

Effect of dietary conjugated linoleic acid on laying hen performance, egg yolk fatty acid composition and egg quality during refrigerated storage

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ABSTRACT

Sixty-four Lohman LSL white layers, 75 weeks of age, kept in individual cages were assigned randomly to four treatments of 16 hens per group. The hens received one of four diets with 0, 0.5, 1 or 2% CLA for 4 weeks. Eggs were collected daily and stored at 4°C for 1 or 42 days. The performance of hens and fatty acid composition, thiobarbituric acid reactive substances (TBARS) levels, pH of yolk and albumen, and yolk colour in eggs were measured.

Feed intake, feed conversion and egg weight were influenced by the dietary CLA level ($P<0.05$). The concentration of CLA in the yolk lipids linearly increased as the dietary CLA increased. The addition of CLA to feed significantly ($P<0.01$) increased the saturated fatty acid (SFA) and polyunsaturated fatty acid (PUFA) and decreased ($P<0.01$) the MUFA content in the egg yolk. The duration of refrigeration increased the proportion of egg yolk but decreased the albumen content. It also increased egg yolk pH but decreased albumen pH. Yolk colour was influenced by storage time but it was not influenced by the CLA level. TBARS values were significantly influenced by the dietary CLA and storage time ($P<0.01$).

KEY WORDS: conjugated linoleic acid, laying hen, fatty acid composition, egg quality

INTRODUCTION

Conjugated linoleic acid (CLA) is a mixture of several geometrical and positional conjugated isomers of linoleic acid (C 18:2 *cis*-9, *cis*-12). CLA is currently recognized as having a beneficial effect on human health, atherosclerosis, hypercholesterolaemia and cancer (Pariza and Hargraves, 1985; Chin et al., 1992;

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Ip et al., 1994). Ha et al. (1990) reported that CLA is an antioxidant almost as effective as butylated hydroxytoluene (BHT).

Ruminant products, such as milk, meat, and cheese, are major CLA sources in human diets (Chin et al., 1992). Foods obtained from non-ruminants, however, contain much less CLA. Poultry products contain relatively low CLA levels. Chicken meat and egg yolks contain approximately 0.9 and 0.6 mg of CLA/g of fat, respectively (Chin et al., 1992). Feeding animals synthetic CLA sources should be a good way to enrich foods in biologically effective CLAs. Ahn et al. (1999) reported that enriching eggs with CLA likely alters egg characteristics. Chamruspollert and Sell (1999) demonstrated that chicken eggs could be enriched in CLA to as high as 11% by feeding hens 5% CLA in the diet.

CLA is incorporated into all lipid classes of egg yolk in amounts proportional to the dietary level of CLA (Chamruspollert and Sell, 1999). Dietary CLA increased the proportion of saturated fatty acids and decreased monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) of yolk (Ahn et al., 1999).

The experiment was designed to determine the effect of dietary CLA levels on the concentration of CLA, other fatty acids and thiobarbituric acid reactive substances (TBARS) of egg yolk lipids at 4°C. The influence of dietary CLA on the productive performance of laying hens and selected egg characteristics of commercial importance also was determined.

MATERIAL AND METHODS

Sixty-four 75-week-old Lohman hens kept in individual cages were used. Each of four dietary treatments was assigned randomly to sixteen hens. The diets contained, %: 0, 0.5, 1 or 2 CLA (Table 1). The CLA source contained, %: 60 CLA (the CLA mixture contained, %: 18.1 c9-t11, 19.87 c10-t12, other CLA isomers 21.34). During the experiment (4 weeks) hens were provided feed and water *ad libitum*. Egg production and feed consumption were recorded daily.

Beginning on day 21, all eggs were labeled according to date of laying and hen number, weighed and stored at 4°C for 1 or 42 days. Eggs used for evaluation of yolks and albumen were broken and the weights of yolks, albumen, and shell, recorded. Yolk colour was determined using a 14-point Improved Roche Colour Fan. The pH of the egg yolk and white was measured after diluting with deionized water.

Chemical analysis

From three eggs in each group, 2 g of yolk was taken and lipids were extracted by the method of Folch et al. (1957). Fatty acids of the lipids were methylated

by adding 10 mL of anhydrous 3N HCL-methanol to 180 to 200 μg of lipid and heating this mixture for 40 min at 60°C (Chin et al., 1992). Analysis of fatty acid methyl esters (FAME) was performed by gas chromatography.

Thiobarbituric acid reactive substances were determined on egg samples as described by Salih et al. (1987) with some modifications (Cherian et al., 1995). Egg samples (2 g) were weighed into 50 mL test tubes, and 18 mL of 3.86% perchloric acid were added. The samples were homogenized. BHT (butylated hydroxyanisole) was added to each sample. The homogenate was filtered through Whatman 1 filter paper. Two mL of filtrate were mixed with 2 mL of 20 mM TBA in distilled water, and incubated. Absorbance was determined at 531 nm. The TBA numbers were expressed as milligrams of malonaldehyde (MA) per kilogram of yolk.

Differences between groups were analysed with one-way and two-way analysis of variance (ANOVA) by using the statistical package SPSS for Windows (1999), version 10.0. Significant means were subjected to a multiple comparison test (Duncan) at $\alpha = 0.01$ and 0.05 level.

TABLE 1

Composition of diets fed to laying hens, g/kg

Indices	Dietary treatments, % CLA			
	0	0.5	1	2
<i>Ingredients</i>				
maize	38	38	38	38
soyabean meal	21	21	21	21
wheat middlings	18	18	18	18
meat-and-bone meal	3	3	3	3
sunflower meal	3	3	3	3
limestone	85	85	8.5	8.5
dicalcium phosphate	0.4	0.4	4.0	4.0
vitamin premix ¹	3	3	3	3
mineral premix ²	3	3	3	3
NaCl	0.5	0.5	0.5	0.5
DL-methionine	1.4	1.4	1.4	1.4
soyabean oil	73.1	64.7	56.4	39.7
CLA source ³	-	8.4	16.7	33.4
<i>Calculated</i>				
crude protein	16	16	16	16
ME, kcal/kg	2730	2730	2730	2730

¹ supplied per kilogram of diet, mg: Mn, 80; Zn, 90; Fe, 50; Cu, 12; Se, 0.15 and sodium chloride, 2.5 g

² supplied per kilogram of diet: vit A, 8000 IU; cholecalciferol, 1580 IU; dl- α -tocopheryl acetate, 12; vit. B₁₂, 16; vit. K, 2; biotin, 75; mg: riboflavin, 4; pantothenic acid, 12.8; niacin, 75; μg : choline, 509; folic acid, 1.62; ethoxyquin, 15

³ CLA source was obtained by Pharmanutrients, Lake Bluff, IL 60044

RESULTS AND DISCUSSION

Hens fed the diet containing 1 or 2% CLA consumed less feed ($P < 0.01$) than hens fed 0 or 0.5% CLA (Table 2). Feed efficiency and egg weight were also affected by feeding 0.5, 1 or 2% CLA. However, no effects of dietary CLA concentration were found on the rate of egg production. No mortality of hens occurred during the experiment. Ahn et al. (1999) and Shang et al. (2004) reported that with increasing dietary CLA supply, feed intake and the rate of egg production decreased linearly.

TABLE 2

Influence of dietary conjugated linoleic acid (CLA) on performance of laying hens

Dietary CLA, %	Feed consumption g/hen/d	Rate of egg production %	Feed efficiency kg:kg	Egg weight g
0	134.72 ^a	75.16	2.63 ^a	68.93 ^b
0.5	129.36 ^b	71.66	2.53 ^{ab}	71.48 ^a
1	126.97 ^c	74.40	2.35 ^b	70.48 ^a
2	125.01 ^c	71.11	2.43 ^{ab}	72.35 ^a
SEM	3.75	2.166	0.18	2.35
Probability	0.000	NS	0.04	0.045

^{a,b} column means with no common superscript differ significantly

Although initially no significant differences were observed between groups with respect to percentage of egg yolk at one day of storage, there were significant differences between percentage increases of egg yolks at the end of the storage period (Table 3). The greatest changes during storage occurred in eggs from hens fed 0.5 or 1% CLA, the dietary CLA by storage time interaction was not significant. Ahn et al. (1999) reported that the yolk percentage increased as dietary CLA increased ($P < 0.001$). The percentage of albumen decreased ($P < 0.001$) with time of refrigerator storage, but it was not affected by the dietary CLA level. The egg shell proportion was not influenced by CLA or storage time.

Egg yolk pH increased during refrigerated storage in all dietary treatments, but the increase was greatest in eggs from hens fed the 1 or 2% CLA diets (Table 4). Ahn et al. (1999) reported that ion movement between the yolks and albumen through the yolk membrane in CLA eggs was greater than that in the control eggs. It is speculated that incorporation of CLA, most of which was in the *trans* form, could have increased the permeability of the yolk membrane due to the *cis-trans* arrangements of CLA, which would have increased membrane fluidity and the size of the space between the fatty acid chains of phospholipids that constitute the membrane bilayer. Albumen pH of eggs from hens fed 0, 0.5 or 1% of CLA was

TABLE 3
Influence of dietary conjugated linoleic acid (CLA) and days of refrigeration on percentage of yolks, albumens and shells of egg

Duration of refrigeration	Yolk, %		Albumen, %				Shell, %					
	0	1	2	0	0.5	1	2	0	0.5	1	2	
1 day	29.0 ^{cd}	29.3 ^{cd}	30.3 ^{cd}	27.5 ^{cd}	60.3 ^{ab}	60.6 ^{ab}	59.8 ^{ab}	61.9 ^a	10.7 ^{abc}	10.1 ^{bc}	9.88 ^c	10.6 ^{abc}
42 days	31.5 ^{bc}	34.4 ^a	33.5 ^{ab}	30.8 ^{bc}	57.4 ^{cd}	54.6 ^c	55.4 ^{de}	58.7 ^{bc}	11.2 ^a	10.9 ^{ab}	11.1 ^a	10.4 ^{abc}
SEM	0.318		0.265		0.103							
Diet	NS		NS		NS		NS					
Days	0.001		0.000		0.05		0.05					
Diet × day	NS		NS		NS		NS					

^{a-d} means with no common superscript differ significantly

TABLE 4
Influence of dietary conjugated linoleic acid (CLA) and days of refrigeration on colour, and pH of yolks and albumens of egg

Duration of refrigeration	Colour, %		pH albumen, %				pH yolk, %					
	0	1	2	0	0.5	1	2	0	0.5	1	2	
1 day	9 ^c	10 ^c	9.6 ^c	8.94 ^a	8.88 ^{ab}	8.81 ^{ab}	8.36 ^c	5.79 ^e	5.93 ^{de}	5.49 ^f	5.49 ^f	
42 days	12.33 ^{ab}	12 ^b	13.66 ^a	12.66 ^{ab}	8.87 ^{ab}	8.53 ^{bc}	8.76 ^{bc}	6.08 ^d	7.62 ^b	7.96 ^a	7.04 ^e	
SEM	0.167		0.037		0.029							
Diet	NS		0.05		0.05							
Days	0.000		0.012		0.000							
Diet × day	NS		0.013		0.000							

^{a-f} means with no common superscript differ significantly

decreased significantly by storage time, but it increased in eggs from hens fed 2% CLA. There was no effect of dietary CLA on yolk colour (Table 4). Yolk colour was significantly affected by refrigerated storage ($P < 0.01$).

The fatty acid composition of yolk lipids was significantly altered by increasing concentrations of dietary CLA, but it was not changed by storage time (Table 5). The proportions of myristic, palmitic, stearic and CLA (9-*cis*, 11-*trans* and 10-*trans*, 12-*cis*) in egg yolk lipids was increased by dietary CLA, but those of palmitoleic, oleic, linoleic, linolenic and arachidonic acid were decreased. These results were similar to those reported by Chamruspollert (1998) and Ahn et al. (1999). Du et al. (1999) reported that n-3 and n-6 precursors have been used for the synthesis of long-chain-PUFA. It has been reported that dietary supplementation of the *cis*-9, *trans*-11-CLA isomer inhibited the activity of Δ 6-desaturase in rat liver (Bretillon et al., 1999). They concluded that decreases in unsaturated fatty acids could be caused by the competitive inhibition of Δ 6-desaturase by CLA. In this research, we increased the dietary supplementation of CLA on the basis of preceding reports and found that CLA in yolk lipids increased linearly. This also confirms earlier findings (e.g., Ahn et al., 1999; Chamruspollert and Sell, 1999; Cherian et al., 2002; Shang et al., 2004). The greatest concentration of CLA, *cis*-9, *trans*-11 plus *trans*-10, *cis*-12 isomers, in yolk lipids (6.04%) was obtained when the diet contained 2% CLA. The most accumulated CLA isomers were 9-*cis*, 11-*trans* observed from the group with 2% CLA (Table 5). Moreover, accumulation of *cis*-9, *trans*-11 was higher than that of *trans*-10, *cis*-12.

Ahn et al. (1999) reported that the decrease in the concentration of linoleic and linolenic acids in yolk lipids of hens fed CLA probably reflects the relatively low concentration of these fatty acids in the CLA source as compared with soyabean oil. The content of arachidonic acid in yolk from hens fed diets enriched in CLA was lower than from the control diet, probably due to competition of metabolites of dietary CLA isomers with metabolites of linoleic acid, particularly in reaction with Δ 6-, Δ 5- desaturases and elongase (i.e. mechanism of anticancer activity of *cis*-9, *trans*-11CLA). Li and Watkins (1998) speculated that CLA reduced the concentration of oleic acid by inhibiting liver Δ 9-desaturase activity and found that dietary CLA decreased the concentrations of palmitoleic and oleic acids, which agrees with the present study. The effects of dietary supplementation of CLA on total saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acid (PUFA) composition of yolk lipids are shown in Table 5. SFA and PUFA was increased ($P < 0.01$) by dietary CLA compared with the control group (0% CLA), whereas the concentration of MUFA decreased ($P < 0.01$). These results are in accordance with the findings of Chamruspollert and Sell (1999) and Szymczyk et al. (2003).

TABLE 5
Influence of dietary conjugated linoleic acid (CLA) and days of refrigeration on fatty acid composition of egg yolk lipids

Fatty acids, %	Duration of refrigeration												SEM	Prob-abilities of diet effect
	1 days						42 days							
	0 % CLA	0.5 % CLA	1 % CLA	2 % CLA	0 % CLA	0.5 % CLA	1 % CLA	2 % CLA	0 % CLA	0.5 % CLA	1 % CLA	2 % CLA		
Myristic	0.40 ^d	0.45 ^c	0.5 ^b	0.65 ^a	0.46 ^c	0.46 ^c	0.49 ^b	0.50 ^b	0.003	0.000				
Palmitic	25.84 ^e	30.03 ^d	31.20 ^{bcd}	32.3 ^{ab}	26.00 ^e	30.15 ^{cd}	31.29 ^{bc}	32.90 ^a	0.138	0.000				
Palmitoleic	2.54 ^a	1.24 ^b	0.79 ^c	0.67 ^c	2.42 ^a	1.19 ^b	0.74 ^c	0.63 ^c	0.018	0.000				
Stearic	7.91 ^d	13.64 ^c	15.19 ^b	16.01 ^a	8.01 ^d	13.62 ^c	15.21 ^b	16.14 ^a	0.046	0.000				
Oleic	35.56 ^a	24.27 ^b	21.33 ^c	19.74 ^d	35.30 ^a	24.20 ^b	21.17 ^c	19.12 ^d	0.146	0.000				
Linoleic	23.44 ^a	23.01 ^{ab}	22.04 ^{bc}	20.94 ^{cd}	22.67 ^{ab}	23.24 ^{ab}	22.17 ^{abc}	20.44 ^d	0.145	0.001				
α -Linolenic	1.20 ^a	1.18 ^a	1.19 ^a	0.92 ^b	1.14 ^a	1.11 ^a	1.19 ^a	1.08 ^a	0.016	0.008				
Arachidonic	1.97 ^a	1.59 ^b	1.36 ^c	0.96 ^d	2.03 ^a	1.51 ^b	1.26 ^c	0.78 ^e	0.016	0.000				
9- <i>cis</i> , 11- <i>trans</i> CLA	ND	1.24 ^c	2.17 ^b	3.98 ^a	ND	1.31 ^c	2.30 ^b	4.21 ^a	0.028	0.000				
10- <i>trans</i> , 12- <i>cis</i> CLA	ND	0.6 ^d	1.10 ^c	2.02 ^a	ND	0.45 ^e	0.99 ^c	1.83 ^b	0.013	0.000				
SFA	34.15 ^d	44.12 ^c	46.89 ^b	48.69 ^a	34.47 ^d	44.23 ^c	46.99 ^b	49.54 ^a	0.131	0.000				
MUFA	38.1 ^a	25.51 ^b	22.12 ^c	20.41 ^d	37.72 ^a	25.39 ^b	24.91 ^b	19.75 ^d	0.149	0.000				
PUFA	26.61 ^{cd}	27.08 ^{bc}	27.86 ^{abc}	28.82 ^a	25.84 ^d	27.62 ^{abc}	27.98 ^{ab}	28.34 ^{ab}	0.139	0.001				

ND: not detected

TABLE 6

Effect of dietary conjugated linoleic acid (CLA) and days of refrigerated storage on the thiobarbituric acid reactive substances (TBARS) values of egg yolk

Duration of refrigeration	CLA, %			
	0	0.5	1	2
1 day	0.42 ^b	0.40 ^c	0.37 ^d	0.32 ^e
42 days	0.48 ^a	0.41 ^{bc}	0.38 ^d	0.37 ^d
SEM			0.002	
Diet			0.000	
Days			0.000	
Diet × day			0.000	

^{a-d} means with no common superscript differ significantly

Inclusion of CLA resulted in a significant ($P < 0.01$) reduction in the TBARS values in eggs. TBARS values were increased during refrigerated storage in all dietary treatments. Supplementation of laying hen's diets with CLA is a novel way of increasing the intrinsic concentration of CLA in eggs. A higher concentration of CLA ameliorated lipid oxidation in eggs, resulting in a dietary CLA by storage time interaction ($P < 0.01$).

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STRESZCZENIE

Wpływ dodatku sprzężonego kwasu linolenowego na wyniki produkcyjne kur niosek, skład kwasów tłuszczowych żółtka jaja oraz jakość jaj podczas przechowywania w chłodni

Sześćdziesiąt cztery nioski Lohman LSL, 75-tygodniowe, utrzymywane w indywidualnych klatkach, podzielono losowo na 4 grupy, po 16 ptaków w każdej. Kury otrzymywały przez 4 tygodnie jedną z czterech diet, zawierających 0; 0,5; 1 lub 2% CLA. Jaja zbierano codziennie i przechowywano w temp. 4°C przez 1 lub 42 dni. Oznaczano wyniki produkcyjne niosek oraz skład kwasów tłuszczowych, zawartość związków reagujących z kwasem tiobarbiturowym (TBARS), pH żółtka i białka, a także kolor jaj.

Dodatek CLA do diety wpłynął na pobranie i wykorzystanie paszy oraz masę jaj ($P < 0,05$). Stężenie CLA w tłuszczu żółtka zwiększało się liniowo w miarę wzrostu udziału CLA w diecie. Dodatek CLA do paszy istotnie zwiększył ($P < 0,01$) zawartość nasyconych (SFA) i wielonienasyconych kwasów tłuszczowych (PUFA), a obniżył ($P < 0,01$) zawartość MUFA w żółtku jaja. W zależności od długości przechowywania zwiększył się udział żółtka, a obniżyła zawartość białka w jajach. Dodatek CLA spowodował także zwiększenie pH żółtka, a obniżenie pH białka. Kolor żółtka zależał od długości przechowywania jaj, lecz nie zależał od ilości dodatku CLA do diety. Wartości TBARS zależały od poziomu CLA w diecie i okresu przechowywania jaj ($P < 0,01$).