Effect of Conjugated Linoleic Acid on Intestinal and Hepatic Antioxidant Enzyme Activity and Lipid Peroxidation in Broiler Chickens*

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ABSTRACT : The present study was designed to define whether dietary conjugated linoleic acid (CLA) could affect antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), and glutathione S transferase (GST), and the level of malondialdehyde (MDA), a marker of lipid peroxidation, in the small intestine and liver from broiler chickens. A total of twenty-four 3 wk-old male broiler chickens were assigned to three dietary treatments (1.5% corn oil, 0.75% corn oil plus 0.75% CLA, and 1.5% CLA, isocalorically), and fed a grower-finisher diet from 22 to 35 days. In the small intestinal mucosae, the specific activities of SOD, GSH-Px, CAT, and GST, and the level of MDA were not substantially influenced by dietary CLA. In the liver, the specific activities of SOD, GSH-Px, and GST, and the level of MDA were also unaffected by dietary CLA at the level of either 0.75% or 1.5% compared with corn oil at the level of 1.5%. However, the broiler chickens fed the diet containing 1.5% CLA resulted in a significant increase in peroxisomal CAT activity and a marked decrease in total lipid and non-esterified fatty acids (NEFA) from liver tissues compared with those fed the diet containing 1.5% corn oil. In conclusion, ability of CLA to increase hepatic CAT activity suggest that dietary CLA may affect, at least in part, antioxidant defense system as well as lipid metabolism in the liver of broiler chickens. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 8 : 1162-1167)***

Key Words : CLA, Broiler Chickens, SOD, GSH-Px, CAT, GST, MDA

INTRODUCTION

Conjugated linoleic acid, a group of positional and geometric isomers derived from linoleic acid, has attracted considerable attention for the last decade due to its myriad of biological actions against cancer, obesity, arteriosclerosis, and oxidative stress in several animal models (Ha et al., 1990; Whigham et al., 2000). Their beneficial effects have raised a great attention to obtain CLA-containing food, since the only small amounts of CLA is naturally existed in meat and dairy products of ruminants (Wang et al., 2002). Thus, the dietary inclusion of synthesized CLA from plant oils rich in linoleic acid (Chin et al., 1992) could be the efficient way to produce meat containing high level of CLA.

Recently, several studies have indicated that dietary CLA was associated with a reduction of fat in pigs and rodents (Rahman et al., 2001; Dunshea et al., 2002). Moreover, the improvement of meat color stability in CLA-fed chickens indicated that CLA might play a crucial role in the prevention of lipid peroxidation in tissues (Du et al., 2001). It has been also reported that lipid peroxidation of pork meat might be associated with the deposited level of CLA partially due to the lower polyenoic fatty acid composition (Joo et al., 2002). Thus, a beneficial effect of CLA-mediated anti-peroxidation was involved in the improvement of meat quality in domestic animals. By contrast, recent several studies with experimental animals have suggested that CLA had a more direct effect on the increased lipid peoxidation, which may contribute to the anti-tumorigenic and anti-obesity (Basu et al., 2000). These evidences provided that dietary CLA, at least somewhat, played a role in changes in antioxidant or lipid peroxidation, depending on the availability of CLA in tissues and circulation in animals. A systemic study regarding tissue distribution of CLA to pigs indicated that the liver is the major site to accumulate CLA absorbed through small intestine (Kramer et al., 1998). In addition, the mucosa of the small intestine was known to be extremely sensitive to free-radicals attacks (Jang et al., 2001). However, little is known about the underlying mechanisms by which dietary CLA modulates antioxidant enzymes and lipid peroxidation, especially in the digestive organs including the intestine and liver.

Therefore, to reveal the effect of dietary CLA on the antioxidant ability, the present study was conducted to examine the specific activity of antioxidant enzymes such as GSH-Px, SOD, CAT, and GST, and the level of MDA in the small intestine and liver from broiler chickens

MATERIALS AND METHODS

Experimental design

Male broiler chickens (ROSS) purchased from Halim,

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Table 1. Formula and chemical composition of experimental diets fed to broiler chickens

Item	Diets*		
	CORN	CLA I	CLA II
	Ingredients, %		
Corn	43.48	43.48	43.48
Wheat	19.70	19.70	19.70
Wheat bran	3.94	3.94	3.94
Animal fat	2.96	2.96	2.96
Corn gluten	3.94	3.94	3.94
Soybean meal (44% CP)	16.26	16.26	16.26
Rapeseed meal	1.97	1.97	1.97
Fish meal	0.99	0.99	0.99
Meat meal	1.97	1.97	1.97
Corn oil	1.50	0.75	-
Conjugated linoleic acid	-	0.75	1.50
Salt	0.23	0.23	0.23
Calcium carbonate	0.20	0.20	0.20
Tricalcium phosphate	1.58	1.58	1.58
Lysine (liquid)	0.65	0.65	0.65
Methionine	0.12	0.12	0.12
Choline-HCl	0.01	0.01	0.01
Vitamin premix[1]	0.20	0.20	0.20
Mineral premix[2]	0.20	0.20	0.20
Antibiotics	0.10	0.10	0.10
	Chemical composition, %		
Dry matter	88.71	89.70	89.51
Crude protein	19.35	19.24	19.64
Crude fat	7.50	7.33	7.23
Crude fiber	8.00	8.00	8.00
Crude ash	5.70	5.41	5.70

[1] contained per kg: vit. A, 5,500,000 IU; vit D3, 1,500,000 IU; vit E, 15,000 mg; vit K, 800 mg; thiamin, 1,000 mg; riboflavin, 4,000 mg; niacin, 25,000 mg; biotin, 30 mg; folic acid, 500 mg pantothenic acid, 5,000 mg, pyridoxine, 1,500 mg; vitamin B12, 15 mg.

[2] contained per kg: Cu, 12,000 mg; Fe, 35,000 mg; Zn, 25,000 mg; Co, 150 mg; I. 500 mg; Co, 150 mg; Se, 120 mg; Mn, 38,000 mg.

* CORN, corn oil 1.5%; CLA I, corn oil 0.75%+CLA 0.75%; CLA II, CLA 1.5%.

Korea were maintained under facility regulated temperature and light/dark cycle (light on 07:00-19:00). Forty eight male chickens (3 day old, 40.66±0.83 g) were fed a commercial diet *ad libitum* for 3 wks. After that, the selected 24 birds having similar body weight and gain were housed two in a steel wire cage, and randomly allotted to three dietary groups consisted of 1.5% corn oil (CORN), 0.75% corn oil +0.75% CLA (CLA I), and 1.5% CLA (CLA II) diets. Corn oil (CJ, Co. Ltd) and a mixture of CLA containing 24.1% *cis*-9, *trans*-11 isomer and 25.6% *trans*-10, *cis*-12 isomer (donated by Gyeongnam Province Advanced Swine Research Institute) were added to the basal diet, isocalorically according to the experimental design. All birds were fed corresponding experimental diets ad libitum for 2 wks, and had free access to water for entire trial. The formula and chemical composition of experimental diet are presented in Table 1. At the end of the feeding trial, eight birds per group were sacrificed by cutting jugular vein. The whole small intestine and liver were carefully removed.

Tissue harvest and preparation

The harvested small intestine was perfused with ice-cold saline, and gently squeezed to remove remaining digesta. After measuring intestinal weight, sixty percent of the upper region was designated as the proximal intestine, and the rest of the region as the distal part. The length of each segment was rinsed in three successive baths containing mannitol buffer (5 mM $MgCl_2$, 150 mM mannitol, 10 mM Tris succinate, 5 mM K_2HPO_4, and 1 mM $MnCl_2$; pH 7.4). The mucosal surface of the proximal and distal regions was removed by gentle scraping with a glass slide, and scrapings were mixed in an aluminum pan seated on a bed of ice. Residual fat and digesta were removed from harvesting mucosal scraping by resuspending twice in equal volumes of mannitol buffer followed each time by centrifugation at 4,500×g at 4°C for 10 min. Immediately after withdrawal of the whole liver, it was rinsed with ice-cold saline and blotted moisture. To isolate the fractions enriched in peroxisomes, microsomes, and cytosol from tissues, the mucosae and liver tissues were homogenized with 1:6 volume of mannitol buffer containing 2% triton X-100, and 0.25 M sucrose buffer, respectively in a glass-glass homogenizer. The crude homogenized mucosae and liver tissues were centrifuged at 600×g for 10 min, and the resulting supernatant was centrifuged at 10,000×g for 20 min to isolate the fractions enriched in peroxisomes and lysosomes from tissues. The remaining supernatant was recentrifuged at 105,000×g for 90 min in a Beckman ultracentrifuge. The pellets obtained from centrifugation were suspended in phosphate buffer containing 150 mM KCl (pH; 7.4) to adjust protein concentration. The obtained cytosol and suspended pellets were frozen liquid nitrogen, and stored at -70°C until assay.

Analytical assays

Protein was assayed by BCA method (Pierce Assay) using an ELISA (Molecular Devices). The activity of superoxide dismutase (Cu-Zn SOD) was determined in cytosolic fractions using xanthine and xanthine oxidase system for production of superoxide radical and subsequent measurement of cytochrome c as a scavenger of the radicals (McCord and Fridovich, 1969). The SOD activity was expressed as units/mg of proteins, where one unit of activity is the mounts of enzymes required to inhibit the rate of reduction of cytochrome c by 50%. Glutathione peroxidase (GSH-Px) was measured at 37°C with cumen hydroperoxide as a substrate (Tappel, 1978). The GSH-Px coupled the reduction of cumen hydroperoxide to the oxidation of NADPH by glutathione reductase and

Table 2. Effect of dietary CLA on weights of body, gains, and digestive organs from broiler chickens at the age of 35 days

Item	Diets*		
	CORN	CLA I	CLA II
Initial body weight (at 22 days), g	791.8±55.7	764.9±59.2	742.2±56.2
Final body weight (at 35 days), g	1,773.7±142.5	1,738.9±42.0	1,701.6± 110.7
Weight gains (22-35 days), g	981.9±120.9	974.0±35.6	959.5±100.5
Liver, g	38.05± 7.0	41.35± 5.09	40.64±4.84
Small intestine, g	30.44±3.11[ab]	32.80±3.68[a]	28.50±3.07[b]
Intestinal mucosae, g	18.42 ± 2.33	21.23±2.87	19.36±2.64

* CORN, corn oil 1.5%; CLA I, corn oil 0.75%+CLA 0.75%; CLA II, CLA 1.5%.

[a, b] Values (Mean±SD, n=8) with different superscripts differ significantly ($p<0.05$).

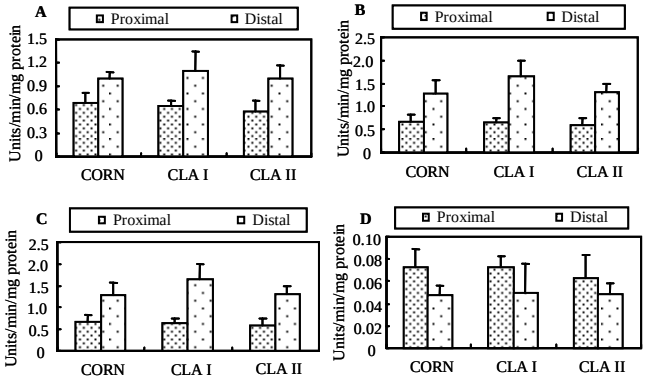

Figure 1. Effect of dietary CLA on the specific activities of SOD (A), GSH-Px (B), CAT (C), and GST (D) in the proximal and distal regions of the small intestine from broiler chickens at the age of 35 days. Bar represents mean±SD (n=8).

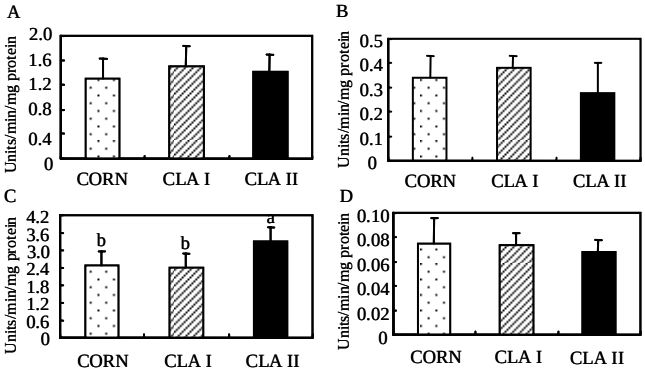

Figure 2. Effect of dietary CLA on the specific activities of SOD (A), GSH-Px (B), CAT (C), and GST (D) in the liver from broiler chickens at the age of 35 days. [a, b] Values with different superscripts differ significantly ($p<0.05$). Bar represents mean±SD (n=8).

concomitant oxidation is monitored in a spectrophotometer with the decrease in absorbance at 340 nm. One unit of GSH-PX is expressed as the amount of GSH-Px needed to oxidized 1 μmol of NADPH per min. Glutathione S transferase (GST) was determined with 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate, and monitored increase in absorbance at 340 nm (Habig et al., 1974). One unit of activity was expressed as the amount of enzyme catalyzing the conjugated CDNB per min. The catalase (CAT) activity was estimated by the decomposition of H_2O_2 to yield H_2O and O_2, and monitored changes in absorbance at 240 nm for 2 min (Aebi, 1974). One unit of activity was expressed as the amount of enzyme catalyzing the decomposition of one μM of H_2O_2 per min at 25°C and pH 7.0. The specific activity of all enzymes assayed was expressed as activity per mg protein. The estimation of lipid peroxidation in the microsomes was quantified by measuring the 2-thiobarbituric acid (TBA) reactive substances with spectrophotometer at 532 nm (Bidlack and Tappel, 1973). TBA-material is described as nM of malondialdehyde (MDA) per milligram of protein. Hepatic total lipid was estimated by extracting with chloroform-methanol (2:1) as described by Folch et al. (1957). Triglycerides were then analyzed spectrophotometrically as glycerol using an enzymatic assay kit (Sigma). Non-esterified fatty acid (NEFA) from extracted total lipid was measured spectrophotometrically using NEFA-Test kit (Wako).

Statistical analysis

Data on diet-associated difference were analyzed by PROC-GLM (SAS package, 1989) procedures appropriate for completely randomized designs. Mean differences among dietary treatments were evaluated by the Duncan multiple test at $p<0.05$.

RESULTS

Effect of CLA on weights of body and digestive organs

Weights of body, gains and digestive organs in broiler chickens fed dietary CLA are presented in Table 2. Without a statistical difference, the broiler chickens fed the diet containing 1.5% corn oil (CORN) showed 2.3% higher weight gains than those fed the diet containing 1.5% CLA (CLA II). Dietary CLA did not affect weights of final body, the liver, and small intestinal mucosae. However, small intestinal weight was significantly ($p<0.05$) lower in the birds fed 1.5% CLA diet than those fed 0.75% CLA plus 0.75% corn oil diet (CLA I).

Table 3. Effect of dietary CLA on the level of MDA (nM/mg protein) in the microsomes of the small intestine and liver from the broiler chickens at the age of 35 days

Item	Diets*		
	CORN	CLA I	CLA II
Liver	1.00±0.18	1.04±0.23	0.91±0.24
Proximal intestine	2.23±0.94	1.77±0.40	1.94±0.93
Distal intestine	6.13±1.90	6.25±2.98	6.64±1.64

* CORN, corn oil 1.5%; CLA I, corn oil 0.75%+CLA 0.75%; CLA II, CLA 1.5%. Mean±SD (n=8).

Table 4. Effect of dietary CLA on lipid contents of the liver from broiler chickens at the age of 35 days

Item	Diets*		
	CORN	CLA I	CLA II
Total lipid, %	2.87±0.70[a]	2.17±0.61[b]	2.10±0.32[b]
Triglyceride, mg/g	417.47±47.42	392.32±95.18	321.09±75.15
NEFA, mEq/l	1.46±0.1[a]	1.19±0.2[b]	1.18±0.12[b]

* CORN, corn oil 1.5%; CLA I, corn oil 0.75%+CLA 0.75%; CLA II, CLA 1.5%.

[a, b] Values (Mean±SD, n=8) with different superscripts differ significantly ($p<0.05$).

Effect of CLA on the specific activity of antioxidant enzymes and lipid peroxidation

To determine whether dietary CLA affects antioxidant ability in the small intestine and liver from the broiler chickens, we determined the specific activities of SOD, GSH-Px, SOD, CAT, and GST, and the level of MDA, a marker of lipid peroxidation. Figure 1 and 2, and Table 3 present changes in the specific activities of antioxidant enzymes and lipid peroxidation in the mucosae of the small intestine (proximal and distal regions) and liver from broiler chickens fed the diets containing either corn oil or CLA. In the small intestine, dietary CLA did not noticeably affect the specific activities of SOD, GSH-Px, CAT, and GST (Figure 1). Also, we did not observe a significant difference in MDA value in the proximal and distal intestine (Table 3). In hepatic tissues, there was no difference in the specific activities of SOD, GSH-Px, and GST in response to dietary CLA (Figure 2). Dietary treatments also did not influence on MDA level in liver microsomes (Table 3). However, a significant ($p<0.05$) increase in the specific activity of hepatic CAT was observed in the birds fed 1.5% CLA diet (CLA II) compared with those fed 0.75% CLA (CLA I) or 1.5% corn oil diet (CORN; Figure 2).

Effect of CLA on lipid contents in the liver

The effect of dietary CLA on hepatic lipid contents from broiler chickens is shown in Table 4. There were remarkably ($p<0.05$) lower total lipid and NEFA contents in the liver from the birds fed the diet supplemented with 1.5% CLA (CLA II) than those fed the diet supplemented with 1.5% corn oil (CORN). Dietary CLA did not markedly affect hepatic triglyceride level, although it tended to be numerically lower by 23% in the birds fed 1.5% CLA diet compared with those fed 1.5% corn oil diet.

DISCUSSION

The survey of the pertinent literatures suggested that diets supplemented with 1-1.5% levels of CLA exhibited the most desirable activity and induction of myriad responses in animals (Evans et al., 2002). However, the responses of dietary CLA to antioxidant defense system in tissues are still a matter of issues, although it has been known that dietary CLA reduced lipid peroxidation and improved the quality of meat colors in the carcass (Du et al., 2001; Joo et al., 2002). Moreover, the effect of dietary CLA on antioxidant capacity seemed to be various according to basal diet components, the feeding regimen, the composition of the CLA mixture, and even species (Stangle, 2000). It was reported that feeding dietary CLA showed the potential role of CLA as an antioxidant under a certain condition (Ha et al., 1990). The lipid peroxidation values and meat color stability of loins from pigs consumed CLA were markedly lower than those of controls (Joo et al., 2002). It could be postulated that the ability of CLA to decrease lipid peroxidation might be partly due to the result of CLA's ability to affect antioxidant defense system. In particular, Leung and Liu (2000) reported that *trans*-10, *cis*-12-CLA isomer showed stronger free radical scavenging capacity than *cis*-9, *trans*-11-CLA isomer, which suggests that discrepancies among the previous results on the properties of CLA may be partly due to the balance of the antioxidant properties of *trans*-10, *cis*-12-CLA and the prooxidant properties of *cis*-9, *trans*-11-CLA. Yu et al. (2002) also reported that individual CLA isomer differently affected its free radical scavenging actions. Especially, oxidative stress induced by lipid peproxidation in the membranes may affect antioxidant defense mechanisms, represented by antioxidant enzymes including GSH-Px, SOD, and CAT (Youdim and Deans, 1999). Excess oxidants are captured by SOD, GSH-Px, and CAT. First, SOD converts superoxide anion to hydrogen peroxide in a cellular antioxidant reaction (McCord and Fridovich, 1969). Thereafter, GSH-Px and CAT independently detoxify produced hydrogen peroxide (Josephy et al., 1997).

Contrary to above studies, it has been reported that dietary CLA induced both non-enzymatic and enzymatic lipid peroxidation *in vitro* and *in vivo* under certain conditions (O'Shea et al., 2000). Thus, increased lipid peroxidation by dietary CLA may contribute to the anti-tumorigenic effects in human and rodents (Basu et al., 2000; Kilian et al., 2002).

From our observation, dietary CLA at the level of either 0.75% or 1.5% did not induce alterations in the activities of SOD and GSH-Px, and lipid peroxidation from the small

intestine and liver, whereas broiler chickens fed 1.5% CLA diet showed a significant increase in hepatic CAT activity compared with those fed 1.5% corn oil diet. However, dietary CLA does not appear to act as a strong enzymatic antioxidant in the liver from broiler chickens, since activities of SOD and GSH-Px, and MDA value in tissues from the birds fed 1.5% CLA diet found to remain similar to those fed 1.5% corn oil diet. If dietary CLA has strong enzymatic antioxidant properties, CLA-induced antioxidant will be expected to change both GSH-Px and CAT activities simultaneously, which in turn lead to the reduction of lipid peroxidation in the liver from broiler chickens. Similar to our observation, Stangl (2000) reported that diet supplemented with 3% of CLA enhanced CAT activity in the liver and decreased cholesterol carrying lipoprotein, suggesting that CLA act as a hepatic peroxisome proliferator to affect lipid metabolism. The increased CAT activity in the peroxisome-enriched cell fraction of the liver may be associated with a proliferation of peroxisomes, which could lead to increase in energy expenditures via lipid oxidation (Stangle, 2000). It has been evidenced that dietary CLA increased peroxisomal activity, which might be associated with fat reduction in the carcass (Evans et al., 2002). Therefore, we examined hepatic lipid contents from the broiler chickens fed the diet containing CLA to understand the relationship between increased CAT activity and hepatic lipid metabolism. Total lipid and NEFA contents in the liver were significantly reduced in the broiler chickens fed 1.5% CLA diet compared with those fed 1.5% corn oil diet, indicating that CLA could be fat reducing agent through increased peroxisomal activity. In addition, that dietary CLA incorporated into membrane, and subsequently results in increasing saturated fatty acids and decreasing non-CLA polyunsaturated fatty acids in tissue lipids of rodents, poultry, and pigs following dietary CLA consumption (Bee, 2000; Stangle, 2000; Badinga et al., 2003). Thus, alteration in the composition of fatty acids in the membranes would lead to decrease the formation of lipid peroxidation products such as MDA (Joo et al., 2002).

Taken together, it is reasonable to assume that dietary CLA could result in changes in hepatic fatty acid composition and peroxisomal CAT activity, which may be also associated with decreasing free radicals in hepatic tissues. Therefore, it is speculated that dietary CLA may exert a favorable influence on antioxidant ability through enhancing CAT activity in the hepatic proxisomes.

In conclusion, the present study gave some evidences that the presence of CLA in tissues may affect, at least somewhat, the hepatic antioxidant defense system as well as lipid metabolism in the liver from broiler chickens. More detailed studies are necessary, however, to elucidate the effect of each CLA isomer on an antioxidant capacity and the relationship between antioxidant defense system and lipid metabolism.

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