

Excessive Dietary Conjugated Linoleic Acid Affects Hepatic Lipid Content and Muscular Fatty Acid Composition in Young Chicks

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ABSTRACT : The effects of dietary conjugated linoleic acid (CLA) on lipid concentrations and fatty acid composition of various tissues were studied in young chicks. From 7 days of age, a total of 160 chicks were divided into 4 groups, placed into 4 pens per group (10 birds per pen) and fed one of four experimental diets containing 6% tallow (TO 6%), 4% tallow plus 2% CLA (TO 4%-CLA 2%), 2% tallow plus 4% CLA (TO 2%-CLA 4%) or 6% CLA (CLA 6%) for 3 weeks. There were no significant differences in growth performances and the relative weights of various organs, but relative liver weight of chicks fed dietary CLA at 4 and 6% levels was significantly higher ($p < 0.05$) than that of TO 6% group. The chemical compositions of leg muscle were not affected by CLA feeding. However, hepatic total lipid of chicks fed 6% CLA diet was significantly higher ($p < 0.05$) than those of TO 6% and TO 4%-CLA 2% groups. The concentrations of various lipid fractions in serum were not affected by CLA feeding. With the increase in dietary CLA levels, *cis* 9-*trans* 11 CLA, *trans* 10-*cis* 12 CLA and total CLA of leg muscle increased linearly. The relative proportions of C18:1 ω -9 and C20:4 ω -6 fatty acids in the leg muscles of chicks fed the CLA containing diets were significantly lower ($p < 0.05$) than those of TO 6% group. These results indicate that the levels of CLA isomers were increased linearly in dose-dependent manner after feeding of synthetic CLA source. But it was also observed that excessive amount of dietary CLA resulted in the possible adversely effects, such as increase of liver weight, hepatic lipid accumulation and serum GOT level. (*Asian-Aust. J. Anim. Sci.* 2003. Vol 16, No. 8 : 1171-1176)

Key Words : Conjugated Linoleic Acid, Hepatic Total Lipid, Concentration of Serum Lipids, Tissue Fatty Acid Composition, Young Chicks

INTRODUCTION

Conjugated dienoic derivatives of linoleic acid (CLA) are a series of positional and geometric isomers of linoleic acid. Results of several studies show that dietary CLA isomers have an anticarcinogenic effect and modulate immune response in experimental animals (Ip et al., 1999; Yamasaki et al., 2000). Cook et al. (1993) showed that CLA improved immune responses in rat and prevented the catabolic effects following immune stimulation in chicks. Furthermore, CLA has been suggested by some animal studies to possess potent antiatherogenic effects (Lee et al., 1994; Nicolosi et al., 1997).

CLA is found predominantly in products from ruminants, including milk, cheese, and beef (Bauman et al., 2000). Foods derived from non-ruminant animals contain much less CLA than those from ruminant. The positive effects related to CLA have intensified the research efforts to increase the level of this fatty acid in animal products because increasing the content of CLA in animal products is a possible way for human to increase CLA intake. Practically, feeding CLA resulted in increased accumulation

of CLA in egg yolk (Chamruspollert and Sell, 1999; Jones et al., 2000) and broiler meat (Du and Ahn, 2002). In addition, large-scale manufacture of synthetic CLA has recently made CLA supplementation into feed economically viable (Dugan et al., 1997).

Aydin et al. (2001) reported that CLA feeding resulted in embryonic mortality by causing the transformation of fatty acid in the egg yolk. In addition, dietary CLA adversely affected the quality characteristics of poultry products. Du and Ahn (2002) found that the hardness of breast muscle from chickens fed CLA containing diets was increased, and this was associated with the change of fatty acid profiles in muscle lipid. Textural changes and color defects were also observed in eggs produced from hens fed CLA (Ahn et al., 1999; Aydin et al., 2001). The objectives of the present study were to investigate the dietary effects of CLA on the lipid concentrations and fatty acid composition in various tissues of young chicks. To identify the possible effects of excess CLA intake, chicks were fed CLA up to 6%, at the expense of tallow.

MATERIALS AND METHODS

Seven-day-old Hy-Line Brown male chicks were used. All chicks were weighed and randomly assigned into four experimental treatments. Each treatment comprised four replicates of 10 birds each. Chicks were housed in wire cages assigned to each replicate and fed one of four

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Table 1. Formula and chemical composition of experimental diets¹⁾

Ingredients	TO6%	TO4%-CLA2%	TO2%-CLA4%	CLA6%
Yellow corn	52.73	52.73	52.73	52.73
Corn gluten meal	4.60	4.60	4.60	4.60
Soybean meal	33.00	33.00	33.00	33.00
Limestone	1.00	1.00	1.00	1.00
Tricalcium phosphate	1.63	1.63	1.63	1.63
Salt	0.13	0.13	0.13	0.13
Tallow	6.00	4.00	2.00	-
Conjugated linoleic acid	-	2.00	4.00	6.00
Choline-chloride (25%)	0.23	0.23	0.23	0.23
Salinomycin	0.10	0.10	0.10	0.10
L-lysine HCl (99%)	0.26	0.26	0.26	0.26
DL-methionine (98%)	0.15	0.15	0.15	0.15
Ethoxyquin (66.6%)	0.02	0.02	0.02	0.02
Mineral mixture ²⁾	0.10	0.10	0.10	0.10
Vitamin mixture ³⁾	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00
Calculated analysis				
DM, %		88.27		
CP, %		22.00		
Ether extract, %		8.52		
Crude fiber, %		2.92		
Ash, %		5.71		
Ca, %		1.00		
Available P, %		0.40		
TME _n , kcal/kg		3,200.00		

¹⁾TO, tallow; CLA, conjugated linoleic acid. ²⁾Mineral mixture provided following nutrients per kg of diet: Fe, 40 mg; Zn, 65 mg; Mn, 87 mg; Cu, 66 mg; I, 1.5 mg; Se, 0.1 mg. ³⁾Vitamin mixture provided following nutrients per kg of diet: vitamin A, 11,000IU; vitamin D₃, 2,250IU, vitamin E, 11 mg; vitamin K₃, 0.6 mg; vitamin B₁, 1 mg; vitamin B₂, 10 mg; vitamin B₆, 1 mg; vitamin B₁₂, 0.02 mg; niacin, 32.5 mg; pantothenic acid, 10 mg; biotin, 0.03 mg; folic acid, 0.5 mg; ethoxyquin, 1,650 mg.

experimental diets containing 6% tallow (TO 6%), 4% tallow plus 2% CLA (TO 4%-CLA 2%), 2% tallow plus 4% CLA (TO 2%-CLA 4%) or 6% CLA (CLA 6%), respectively for 3 weeks. The control diet contained 6% tallow, and the CLA source was substituted for the tallow on a weight basis. CLA triglyceride used in this study contained about 27% *cis* 9-*trans* 11 isomer and 26% *trans* 10-*cis* 12 isomer of fatty acids. The formula and chemical composition of experimental diets are shown in Table 1. The diets were formulated to meet or to exceed the recommendation of NRC (1994). Animal facilities and husbandry were similar to conditions described by An et al. (1995). All chicks were provided free access to feed and water. Room temperature was maintained at 25±3°C and 23/1 h light/dark cycle was kept throughout the experimental period. Feed intake and body weight were recorded weekly.

At the end of the experimental period, all chicks were weighed individually. Eight chicks from each group were selected, and blood was drawn from the jugular vein using syringe for determination of the concentration of various lipid fractions and components. At necropsy, the liver, abdominal fat and right leg were immediately removed and weighed. The leg muscle (thigh and drumstick) and an

aliquot of the liver was homogenized and used for analyzing chemical compositions (AOAC, 1990) and fatty acid profiles. The serum was separated from each blood sample by centrifugation and stored at -30°C until use. Total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triacylglycerol and phospholipid in serum were measured enzymatically using Diagnostic-kit (Wako Chemicals, Wako Pure Chemical Ltd., Osaka, Japan). Various serum components were determined by CH-100^{PLUS} Chemistry Analyzer (Seac Radium Group, Ltd., Florence, Italy).

The total lipids of liver and leg muscle were extracted with a mixture of chloroform and methanol (2:1, v/v) by the method of Folch et al. (1957). Lipid extracts obtained were methylated according to the methods of Takenoyama et al. (1999) with some modification. In brief, about a 3 ml of sample was transesterified to fatty acid methyl esters in benzene using 0.5 M KOH/methanol for 10 min at 100°C. After cooling, the turbid preparation was neutralized with HCl/methanol and then reheated. Fatty acid methyl esters were extracted with hexane and measured by gas-liquid chromatography (HP 5890 Series, Hewlett-Packard, Atlanta, USA) using 0.32 mm I.D.×60 m capillary column (SUPELCOWAX-10, Supelco Ltd., Pennsylvania, USA).

Table 2. Effects of dietary CLA on the growth parameters and the relative weights of various organs¹⁾

Ingredients	TO6%	TO4%-CLA2%	TO2%-CLA4%	CLA6%
Initial BW, g	92.2±0.1 ²⁾	92.3±0.1	92.3±0.1	92.3±0.1
Final BW, g	361.3±11.3	345.0±5.4	357.5±7.8	348.8±2.4
Gained BW, g	269.1±11.2	252.7±5.4	265.3±7.8	256.4±2.4
Feed intake, g/bird	541.1±10.2	539.4±6.8	550.6±1.6	540.4±5.1
Feed conversion rate	2.02±0.05	2.13±0.04	2.08±0.04	2.11±0.01
Liver, g/100 g BW	2.69±0.10 ^c	2.77±0.10 ^{bc}	3.08±0.10 ^{ab}	3.30±0.11 ^a
Leg, g/100 g BW	8.28±0.11	8.38±0.12	8.59±0.11	8.62±0.11
Abdominal fat, g/100 g BW	0.82±0.22	0.97±0.21	0.93±0.21	0.78±0.12

¹⁾ TO, tallow; CLA, conjugated linoleic acid. ²⁾ Mean±SE. (n=8 for each diet), ^{a-c} Mean values in a row with no common superscript are significantly different (p<0.05).

Table 3. Effects of dietary CLA on the chemical composition of leg muscle and liver¹⁾

	TO6%	TO4%-CLA2%	TO2%-CLA4%	CLA6%
Leg muscle				
Moisture, %	72.44±0.32 ²⁾	70.81±3.32	74.25±0.21	74.86±0.41
Crude protein, %	21.12±0.41	20.99±0.22	21.59±0.43	21.62±0.42
Ether extract, %	0.91±0.11	0.83±0.11	0.82±0.12	0.72±0.11
Ash, %	1.20±0.01	1.19±0.01	1.22±0.01	1.20±0.01
Ca, %	0.0056±0.001	0.0057±0.001	0.0059±0.001	0.0056±0.001
P, %	0.20±0.01	0.20±0.01	0.20±0.02	0.21±0.01
Liver				
Lipid, %	3.50±0.13 ^b	3.86±0.14 ^b	4.09±0.42 ^{ab}	5.05±0.41 ^a

¹⁾ TO, tallow; CLA, conjugated linoleic acid. ²⁾ Mean±SE. (n=8 for each diet). ^{a, b} Mean values in the same row having different superscripts are significantly different (p<0.05).

The initial column temperature was programmed at 170°C and increased to 220°C at 2°C/min. The injector and detector were set at 250°C and 260°C, respectively. The peaks were identified by comparison with standard mixture of fatty acid methyl esters (Lipid standard and Linoleic acid methyl ester, *cis/trans*-isomers, Sigma Ltd., St. Louis, USA). Fatty acid composition of free fatty acid fraction was expressed as a weight percentage of total fatty acids.

The main effects between treated groups were subjected to ANOVA using the general linear models procedure of SAS (1986), and significant differences were determined using Duncan's multiple range test at the level of p<0.05 (Duncan, 1955). Percentage data were transformed to arc sine percentages before square root percentages ANOVA was performed.

RESULTS AND DISCUSSION

There were no significant differences in body weight gain, relative weights of leg and abdominal fat (g/100 g body weight) of chicks fed the experimental diets as shown in Table 2. Relative liver weights of chicks fed dietary CLA at 4 and 6% levels were significantly higher (p<0.05) than that of TO 6% group. The diet treatment did not have significant effects on feed intake and feed conversion rate.

The chemical compositions of leg muscle and liver of chicks fed the experimental diets are shown in Table 3. There were no significant differences in the contents of

moisture, crude protein, ether extract, ash, Ca, and P among the dietary groups. Crude protein content of leg muscle ranged from 20.99% to 21.62%. Although there was no significant difference, the fat content of leg muscle showed a decreasing trend as the dietary CLA levels increased. Liver lipid was significantly increased in chicks fed the diet containing CLA 6% than those of the TO 6% and TO 4%-CLA 2% group.

The lack of effect of dietary CLA on chemical composition in leg muscle was somewhat unexpected, because CLA supplementation has been reported to lower fat accumulation and to alter body partitioning (Dugan et al., 1997; Park et al., 1997). Dugan et al. (1997) reported that subcutaneous fat accumulation was decreased in pigs fed CLA. Du and Ahn (2002) also observed that fat content in whole carcass of broiler chickens was not influenced by dietary CLA level of up to 1%, but was significantly decreased after feeding 2 or 3% dietary CLA. It is assumed that differences in fat accumulation between species and breeds may account for the lack of effects of dietary CLA in this study using Hy-Line Brown male chicks. On the other hand, extractable total lipid in liver was significantly increased in chicks fed the diet containing CLA 6% than those of chicks fed the diet without CLA. Belury and Kempa-Streczko (1997) reported that CLA feeding increased the extractable total lipid in livers compared with mice fed diets without CLA. No comparable data on the influence of CLA in relation to hepatic lipid accumulation

Table 4. Effects of dietary CLA on the concentration of various lipid fraction and components in serum¹⁾

	TO6%	TO4%-CLA2%	TO2%-CLA4%	CLA6%
Total cholesterol, mg/dl	151.6±7.7 ²⁾	170.2±7.7	171.0±2.5	175.4±7.4
HDL-C, mg/dl	86.0±8.8	95.2±8.8	92.5±6.7	91.8±6.0
LDL-C, mg/dl	48.5±9.2	54.5±9.2	56.6±8.6	60.8±7.2
Triacylglycerol, mg/dl	85.8±9.8	112.8±9.8	109.4±2.1	111.4±5.4
Phospholipid, mg/dl	246.6±1.7	254.2±11.7	262.0±5.2	236.4±9.7
GOT, U/L	166.6±10.1 ^b	177.8±14.5 ^b	194.0±13.2 ^{ab}	224.6±10.8 ^a
GPT, U/L	10.0±3.2	8.4±1.9	9.9±2.1	8.9±2.5
Creatinine, U/L	0.5±0.1	0.5±0.0	0.5±0.0	0.5±0.0
BUN, mg/dl	5.8±1.0	6.3±1.0	6.2±0.9	5.9±0.9
Total bilirubin, mg/dl	0.4±0.0	0.4±0.0	0.4±0.0	0.3±0.0
Total protein, g/dl	6.6±0.2	6.6±0.2	6.8±0.2	6.5±0.2
Albumin, g/dl	1.6±0.2	1.8±0.1	1.9±0.0	1.8±0.1
Globulin, g/dl	5.1±0.3	4.8±0.2	4.9±0.1	4.6±0.1
Ca, mg/dl	11.6±0.6	12.8±0.7	11.9±0.4	12.0±0.4
P, mg/dl	11.2±0.7	10.4±0.4	10.6±0.4	10.6±0.3

¹⁾TO, tallow; CLA, conjugated linoleic acid; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase; BUN, blood urea nitrogen. ²⁾Mean±SE. (n=8 for each diet).

^{a, b} Mean values in the same row having different superscripts are significantly different (p<0.05).

Table 5. Effects of dietary CLA on the fatty acid composition of leg muscle¹⁾

Fatty acids ²⁾	TO6%	TO4%-CLA2%	TO2%-CLA4%	CLA6%
C14:0	0.86±0.01 ^{a3)}	0.88±0.02 ^a	0.69±0.12 ^b	0.67±0.14 ^b
C16:0	20.22±0.42	22.96±1.01	20.86±1.43	21.68±0.82
C16:0 ω-7	2.58±0.21 ^a	2.06±0.23 ^b	1.44±0.22 ^c	1.28±0.13 ^c
C18:0	10.62±0.30 ^b	12.90±0.21 ^a	13.08±0.73 ^a	13.89±0.31 ^a
C18:1 ω-9	31.03±0.72 ^a	25.11±1.02 ^b	22.71±0.42 ^c	19.68±0.43 ^d
C18:2 ω-6	17.54±0.61	16.74±0.32	17.14±0.84	16.65±0.54
C18:3 ω-6	0.16±0.10	0.08±0.01	0.10±0.02	0.16±0.11
C18:3 ω-3	0.64±0.01 ^a	0.60±0.11 ^a	0.47±0.01 ^b	0.45±0.02 ^b
<i>cis</i> 9- <i>trans</i> 11 CLA	nd ^{d4)}	2.49±0.42 ^c	4.18±0.34 ^b	5.85±0.34 ^a
<i>Trans</i> 10- <i>cis</i> 12 CLA	nd ^d	1.83±0.40 ^c	3.12±0.32 ^b	4.34±0.33 ^a
C20:3 ω-9	0.36±0.01	0.38±0.11	0.34±0.01	0.39±0.02
C20:3 ω-6	0.25±0.01	0.26±0.01	0.27±0.01	0.25±0.02
C20:4 ω-6	3.28±0.21 ^a	2.72±0.32 ^{ab}	2.43±0.23 ^b	2.38±0.32 ^b
Total SFA	31.54±0.42 ^b	36.90±0.91 ^a	34.68±2.02 ^{ab}	36.28±1.03 ^a
Total MUFA	32.61±0.93 ^a	27.17±1.15 ^b	24.15±0.63 ^c	20.96±0.40 ^d
Total PUFA	22.23±0.72 ^c	25.11±1.01 ^b	28.05±1.34 ^a	30.48±0.71 ^a
Total ω-6	17.06±0.61	17.08±0.31	17.51±0.82	17.06±0.62
Total CLA	nd ^d	4.32±0.83 ^c	7.30±0.73 ^b	10.19±0.53 ^a
C18:0/C18:1 ω-9	0.34±0.01 ^c	0.52±0.02 ^b	0.58±0.01 ^b	0.71±0.02 ^a

¹⁾TO, tallow; CLA, conjugated linoleic acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. ²⁾Number of carbon atom: number of double bonds, followed by the position of the first double bond relative to the methyl end. ³⁾Mean±SE. (n=6 for each diet).

^{a-d} Mean values in a row with no common superscript are significantly different (p<0.05). ⁴⁾nd, not detected.

in chicken were found in the literature. Thus, more research on this aspect of feeding CLA to chickens is needed to determine the reasons for changes in liver lipid.

The concentrations of various lipid fractions and components in serum of chicks fed the experimental diets are shown in Table 4. The dietary treatments did not have significant effects on total cholesterol, HDL-C, and LDL-C. The concentrations of triacylglycerol and phospholipid were not influenced by CLA feeding. The level of GOT in chicks fed diet containing CLA 6% was significantly higher than

those of the TO 6% and TO 4%-CLA 2% group. In addition, the level of GOT tended to be increased by CLA feeding. There were no significant differences in the levels of GPT, creatinine, BUN, blood proteins, Ca and P of chicks fed experimental diets.

CLA has been suggested by some animal studies to possess potent antiatherogenic effects (Lee et al., 1994). Nicolosi et al. (1997) found that CLA reduced plasma LDL-C concentration and development of atherosclerosis in hamsters. However, Benito et al. (1999) reported that CLA

supplementation for 63 d had no effect on plasma total cholesterol, LDL-C, HDL-C, and triacylglycerol in humans. Therefore, it is likely that the antiatherogenic effect by dietary CLA does not always occur because differences in the species and the type of diet used. There was significant difference in the serum GOT level from the four dietary groups; the level of GOT was highest with CLA 6% diet and lowest TO 6% diet. Serum GOT level is the most sensitive indicator of tissue damage in avian species (Lumeij, 1997). Feeding of diet containing CLA to the rats injected hepatoma resulted in marked elevation of serum GOT level (Yamasaki et al., 2001). Further studies are required to clarify whether the increase in serum GOT level is associated with the accumulation of hepatic lipid or not.

The fatty acid composition in leg muscle of chicks fed the experimental diets is presented in Table 5. The level of C18:0 (% of total methyl esters of fatty acids) in leg muscle of chicks fed tallow 6% was significantly lower than that of chicks fed CLA containing diets, whereas C18:1 ω -9 decreased linearly by CLA feeding. Therefore, the ratio of C18:0/C18:1 ω -9 significantly increased by elevating the levels of dietary CLA. As the dietary CLA levels increased, the levels of *cis* 9-*trans* 11 CLA and *trans* 10-*cis* 12 CLA were increased linearly. The relative proportion of CLA isomers was highest with CLA 6% diet. No CLA isomers were found in leg muscle from chicks fed the diet containing tallow only. The level of C20:4 ω -6 was highest with TO 6% diet and lowest with CLA 6% diet. Total monounsaturated fatty acids significantly reduced by CLA feeding, whereas total saturated and polyunsaturated fatty acids were significantly increased. The fatty acid profiles in liver were similar to those of leg muscle (data were not shown).

In the present study, the ratio of C18:0/C18:1 ω -9 in leg muscle was significantly increased by CLA feeding ($p < 0.05$). It is well known that CLA inhibits the actions of Δ -9 desaturase, the enzyme involved in the conversion of C18:0 to C18:1 ω -9 (Lee et al., 1998). Perhaps the increase of C18:0 in leg muscle of chicks fed CLA can be partially explained by the modification of Δ -9 desaturation. The dietary CLA was easily incorporated into edible meat at the expense of C18:1 ω -9, which confirmed many previous studies (Chamruspollert and Sell, 1999; Jones et al., 2000; Du and Ahn, 2002). Feeding animals synthetic CLA sources should be a good way to enrich beneficial CLA in food, and such poultry products could be valuable CLA sources for human consumption. But it was also observed that excessive amount of dietary CLA resulted in the possible negative effects on liver, such as increase of liver weight and accumulation of hepatic lipid. The reason for this finding requires further investigation.

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