Effect of Dietary Conjugated Linoleic Acid on Lipid Characteristics of Egg Yolk

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ABSTRACT : A total of 250 laying hens were fed a diet containing 0, 1, 2.5 or 5% conjugated linoleic acid (CLA), and 5% Safflower seed oil (SSO) for 5 weeks, and eggs were collected by week to analyse lipid characteristics of egg yolk. Egg yolk from CLA-fed groups showed significant increase in CLA content with increased CLA in the diet. Dietary CLA also increased the ratio of saturated fatty acids and decreased unsaturated fatty acids in the egg yolk. The proportion of myristic, palmitic, stearic and CLA were increased, while those of oleic, linoleic, linolenic and arachidonic acid were decreased. The cholesterol content in egg yolk was significantly decreased by dietary CLA for 5 weeks feeding. After 7 days of feeding, 5% CLA-fed group showed the lowest cholesterol content in egg yolk. CLA-fed groups showed significantly lower 2-thiobarbituric acid-reactive substances (TBARS) values compared to control and SSO-fed group after 14 days of storage. No significant differences in TBARS values among CLA-fed groups were observed at the 28 days of storage. Results suggested that lipid oxidation of egg yolk during cold storage could be inhibited by dietary CLA due not only to changes in fatty acid composition but also to the high concentration of CLA in egg yolk. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 8 : 1165-1170)***

Key Words : Egg Yolk, Conjugated Linoleic Acid, Fatty Acid, Cholesterol, Lipid Oxidation

INTRODUCTION

CLA is a mixture of octadecadienoic acids with different locations of conjugated double bonds and different *cis*, *trans* combination. Up to 8 CLA isomers are normally identified in commercially available CLA sources, with two main isomers (*cis*-9, *trans*-11 and *trans*-10, *cis*-12) that constitute about 80% of the total CLA. The CLA has been recognized as having anticarcinogenic and antioxidative properties (Ha et al., 1990). Also, CLA has been shown to have beneficial effects on human health (Ip et al., 1995).

Lee et al. (1994) reported that feeding rabbits with CLA (0.5 g CLA/rabbit per day) for 12 weeks markedly reduced total and low density lipoprotein (LDL) cholesterol and triglycerides in serum. Nicolosi et al. (1997) also reported that animals fed the CLA containing diets collectively had significantly reduced levels of plasma total cholesterol and low density lipoprotein cholesterol, and triglycerides. However, in pigs, Stangl et al. (1999) showed that the LDL cholesterol to high density lipoprotein (HDL) cholesterol ratio was significantly increased by CLA. The effects of dietary CLA on the reduction of cholesterol contents in animal products have been not understood yet. Also the effect of CLA on lipid oxidation has been debated although many researchers reported that dietary CLA improved the oxidative stability of beef patty (Shantha et al., 1995), chicken meat (Du et al., 2000a,b) and pork loin (Joo et al., 2002a).

Small amount of CLA are present in egg yolk. Because CLA content in the egg is not increased by a conventional feed, the only possible way to increase effectively CLA content is to use the chemically synthesized CLA as diet additives. Increasing the CLA content and changing the fatty acids composition in egg yolk by dietary CLA may provide a value-added egg. The development of CLA-enriched eggs could have implications in the poultry industry by improving immunity and health, increasing growth and improving feed efficiency. However, there is little information on the effects of dietary CLA or SSO on changes of lipid metabolism in egg. Therefore, in this study, the effects of dietary CLA on fatty acid composition, cholesterol content, and lipid oxidation in egg yolk during storage were investigated.

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Received December 9, 2002; Accepted March 4, 2003

MATERIALS AND METHODS

Hen feeding and sample preparation

Two hundred fifty, 28-week-old Isa Brown hens were assigned to one of the five dietary treatments containing 0, 1, 2.5 and 5% CLA, and 5% safflower seed oil (SSO). Each hen was kept in individual wire cages (40×40×40 cm) placed in a temperature (25°C) and humidity (70% RH) controlled room. Hen was subjected to a basal diet obtained from a commercial company (Table 1). Treatment diets (1, 2.5 and 5% CLA) were prepared by mixing an appropriate amount of the chemically synthesized CLA with the basal diet by weight. A positive control diet (5% SSO) was also prepared by mixing an appropriate amount of SSO with the basal diet by weight. Each group of hens was adapted to the basal diet for a week, and then subjected to one of the five treatment diets for 5 weeks. Five eggs were collected randomly per treatment by each week for analysis of cholesterol content in the yolk during experimental period of 5 weeks. Eggs that were collected at feeding 5 weeks

Table 1. Composition of the experimental basal diet for laying-hens[1)]

Ingredient	Content (%)	Chemical composition (%)
Yellow corn	45.0	Crude protein<15.0
Wheat	12.0	Crude fat<3.0
Rice bran	8.0	Calcium<3.0
Wheat bran	10.0	Phosphorus<0.3
Soybean oil meal	9.0	Crude fiber<6.0
Fish meal	7.0	Crude ash<15.0
Brown seaweed[2)]	0.2	
Animal fat	3.0	
Bone meal	1.0	
$CaCO_3$	3.8	
MgO	0.5	
Salt	0.2	
Vitamin premix	0.3	

[1)] Diet contains amino acids and minerals identical to the NRC (1994) Specifications for laying hen diet. Diet contains 2,912 ME kcal/kg, and various amount of vitamins (A: 6,893 IU, D: 562 IU, E: 40 IU, K: 0.75 mg, riboflavin: 8.6 mg, pantothenic acid: 19.5 mg, niacin: 8.8 mg and B_{12}: 0.023 mg/kg). [2)] Brown seaweed was roughly ground after washing and drying.

were stored for 28 days at 4°C to investigate the changes in fatty acid composition and lipid oxidation of the egg yolk during cold storage at 4°C.

Synthesis of CLA

The CLA used in this study was chemically synthesized by alkaline isomerization of safflower oil and purified by the low-temperature precipitation method of Ha et al. (1990). Purity of the CLA was 95%, consisting of four major CLA isomers (46% *cis*, 9-*trans*, 11, 45% *trans*, 10-*cis*, 12, 2% *trans*, 9-*trans*, 11 and 2% *trans*, 10-*trans*, 12 CLA). The purity and composition were confirmed using the method of Kim et al. (2000). All diets were freshly prepared and stored at the cold room temperature (4°C). Peroxide value of the diets containing CLA or SSO was not different from that of basal diet when determined before and after feeding.

Cholesterol analysis

Two grams of egg yolk were added into 50 ml tube with 10 ml of saponification reagent (30% KOH and ethanol with the ratio of 6:94) and 0.5 ml internal standard (2 mg 5 α-cholestane/sample), and then homogenized with a Polytron homogenizer (IKA Labortechnik T25-B, Selangor, Malaysia) for 10 sec, caped and incubated for 1 h at 60°C. After cooling the sample, 8 ml of deionized distilled water and 3 ml hexane were added and mixed thoroughly to allow separating. Top layer (hexane layer) was taken out and dried in scintillation vials, and 100 μl of *bis*-[trimethylsilyl] trifluoroacetamide+1% trimethylchlorosilane and 200 μl of pyridine were added and mixed, and set overnight and then analyzed by gas chromatography (Shimadzu GC-14A; Tokyo, Japan). A ramped oven temperature condition (180°C for 2.5 min, increased to 230°C at 2.5°C/min, then held at 230°C for 7.5 min) was used. Temperatures of both the inlet and detector were 280°C. Helium was the carrier gas at linear flow of 1.1 mL/min. Dector (flame ion detector) air, H_2, and make-up gas (He) flows were 350, 35 and 43 mL/min, respectively.

Fatty acid analysis

Lipids were extracted with chloroform and methanol as described by Folch et al. (1957). The extracted lipids were concentrated using an evaporator (Zymark turbovap 500, Hopkinton, MA, USA). For lipid hydrolysis, an aliquot of lipid extract (30 mg) and 3 ml of 4% H_2SO_4 in methanol were combined in a screw-capped test tube. The test tube was placed in boiling water (100°C) for 20 min and subsequently cooled at room temperature. The resulting free fatty acids were methylated with 1 ml of 14% boron trifluoride in methanol at room temperature for 30 min. Water (1 ml) and hexane (5 ml) were added. Samples were vortexed and centrifuged at 500×g for 10 min. The upper organic solvent layer was used to determine fatty acids composition. Fatty acid methyl esters were analyzed on a gas chromatography equipped with an on-column injector port and flame-ionization detector. A Silar capillary column (30 m×0.32 mm×0.25 μm; Shimadzu, Tokyo, Japan) was used for the separation of the fatty acid methyl esters. The gas chromatography oven temperature was 140°C and increased at a rate of 2°C/min to a final temperature of 230°C. The temperatures of injector port and detector temperatures were set at 240°C and 250°C, respectively. Fatty acid methyl ester (1 ml) was injected onto the split injection port (100:1 split ratio). The flow rate for He carrier gas was 50 ml/min. Each fatty acid was detected by the standards' retention time.

Lipid oxidation (2-thiobarbituric acid reactive substances; TBARS) analysis

Five grams of egg yolk were weighed into a 50 mL test tube and homogenized with 15 mL of deionized distilled water using the polytron homogenizer for 10 seconds at the highest speed. One mL of egg yolk homogenate was transferred to a disposable test tube (13×100 mm), and butylated hydroxyanisole (50 μl, 10%) and thiobarbituric acid/trichloroacetic acid (TBA/TCA) (2 mL) were added. The mixture was vortexed and then incubated in a boiling water bath for 15 min to develop color. The sample was cooled in cold water for 10 min, vortexed again, and centrifuged for 15 min at 2,000×g. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 mL of deionized distilled water

Table 2. Effect of dietary CLA and SSO on fatty acid composition of egg yolk

Treatment	Fatty acid composition								
	14:0	16:0	18:0	18:1	18:2	18:3	CLA	20:4	SFA/ USFA[1)]
	%								
Basal	0.20^{CD}	25.16^{CD}	11.10^{C}	40.00^{A}	20.00^{B}	0.92^{A}	Trace	2.65^{B}	36.46/63.57
1% CLA	0.25^{C}	26.20^{C}	12.49^{B}	37.10^{B}	17.22^{C}	0.70^{C}	3.77^{C}	2.28^{C}	38.94/61.07
2.5% CLA	0.35^{B}	29.80^{B}	13.14^{A}	32.25^{C}	17.08^{C}	0.53^{D}	5.31^{B}	1.54^{D}	43.29/56.71
5% CLA	0.46^{A}	32.58^{A}	13.31^{A}	25.60^{D}	15.55^{D}	0.49^{D}	10.35^{A}	1.66^{D}	46.35/53.65
5% SSO	0.17^{D}	25.23^{D}	11.34^{C}	33.78^{C}	25.65^{A}	0.60^{B}	Trace	3.22^{A}	36.74/63.25
SEM	0.04	0.33	0.50	1.32	1.20	0.11	1.18	0.14	-

^{A,B,C,D} Different letters within a column indicate significant differences between mean values ($p<0.05$).

[1)] SFA: Saturated fatty acid, USFA: Unsaturated fatty acid, CLA: Conjugated linoleic acid, SSO: Safflower seed oil, N=10.

and 2 mL of TBA/TCA solution. The amounts of TBARS were expressed as milligrams of malondialdehyde per kilogram of egg yolk.

Statistical analysis

Analysis of samples was conducted in triplicate. The effects of supplemented CLA levels of diet on the content of cholesterol, fatty acids composition and lipid oxidation of egg yolk were analyzed using ANOVA with SAS (SAS Institute, 1996) at 5% level of significance.

RESULTS AND DISCUSSION

Changes in fatty acid composition

Dietary CLA significantly changed the fatty acid composition of egg yolk (Table 2). Basal group showed no significant difference in CLA content with SSO-fed group. However, the egg yolk from CLA-fed groups showed significantly higher CLA concentration compared to those of control or/and SSO-fed groups. Also data showed that CLA concentration in egg yolk depended on the level of CLA in diet. The CLA concentration in egg yolk increased with increased CLA in the diet. These results confirmed the early reports that dietary CLA was efficient to elevate CLA content in egg (Du et al., 1999) as in pig muscle (Joo et al., 2002a).

Dietary CLA increased the ratio of saturated fatty acids (SFA) and decreased unsaturated fatty acids (USFA) in the egg yolk (Table 2). The proportion of myristic, palmitic, stearic and CLA were increased by dietary CLA, but those of oleic, linoleic, linolenic and arachidonic acid were decreased. These changes in fatty acid composition of egg yolks are similar to those reported by Ahn et al. (1999). It might be possible that CLA inhibited the △6-desaturase enzyme system in the liver, thereby increasing saturated fatty acid concentration (Lee et al., 1995). It is not known, at this stage, whether the changes in fatty acid profiles of yolk are the result the effect of a mixture of CLA isomers or specific isomers.

In this study, the concentration of linoleic acid in egg yolk was, in the following order: 5% SSO>basal diet>1% CLA and 2.5% CLA>5% CLA. This result was expected because the amount of linoleic acid, as CLA precursors, decreased as CLA content increased (Ha et al., 1989; Chin et al., 1994). Similarly, in an earlier study, we reported that linoleic acid concentration of pork loin intramuscular fat was significantly decreased by dietary CLA (Joo et al., 2002a). There were significant differences in the arachidonic acid concentrations in egg yolk among treatments. Different concentration of arachidonic acid might be related to the linoleic acid concentration because it is a substrate for arachidonic acid synthesis, and could also be related to the inhibiting effects of CLA on △6-desaturase activity (Bretillon et al., 1999). Concentration of oleic acid in egg yolk from CLA-fed groups was much lower than those of basal. Several researches (Du et al., 2000a,b; Li and Watkins, 1998) suggested that CLA reduced the concentration of oleic acid by inhibiting liver △9-desaturase activity and found that dietary CLA decreased the concentrations of palmitoleic and oleic acid.

These changes of fatty acid composition could influence on the quality characteristics of eggs. Ahn et al. (1999) reported that dietary CLA and storage of CLA eggs increased the firmness of hard-cooked egg yolk. They also reported that the texture of yolks from hard-cooked CLA eggs was rubbery and elastic. In our previous research, it was observed that egg yolk from CLA-fed hens showed more yellow color and harder when eggs were boiled (Joo et al., 2002b). It was not clear whether the changes in quality characteristics of egg were the result of increasing SFA ratio of egg yolk. Further research is needed to determine the effect of dietary CLA on fatty acid changes and their relation to the changes in egg quality of hens.

Changes in cholesterol content

The cholesterol content in egg yolk was significantly decreased by a supply of dietary CLA for 5 weeks feeding (Table 3). However, the cholesterol content was slightly increased during feeding periods. At the 7 days of feeding, 5% CLA-fed group showed the lowest cholesterol content

Table 3. Effect of dietary CLA and SSO on cholesterol content in egg yolk

Treatment	Feeding period (weeks)					
	1	2	3	4	5	SEM
	mg/g					
Control	13.71^{Ab}	13.81^{Aab}	13.98^{Aab}	14.23^{Aa}	14.26^{Aa}	0.79
1% CLA	13.38^{Ab}	13.59^{ABab}	13.60^{ABab}	13.89^{Ba}	13.90^{Ba}	0.58
2.5% CLA	13.62^{Aab}	13.49^{ABb}	13.62^{ABab}	13.77^{BCa}	13.86^{Ba}	0.89
5% CLA	12.86^{Bb}	13.20^{Bab}	13.35^{Bab}	13.43^{Cab}	13.85^{Ba}	0.65
5% SSO	13.60^{Ab}	13.70^{Ab}	14.00^{Aab}	14.20^{Aa}	14.23^{Aa}	0.57
SEM	0.56	0.6	0.56	0.70	0.47	-

^{A,B,C} Different letters within a column indicate significant differences between mean values ($p<0.05$).

^{a,b,c} Different letters within a row are significantly different ($p<0.05$). CLA: Conjugated linoleic acid. SSO: Safflower seed oil. N=10.

in egg yolk than those of other treatments. but there was no significant difference in the cholesterol content between control and SSO-fed group during 5 weeks of feeding period. The cholesterol content was slightly decreased as increased CLA level in diet. but there were no significant difference among CLA-fed groups at the end of feeding.

This reducing cholesterol concentration by dietary CLA was similar to the results of Lee et al. (1994) who first reported that. feeding rabbits with CLA (0.5 g CLA/rabbit per day) for 12 weeks. total and LDL cholesterol and triglycerides in serum were markedly lower in the CLA-fed group. Stangl (2000) also reported that feeding CLA mixture with a level at 3% and 5% of diet exhibited marked reductions of plasma cholesterol in the low and high density lipoproteins in rats. However. it has been reported that the LDL cholesterol to HDL cholesterol ratio in plasma was significantly increased by CLA in pigs (Stangl et al., 1999). Nicolosi et al. (1997) showed that animals fed diets containing a mixture of CLA had significantly reduced levels of plasma total cholesterol. low density lipoprotein cholesterol. and triglycerides. but with no effect on high density lipoprotein cholesterol. Also de-Deckere et al. (1999) observed that dietary CLA decreased fasting values of LDL- and HDL-cholesterol. but increased the serum triglyceride level.

There were both positive and negative effects of CLA on the cholesterol contents in blood. Because high dietary cholesterol promotes the formation of atherosclerotic plaques in arteries. leading to coronary heart disease. many efforts have been made to reduce egg yolk cholesterol (Lv.. 2002). However. the mechanism for the reduction of cholesterol contents in egg yolk has not been understood yet. Generally. all of the cholesterol found in avian egg yolk originates from liver. where it is synthesized. incorporated into very low density lipoprotein (VLDL) particles. and transported to the ovary (Elkin et al.. 1997). In this study. it

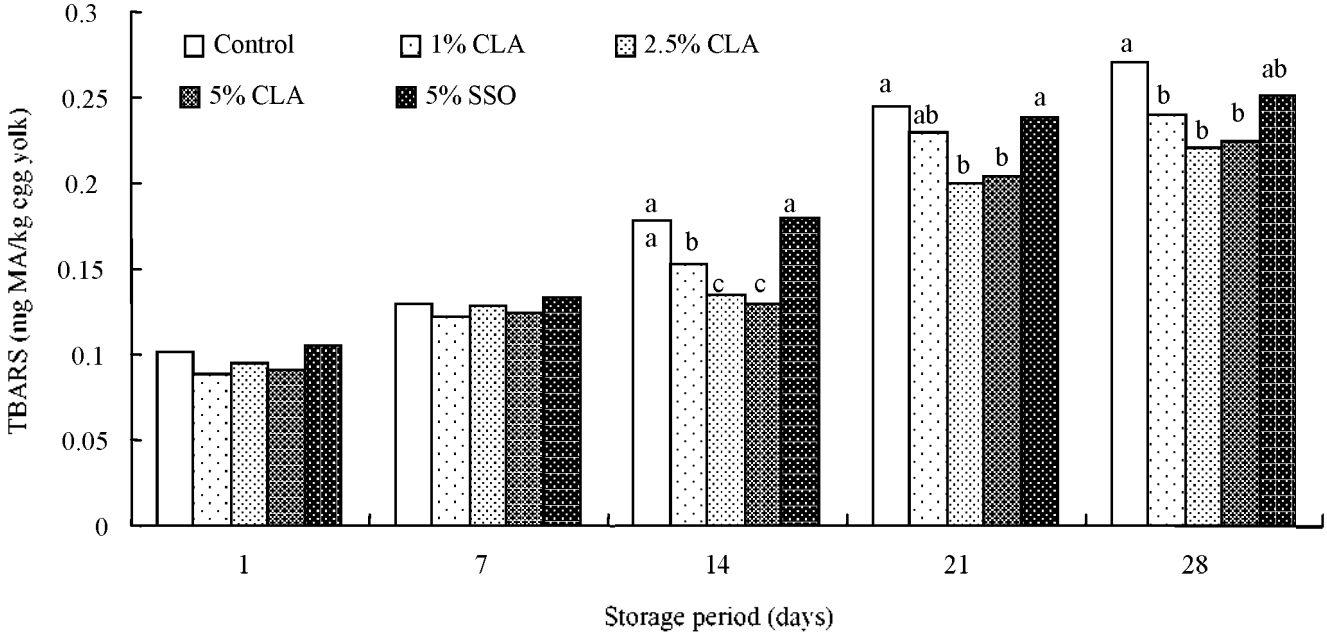

Figure 1. Effect of dietary CLA on lipid oxidation of egg yolk during cold storage. Different letters denote a difference ($p<0.05$) in means between the diets. CLA: Conjugated linoleic acid, SSO: Safflower seed oil. N = 10.

was assumed that the reduction of cholesterol content in egg yolk by dietary CLA might be associated with decreasing of plasma VLDL, LDL and triglyceride concentrations.

Du et al. (1999) reported that the incorporation rates of different CLA isomers into various classes of lipids were significantly different. CLA mix and *trans*-10, *cis*-12 CLA isomer also decreased fasting values of LDL- and HDL-cholesterol, increased VLDL-triacylglycerol, and reduced epididymal fat pad weights, whereas *cis*-9, *trans*-11 CLA isomer had no significant effects (de-Deckere et al., 1999). In this present study, we used the CLA mixture consisting of four major CLA isomers (*cis*, 9-*trans*, 11, *trans*, 10-*cis*, 12, *trans*, 9-*trans*, 11 and *trans*, 10-*trans*, 12 CLA). Therefore, our result of cholesterol reduction in egg yolk might not completely agreement with other results by different mixture of CLA isomers.

Changes in lipid oxidation

Lipid oxidation of egg yolk during cold storage was also affected by dietary CLA (Figure 1). Although there were no significant differences in TBARS values among the treatments until 7 days of cold storage, CLA-fed groups showed significantly lower TBARS values compared to control and SSO-fed group after 14 days of storage. No significant differences in TBARS values among CLA-fed groups were observed at the 28 days of storage. These results suggested that egg yolk from CLA-fed hen was more stabilized from lipid oxidation during cold storage compared to that of non-CLA fed hen.

In this study, dietary CLA reduced arachidonic acid, linoleic acid, and oleic acid content in egg yolk and shifted the whole fatty acid composition to more saturated side. Our previous study with pork loin also showed that dietary CLA reduced the content of unsaturated fatty acids in lipids (Joo et al., 2002a). Therefore, fed CLA to hens might be less susceptible to lipid oxidation of egg yolk due to changes in fatty acid composition to more saturated side. This result agrees with Du et al. (2000a) demonstrating that the increased storage stability of CLA meat could be caused by observed increase in saturated fatty acids and decreased non-CLA polyunsaturated fatty acids.

It was reported that CLA was a stable component in beef patty (Shantha et al., 1995), and the concentration of CLA in pork loin did not change during cold storage (Joo et al., 2002a). Thus, the high concentration of CLA in egg yolk observed in this study could reduce the formation of fatty acid free radicals and subsequent oxidation reaction. Du et al. (2000a) also suggested that conjugated structure made CLA less susceptible to free radical attacks without CLA itself acting as an antioxidant. They showed decreased TBARS values and hexanal content of meat patties after storage as CLA levels increased, and concluded that the CLA improved the oxidative stability of chicken meat (Du et al., 2000a,b). In addition, Ha et al. (1989) reported that oxidative reactions could influence CLA concentrations by either causing the formation of linoleic acid radicals, which in turn could be converted to CLA by hydrogen donors, or causing the oxidative destruction of the conjugated double-bond system of CLA. Therefore, it can be claimed from results of this study, that lipid oxidation of egg yolk during cold storage could be inhibited by dietary CLA due not only to changes in fatty acid composition but also to the high concentration of CLA in egg yolk.

ACKNOWLEDGMENT

The authors would like to thank the Ministry of Education for the financial support of this project (BK-21) in Korea.

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