n-6)	1.29 ± 0.02	0.95 ± 0.03
c-9, t-11 CLA	0.13 [b] ± 0.00	1.30 [a] ± 0.01
t-10, c-12 CLA	n.d.	0.52 ± 0.03
ΣSFA	38.92 [b] ± 1.23	59.42 [a] ± 1.73
ΣMUFA	44.35 [a] ± 1.35	24.22 [b] ± 0.08
ΣPUFA	16.60 ± 0.09	14.54 ± 1.47
ΣUFA	60.95 [a] ± 1.22	38.76 [b] ± 1.95

[1] For fatty acid content analysis, the eggs were obtained on the 8th day of feeding experimental diets
[a, b, c] Means (three egg yolks per treatment) without common superscripts in a row are significantly different ($P < 0.05$)
FAME - fatty acid methyl esters; ΣSFA- total saturated fatty acids; ΣMUFA - total monounsaturated fatty acids; ΣPUFA - total polyunsaturated fatty acids (excluding CLA); ΣUFA - total unsaturated fatty acids (excluding CLA)

Table 3 shows the effects of dietary treatment on egg weights and relative proportion of yolk and albumen of eggs stored at 4 ºC for 30 days. The weight of eggs from hens fed CLA was lower ($P < 0.05$) compared to that of the control group group. When the eggs from CLA-fed hens were stored at 4 ºC for 30

days, the proportion (%) of yolk increased ($P < 0.05$) and that of albumen decreased ($P < 0.05$) compared to the control. Shell weight as a proportion of egg weight was not affected by treatment.

Table 3 Effect of including 0.5% maize oil or 0.5% conjugated linoleic acid (CLA) in the diets of hens on the relative weights of the components of their eggs when stored at 4 ºC for 30 days

	Group A (Basal diet with maize oil)	Group B (Basal diet with CLA)	s.e.m.
Whole egg (g)	62.3 [a]	59.6 [b]	0.9
Yolk (%)	29.2 [b]	34.6 [a]	0.4
Albumen (%)	60.6 [a]	55.1 [b]	0.5
Shell (%)	10.2	10.3	0.2

[a, b] Means (30 samples/treatment) with different superscripts within a row are significantly different ($P < 0.05$)
s.e.m. – standard error of mean

Results of the egg quality evaluation are shown in Tables 4 and 5. When eggs were stored at 4 ºC for one, two or three weeks, the pH of egg yolk was higher and that of albumen lower in eggs from the CLA-fed hens compared those to from the control group. However, when the eggs were stored at room temperature (21-24 °C) the pH of the yolk and albumen did not differ between the dietary treatments. The inclusion of CLA in the diet significantly intensified the colour indices of yolks of eggs stored for 28 days at 4 ºC and 15 ºC (Table 5). However, at the warmer temperature (21-24 ºC) dietary CLA had no effect on the colour indices in the yolk. Egg yolk from the maize oil-fed hens had higher b* values, corresponding to a more yellow yolk, compared to the CLA group.

Table 4 Effect of including 0.5% maize oil or 0.5% conjugated linoleic acid (CLA) in the diet of hens on the pH (mean ± s.e.) of the albumen and yolk of their eggs stored at 4 °C or 21-24 °C for 1, 2 or 3 weeks

Weeks	Temperature (°C)	Yolk pH		Albumen pH	
		Group A	Group B	Group A	Group B
1	4	5.73 [b] ± 0.02	6.15 [a] ± 0.03	9.09 [a] ± 0.01	9.01 [b] ± 0.02
2	4	5.73 [b] ± 0.05	6.89 [a] ± 0.10	9.09 [a] ± 0.05	8.89 [b] ± 0.06
3	4	5.89 [b] ± 0.01	7.22 [a] ± 0.08	9.25 [a] ± 0.06	8.83 [b] ± 0.08
1	21-24	6.00 ± 0.03	6.05 ± 0.05	9.15 ± 0.06	9.10 ± 0.06
2	21-24	6.05 ± 0.03	6.08 ± 0.06	9.20 ± 0.03	9.24 ± 0.05
3	21-24	6.28 ± 0.04	6.30 ± 0.08	9.38 ± 0.08	9.43 ± 0.06

[a,b] Means with different superscripts within a row period differ at $P < 0.05$

Table 5 Effect of including 0.5% maize oil (Group A) or 0.5% conjugated linoleic acid (CLA) (Group B) in the diet of hens on the quality of their eggs stored for 28 days at different temperatures[1]

	Temperature (°C)	L* value	a* value	b* value
Group A	4	71.03 [a] ± 0.31	-4.88 [a] ± 0.01	59.82 [a] ± 0.28
Group B	4	59.08 [b] ± 0.76	-3.44 [b] ± 0.11	38.74 [b] ± 0.56
Group A	15	70.82 [a] ± 0.36	-4.87 [a] ± 0.01	59.83 [a] ± 0.16
Group B	15	59.99 [b] ± 0.65	-3.36 [b] ± 0.05	37.27 [b] ± 0.86
Group A	21-24	70.53 ± 0.21	-4.86 ± 0.00	59.55 ± 0.32
Group B	21-24	70.66 ± 0.12	-4.84 ± 0.02	58.57 ± 0.67

[1] L*= lightness (higher values indicate a lighter colour); a* = redness (higher values indicate a more red colour); b* = yellowness (higher values indicate a more yellow colour)
[a, b] Means were derived from three readings per sample for 10 egg yolks per group and means within columns with no common superscript differ significantly at $P < 0.05$

Discussion

Dietary CLA was shown to influence fatty acid composition of eggs and to cause adverse effects on the quality of eggs stored at 4 °C (Aydin *et al*., 2001). CLA in a low-fat diet increased the level of SFA (mainly C16:0 and C18:0) and decreased the level of MUFA (mainly C16:1, n-7 and C18:1, n-9) in the egg yolk. Similarly, in the present study CLA in a low-fat diet (Group B) increased the ratio of SFA to UFA significantly. Eggs from the CLA group had 10-fold higher c-9, t-11 CLA concentrations compared to the control group. The level of t-10, c-12 CLA isomer in the eggs from the CLA-fed hens was 0.52, but it was not detectable in eggs from the Group A. When the eggs from CLA-fed laying hens were stored at 4 °C, abnormal colour and pH changes occurred (Aydin *et al.*, 2001). It was reported that there was an apparent migration of minerals between the yolk and albumen of eggs from hens fed a diet supplemented with 0.5% CLA when eggs were stored in 4 ºC for 10 weeks. CLA eggs had higher iron, calcium and zinc concentrations and lower magnesium, sodium and chloride concentrations in albumen relative to those from laying hens fed the control diet (Aydin *et al.*, 2001). Recently, Shang *et al.* (2004) showed that there was a linear increase in the levels of sodium, potassium, and magnesium of the yolk of the eggs stored at 4 °C for 28 days. Interestingly, the colour changes seen in yolk and albumen were only associated with cold storage temperatures. In the present study no abnormal colour changes were observed in the eggs from the CLA-fed laying hens when they were stored at the warmer temperatures. The actual cause for discoloration of yolk and albumen of eggs at cold temperature has yet to be determined, but is believed to be the result of yolk hardening during cold storage which consequently results in a disrupted vitelline membrane (Abo-ashour & Edwards, 1970). A pink discoloration of the albumen may develop due to the diffusion of iron, stored in the yolk, to the albumen where it binds ovotransferin (Bandemer & Schaible, 1946). A recent study showed that dietary CLA increased the level of C18:0 in the vitelline membrane (Watkins *et al.*, 2003). The CLA isomer primarily involved in these adverse effects is thought to be the t-10, c-12 CLA isomer. Lee *et al*. (1998) showed that the t-10, c-12 CLA inhibited liver stearoyl-CoA desaturase enzyme activity, an enzyme responsible for the insertion of a double bond in C16:0 or C18:0 in the formation of C16:1(n-7) and C18:1(n-9). Hence, SFA accumulates in the yolk as it is in liver or muscle. An addition of 10% olive oil prevented CLA-induced colour changes in the yolks and albumen of eggs stored at 4 °C (Aydin *et al.*, 2001). This is probably due to decreased *de novo* lipogenesis in the liver of laying hens when fed a high fat diet (Naber & Biggert, 1989). Therefore, in the presence of high fat levels in the diet, laying hens would prefer to use dietary fat instead of hepatic synthesized fat for yolk formation.

As dietary CLA has an effect on the fatty acid metabolism, it also affects the size of chicken egg. In a previous study conducted in quail, dietary CLA at the levels of 2% or 3% caused a reduction in the size of eggs compared to the control group (Aydin & Cook, 2004). This could be due to the inhibition of stearoyl-CoA desaturase. As the stearoyl-CoA is inhibited, hepatic triacylglycerol secretion as VLDL declines (Legrand *et al.*, 1997). However, when quail were fed 0.25% CLA in low-fat diet, the size of eggs increased relative to the control group. In the present study, feeding laying hens CLA significantly reduced the egg size.

In the present study, when eggs from the CLA group were stored at 4 °C for 30 days, the percentage of yolk increased and the percentage of albumen decreased relative to the control group. Fresh eggs from the laying hens fed 0.5% CLA or eggs from the CLA group not stored at cold temperatures (or stored at room temperatures) had no increase in the relative size of egg yolk when compared to the control group (R. Aydin, unpublished data). These findings suggested that under cold room temperature some ingredients of albumen diffuse into yolk or *vice versa*. The increase in yolk weight of eggs from hens fed CLA was much higher than the control group (Ahn *et al.*, 1999). A recent study showed that dietary CLA increased yolk water content linearly (Shang *et al.*, 2004). Similarly, in the present study, when eggs from CLA-fed laying hens were stored at 4 °C for 30 days, there was a significant increase in the proportions of yolk weight and a decrease in the proportions of albumen compared to the control group. Ahn *et al.* (1999) showed that the yolks of eggs from CLA group had higher water content than those from the control group. This may partly explain why dietary CLA caused negative effects on the quality (i.e. colour defects and abnormal pH changes in yolk and albumen of eggs) of the eggs stored at 4 °C. As the level of CLA was increased in the diet, yolk pH increased and albumen pH decreased in the eggs stored at 4 °C (Shang *et al.*, 2004). Similar adverse effects were observed in the eggs from laying hens fed cyclopropene fatty acids (Schaible & Bandemer, 1946). In addition to water passing into yolk from albumen, it was shown that solutes including proteins of the albumen passed into yolk so that proportion of albumen decreased (Schaible & Bandemer,

1946). Therefore, the increase in yolk pH and the decrease in albumen pH may be related to the migration of minerals, water and small albumen proteins. Changes in the activity of stearoyl-CoA desaturase in tissues are reflected in cell membrane phosholipids and triacylglycerol composition (Ntambi *et al.*, 1999). It can be speculated that CLA increases SFA content of the vitelline membrane so that during cold storage the vitelline membrane is disrupted, causing leakage of minerals and water from albumen into egg yolk.

Conclusion

The present study confirmed that CLA in low-fat diets caused significant alteration in the yolk fatty acid composition and changed the quality of eggs stored at cold temperatures. This study also suggests that CLA-related colour changes observed in the yolk and albumen of eggs stored at cold temperature depend on the increase in the ratio of SFA to UFA, probably by changing the permeability of the vitelline membrane of the egg yolk.

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