Conjugated Linoleic Acid : More Than an Anticancer Factor

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Definition and Sources of Conjugated Linoleic Acid

Conjugated linoleic acid (CLA) is a collective term describing a mixture of positional and geometrical isomers of linoleic acid (LA, C18:2) involving 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 configuration (Eulitz et al., 1999). Conjugated linoleic acid is produced in the rumen as a result of incomplete biohydrogenation of LA as well as during the commercial manufacture of dairy products (Chin et al., 1992; Kepler et al., 1966). In the rumen, dietary lipids are rapidly hydrolyzed and the resulting unsaturated free fatty acids can undergo biohydrogenation by the rumen microorganisms (Figure 1). As a result, ruminant animals absorb mainly saturated fatty acids, and foods made from ruminants contain mainly saturated fatty acids, regardless of the fatty acid composition of the ruminant's diet. However, when biohydrogenation is not complete, CLA can escape the rumen and be absorbed from the gastrointestinal tract, thereby providing the peripheral tissues with various isomers of CLA (Kelly et al., 1998).

Conjugated linoleic acid is present in essentially all foods (Chin et al., 1992), but the principal dietary sources in human diets are dairy products and other foods derived from ruminant animals (Bartlet and Chapman, 1961; Chin et al., 1992). The predominant CLA in ruminant fats is the *cis*-9, *trans*-11 isomer which accounts for more than 80% of total CLA isomers in dairy products (Chin et al., 1992). It originates from CLA produced by the rumen bacteria as an intermediate in the biohydrogenation of LA (C18:2) or from tissue synthesis of CLA by Δ^9-desaturase conversion of *trans*-11 fatty acid. Milk fat concentration of CLA varies widely among dairy herds (Kelly et al., 1996; Kelly et al., 1998) and is affected by a number of dietary factors (Table 1) including the type and amount of fatty acid substrate (Noble et al., 1974), the forage to grain ratio (Gerson et al., 1985), and the nitrogen content of the diet (Gerson et al., 1983). Kelly et al. (1998) reported that feeding a high LA oil (sunflower) increased CLA concentration to 24.4 mg/g of milk fat compared with values of 13.3 and 1.7 mg/g of fat for high oleic (C18:1) and high linolenic (C18:3) acids, respectively. Recently, Dhiman and coworkers (1999) found that grazing cows had a 5.7 times higher concentration of CLA in milk than did cows fed diets containing preserved forage. In another study, Jiang et al.(1996) reported that, for a constant supply of LA, CLA content could be affected by the ratio of forage to concentrate. A review of country and seasonal variations in CLA concentrations of milk fat showed a range of 8.6 to 100 μmol/g (Riel, 1963). Seasonal variation was very marked, with values during the summer period often up to three to four times higher than winter values. These observations collectively suggest that,

given an adequate dietary intake of LA, dietary constituents which provide substrates for the optimal growth of bacteria producing LA isomerase will maximize CLA output. However, the recent observation (Kelly et al., 1998) that milk CLA concentrations vary substantially among individual cows (9.9 to 51.7 mg CLA/g of fat) that consume the same diet and are subjected to the same management regimen suggests that additional factors such as individual genetic regulation of rumen microflora may also operate in the control of ruminal CLA synthesis (Moore et al., 1993).

CLA and Prevention of Cancer

Interest in CLA as an anticarcinogen stemmed from the original observation by Pariza and colleagues that both raw and grilled ground beef contained a component that could inhibit mutagenesis (Ha et al., 1987). The inhibitor, which was later shown to possess anticarcinogenic properties (Pariza, 1997), was purified and identified as four isomers of LA with conjugated diene unsaturations (Ha et al., 1987). Dietary studies with rat mammary tumor models have established CLA as a potent anticarcinogen (Figure 2). In a seminal study, Ip et al. (1991) fed CLA to 37-d old rats two weeks before the administration of the carcinogen 7,12- dimethylbenz(a)anthracene (DMBA). Supplementation of a basal diet with 0.5, 1.0 or 1.5% CLA resulted in a reduction of tumor incidence of 17, 42 and 50%, respectively. A follow up study (Ip et al., 1994a) showed that when the dose of DMBA was halved and tumors took longer to occur, dietary CLA concentrations between 0.05 to 0.5% produced dose-dependent inhibition of tumor incidence. In another study (Ip et al. 1994b), short-term feeding of CLA from weaning (21 d of age) to time of carcinogen administration (50 d) also resulted in suppressed tumor production when either DMBA or methylnitrosourea (MNU) was used as carcinogens (Figure 3). Inhibition of mammary tumors by CLA was not influenced by the amount or type of fat in the diet (Ip et al., 1996). Using a different model, Visonneau et al. (1997) fed severely combined immunodeficient mice with 1% CLA two weeks before subcutaneous injection of human breast adenocarcinoma (MDA-MBA 468) cells. Dietary CLA inhibited local tumor growth by 73% and prevented metastatic spread to the lungs, peripheral blood and bone marrow. In cell culture studies, physiological concentrations of CLA inhibited the proliferation of human malignant melanoma colorectal and breast cancer cells (Shultz et al., 1992). In contrast, LA had no inhibitory effects on the cell lines.

Mechanisms by which CLA affects carcinogenesis are largely unresolved and may vary for different sites, age, duration of exposure and stage of carcinogenesis. Various studies suggest that CLA may act by antioxidant mechanisms (Ha et al., 1990; Ip et al., 1991), prooxidant cytotoxicity (Schonberg and Krokan, 1995), inhibition of nucleotide synthesis (Shultz et al., 1992), reduction of proliferative activity (Ip et al., 1994) and inhibition of both DNA-adduct formation (Zu and Schut, 1992) and carcinogen activation (Liew et al., 1995).

Effects of CLA on Milk Yield and Composition

Because of the potential health benefits arising from CLA consumption, there has

been a considerable research effort directed to increasing CLA content of milk and meat products through dietary manipulations (Parodi, 1999). Field experiments with lactating dairy cows have generally failed to detect stimulatory effects of dietary CLA on dry matter intake, milk yield and milk protein content (Baumgard et al., 2000; Chouinard et al., 1999; Dhiman et al., 2000; Tables 2 and 3). In contrast, trans-isomers of unsaturated fatty acids, whether derived from the diet or from incomplete biohydrogenation of unsaturated fatty acids, depress milk fat content (Griinari et al., 1998; Wonsil et al., 1994; Tables 2 and 3). In 1996, Romo and coworkers (1996) reported that abomasal infusion of trans-vaccenic acid (C18:1) decreased milk fat content from 4.1 to 3.1%. Similar results were later reported by Loor and Herbein (1998) who found that abomasal infusion of CLA reduced milk fat by 34% compared with LA. The CLA treatment resulted in significant reduction in the concentrations and yields of fatty acids with six to 16 carbons, indicating depressed de novo synthesis in the mammary gland. Using an *in vitro* model, Dawson and Herbein (1996) found that bovine mammary cell cultures incorporated CLA in an amount proportional to the amount available in the medium, and that de novo synthesis of palmitic acid and desaturation of stearic acid decreased as CLA uptake by the mammary cells increased.

In an effort to identify the CLA isomer that inhibits milk fat synthesis, Baumgard and coworkers (2000) examined milk fat contents and yields in Holstein dairy cows receiving an abomasal infusion of either skim milk, *c*9, *t*11 CLA supplement, or *t*10, *c*12 CLA supplement. The *t*10, *c*12 CLA supplement caused a 42% reduction in milk fat, whereas infusion of similar amounts of the *c*9, *t*11 CLA isomer had no effect on milk fat (Table 3). Consistent with these findings, Griinari et al. (1998) established that dietary-induced milk fat depression corresponds to an increase in milk fat content of *trans*-10 unsaturated fatty acid (C18:1), and suggested that this specific *trans* isomer or related metabolites might be responsible for fatty acid-induced milk fat depression.

Specific mechanisms by which CLA alters mammary lipid metabolism are not clear, but changes in milk fatty acid profiles suggest an inhibition of the pathways in de novo lipogenesis and Δ^9-desaturase activity (Baumgard et al., 2000; Choi et al., 2000; Loor and Herbein, 1998). The initial reaction in de novo synthesis of fatty acids in animal tissues is catalyzed by acetyl CoA carboxylase (ACC; Wakil et al., 1983), and this appears to be a point at which control can be exerted. Emken et al. (1987) reported that LA and its *trans* isomers Δ^9 *trans*, Δ^{12} *trans*) reduced the activity of ACC and other lipogenic enzymes in mouse liver. Together, these findings suggest that CLA-mediated suppression of milk fat is due mainly to inhibition of de novo lipogenesis in the mammary gland.

Effects of CLA on Body Composition

Dietary CLA has been shown to affect body composition in several animal species including mice, rats and pigs (Chin et al., 1994; Ostrowska et al., 1999; Park et al., 1997; West et al., 1998). In AKR/J mice, dietary CLA reduced body fat content by 22 to 40% without affecting energy intake (DeLany et al., 1999; Figure 4). These findings confirmed a previous report by Park et al. (1997) who suggested that the

reduction in body fat accumulation by dietary CLA was due to increased β-oxidation of fatty acids, as reflected by enhanced activity of the enzyme carnitine palmitoyl transferase-1 in fat pads and skeletal muscle. In another study, livers from CLA-fed rats produced more ketone bodies, but synthesized significantly less triacylglycerol and cholesterol than control rats (Sakono et al., 1999), suggesting that, in the rodent model, dietary CLA may lower blood and tissue lipid levels through enhanced oxidation of fatty acids at the expense of reesterification of fatty acids in the liver.

Using a food animal model, Dugan et al (1997) found that pigs fed CLA had reduced feed intake (5.2%), improved feed efficiency (5.9%) and had similar rates of gain relative to sunflower oil-fed pigs. In addition, pigs fed CLA deposited less subcutaneous fat (6.8%) and gained more lean tissue (2.3%) than pigs fed sunflower oil. In finisher pigs, feed efficiency was improved and fat deposition decreased linearly with increasing concentration of CLA in the diet (Ostrowska et al., 1999; Dunshea et al., 1998; Figure 5). The carcass lean tissue deposition was quadratic in nature and was maximized at 5 g of dietary CLA per kg of body weight. Taken together, these observations suggest that dietary CLA can be used as a nutrient partitioning agent that favors lean tissue deposition over fat accretion, further enhancing the quality and health benefit of animal products.

To identify the CLA isomer that induces body composition changes, Park et al. (1999) investigated the effects of CLA preparations, which were enriched for the *c*9, *t*11 CLA isomer or the *t*10, *c*12 CLA isomer, on body composition in mice. Consistent with the milk fat data (Baumgard et al., 2000), body composition changes (reduced body fat, enhanced body water, enhanced body protein, and enhanced body ash) were associated with feeding the *t*10, *c*12 CLA isomer. In contrast, the *c*9, *t*11 and *t*9, *t*11 CLA isomers did not affect these biochemical changes, suggesting that the *t*10, *c*12 CLA isomer is the physiologically active CLA isomer in tissue lipid metabolism.

CLA Modulation of Immune Response

Considerable evidence indicates that aging is associated with a decline in the immune response in mammals (Hausman and Weksler, 1985; Miller, 1994) and that intervention with antioxidant nutrients (e;g; Vitamin E, β-carotene and glutathione) can enhance the immune response in rodents and humans (Beharka et al., 1997; Furukawa et al., 1987; Wu et al., 1994). Based on the well documented antioxidant properties of CLA, Cook et al. (1993) hypothesized that this LA isomer may have an impact on the immune response in aging mammalian species. Chicks fed CLA and injected with the endotoxin lipopolysaccharide (LPS) continued to grow, whereas those not fed CLA either failed to grow or lost weight following LPS injection (Cook et., 1993; Figure 6). In addition, dietary CLA enhanced the phytohemagglutinin response and alleviated the catabolic effect of immune stimulation in rats (Cook et al., 1993; Figure 7). Using a different model, Hayek et al. (1999) found that young and old mice fed 1% CLA had greater splenocyte proliferation in response to concanavalin A and phytohemagglutinin A (PHA) than mice fed the control diet. Conjugated LA-supplemented young mice had significantly higher splenocyte interleukin-2 production than those fed the control diet.

These findings suggest that CLA is effective in preventing the catabolic effect of immune stimulation, and possesses a potent immunostimulatory effect in mammalian species. The potential of preventing the catabolic losses without affecting the generation of adaptive immunity could provide benefit to growth and development.

Summary

Because of the potential health benefits arising from CLA consumption, there is a considerable research effort directed to increasing the CLA content of milk and meat products. Conjugated linoleic acid is an intermediate in the biohydrogenation of linoleic acid by rumen bacteria with potent anticarcinogenic, antiatherogenic and antidiebetogenic actions in rodents and humans. Experiments using several animal models indicate that dietary CLA is a potent nutrient partitioning agent that favors lean tissue deposition over body fat accretion. Additionally, dietary CLA alleviates the catabolic response to immune stimulation, which could provide benefit to growth and development. While exact mechanisms by which CLA exerts its beneficial health and growth promoting effects are not fully elucidated, current experimental evidence indicates that dietary CLA is more than an anticancer factor and may provide a novel nutritional strategy for improving food animal milk and carcass characteristics through dietary management that is readily applicable to producers.

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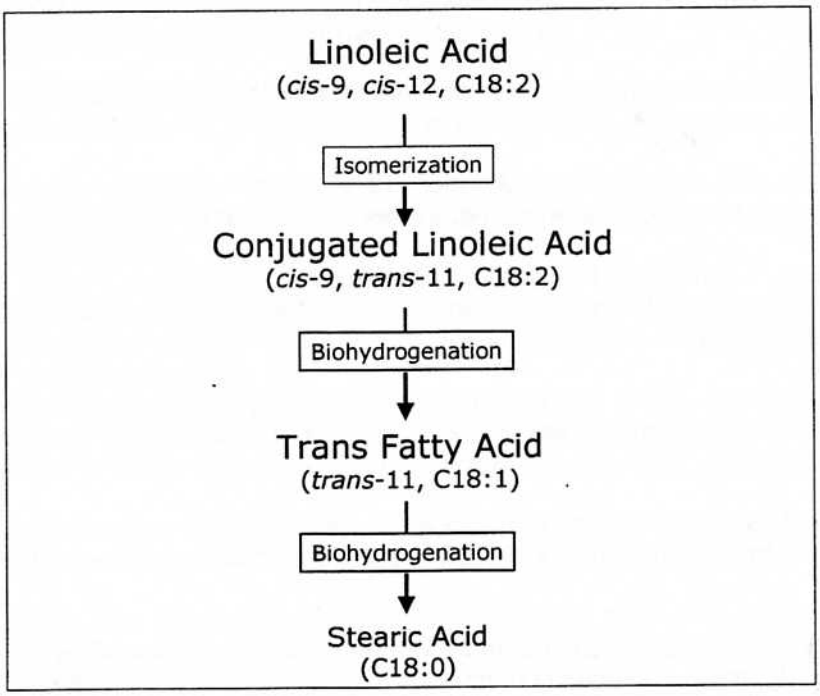

Figure 1. Pathway of biohydrogenation of linoleic acid to stearic acid by rumen microorganisms.

Table 1. Listing of some factors which affect CLA concentrations in milk fat

Dietary Factors	Effect on CLA content of milk
Rumen Environment	
Pasture vs TMR	Increased with pasture
Forage:Concentrate ratio	Increased with high F:C ratio
Growth stage of forage	Increased with less mature forage
Intake level	Increased by underfeeding
Nonstructural Carbohydrate level	Minor effect
Lipid Substrate	
Unsaturated vs Saturated fat	Increased by unsaturated fat
Level of plant oils	Increased by higher levels
Plant oil type	Greatest with oils high in C18:2
Ca salts of plant oils	Increased as with free oils
Fish oils	Increased in relation to level
Processing of soybeans	Increased over raw beans
High oil corn (grain and silage)	Minimal increase
Animal fat byproducts	minimal effect

Adapted from Bauman et al. (1996)

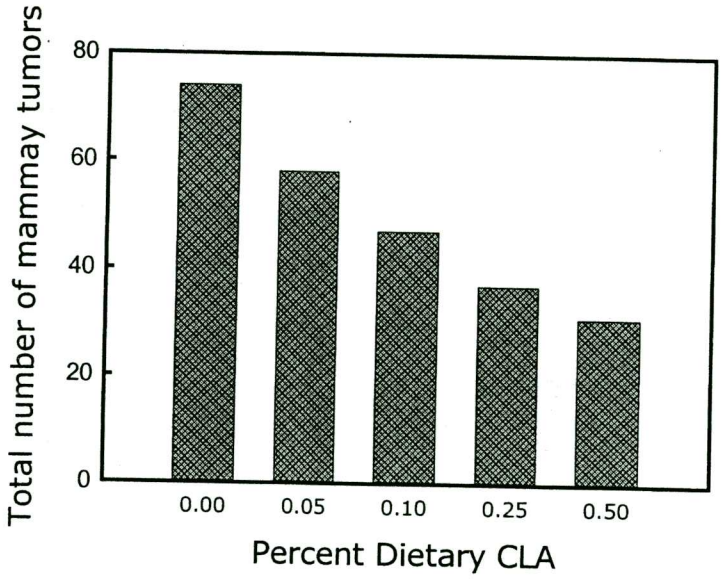

Figure 2. Mammary cancer prevention by dietary CLA supplementation in rats (Ip et al., 1994b).

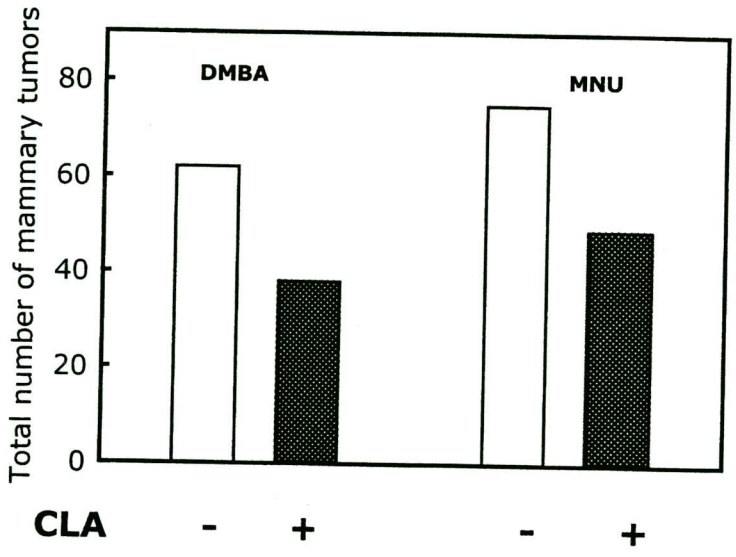

Figure 3. Effects of dietary CLA on DMBA- or MNU-induced mammary tumors in rats (Ip et al., 1994b).

Table 2. Dry matter intake, milk yield, and concentration and yield of milk constituents during abomasal infusion of different CLA supplements (Chouinard et al., 1999).

Variable	Treatment			
	Control	CLA-mix1	CLA-mix2	CLA-mix3
DMI, kg/d	21.1	22.1	20.7	19.6
Milk yield, kg/d	26.9	29.4	26.8	27.5
Fat				
%	3.34[a]	2.36[b]	2.43[b]	2.40[b]
g/d	883[a]	691[b]	633[b]	655[b]
CP				
%	3.14	3.04	3.15	3.03
g/d	831	882	829	826

[a,b]Means within row with different superscripts differ.
CLA-mix1 = 28.8 g/d; CLA-mix2 = 48.5 g/d; CLA-mix3 = 16.3 g/d.

Table 3. Performance of lactating dairy cows infused with either skim milk (control), *c*9,*t*11 CLA supplement or *t*10,*c*12 CLA supplement (Baumgard et al., 2000.

Variable	Treatment		
	Control	9,11 CLA Isomer	10,12 CLA Isomer
DMI, kg/d	24.6	24.4	20.2
Milk, kg/d	35.2	36.9	36.2
Milk fat			
%	3.04[a]	2.94[a]	1.92[b]
g/d	1068[a]	1008[a]	930[b]
Milk protein			
%	2.74[a]	2.73[a]	2.57[b]
g/d	965	1008	930

[a,b]Means within a row with different superscripts differ.

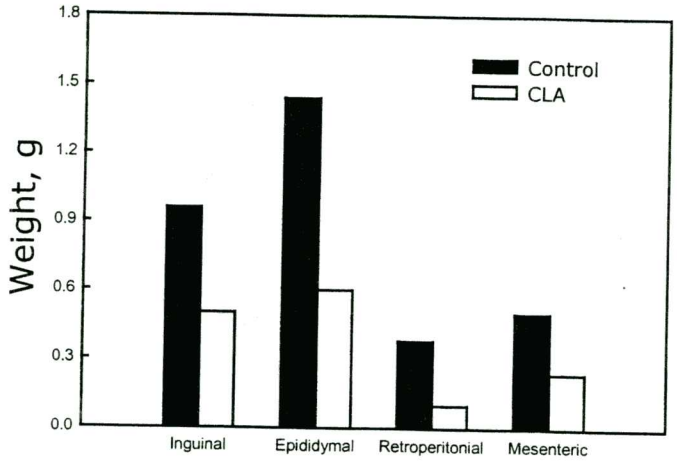

Figure 4. Weight of four adipose depots in mice fed a high fat diet containing 1% CLA or no CLA (West et al., 1998).

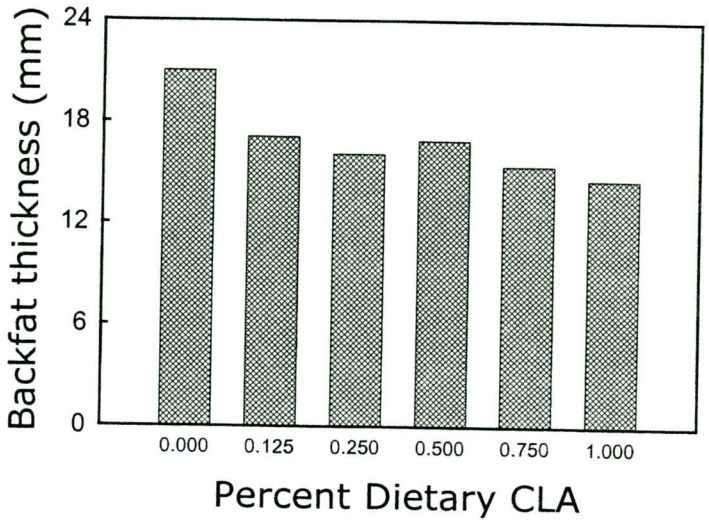

Figure 5. Effects of dietary CLA on body fat of growing gilts (Dunshea et al., 1998).

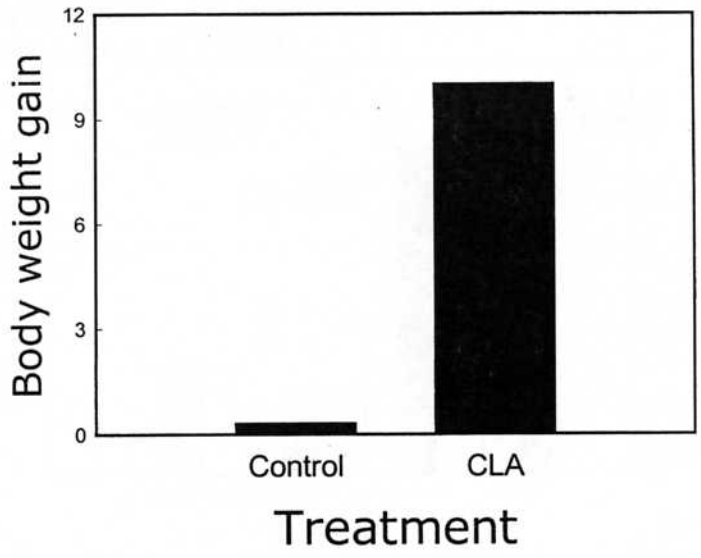

Figure 6. Effects of feeding CLA on the 24-h weight gain following lipopolysaccharide (LPS) injection in growing chicks (Cook et al. 1993).

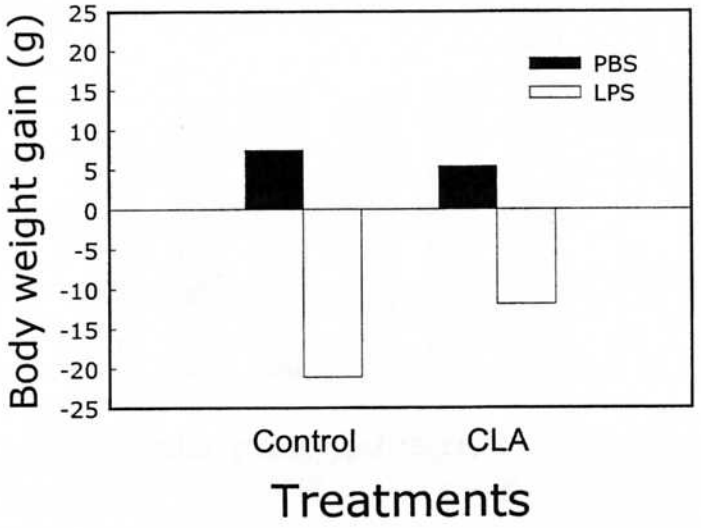

Figure 7. Dietary CLA alleviates the catabolic response to immune stimulation in rats (PBS = phosphate buffered saline as control; LPS = lipopolysaccharide) (Cook et al., 1993).