CONJUGATED LINOLEIC ACID: SOURCES, SYNTHESIS AND POTENTIAL HEALTH BENEFITS- AN OVERVIEW

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ABSTRACT: Conjugated linoleic acid is a group of positional and geometrical isomers of linoleic acid (C18:2, cis-9, cis-12), an essential fatty acid for human and animals. It is produced in ruminants via biohydrogenation of poly-unsaturated fatty acids and during the mechanical processing of dairy products. Meat and dairy products from ruminant animals (such as milk, butter, yogurt and cheese) are the principal natural sources of CLA in the human diet. Because of its reported biological activities (i.e. anticarcinogenic, antiatherogenic, immune-enhancing and weight-reducing properties) in animal models, CLA has become a subject of interest as a supplement for human nutrition. However, further research is required to check the efficacy and role of CLA, the good fat, in cancer and other disease prevention; and to form the basis of evaluating its effect in humans by observational studies and clinical trials.

KEYWORDS: CLA, Dairy, Health benefits, Milk

INTRODUCTION

Conjugated linoleic acid (CLA) is a collective term used to describe the different isomers (positional and geometric) of octadecadienoic (linoleic) acid. The two primary isomers of CLA are cis-9, trans-11 CLA (c9, t11-octadecadienoic acid) and trans-10, cis-12 CLA (t10, c12-octadecadienoic acid) (Pariza et al., 2000) as shown in figure 1. CLA occurs naturally in human plasma phospholipids, majority of dairy products and meats of different origin (Table 1). It is mainly synthesized by microbial biohydrogenation process in the rumen by bacteria (Butyrivibrio fibrisolvens) that produces linoleic acid isomerase; hence it is primarily found in ruminant and dairy products. Milk fat is the richest and natural dietary source of CLA (30mg/g), mainly the cis-9, trans-11 isomer (Parodi, 1997). In response to the anticarcinogenic and antioxidant effects (Cunningham et al., 1997; Pariza et al., 1991; Schonberg and Krokan, 1995), CLA has been widely recognized as a supplement for human nutrition. Since, CLA is a potential promoter of leanness, hence, cardiovascular health of consumer will be improved by lowered risk of developing diet-based disorders. CLA affects cancer at various stages of development, progression and metastasis via reduction in cell proliferation, vitamin A metabolism and prostaglandin metabolism. CLA significantly reduce prostaglandin E synthesis in mouse epidermis, ultimately inhibiting tumor formation. CLA also serves as a protection from catabolic effects of immune stimulation and its supplementation shows various responses on lipid profiles. CLA is also reported to have anti-diabetic effects by improving insulin sensitivity (Rainer and Heiss, 2004).

FIGURE 1. Chemical structure of the most common isomers of CLA (Gregory, 2001).
### TABLE 1. Total CLA (mg/g fat) content of different types of selected foods (Chin et al., 1992).

<table>
<thead>
<tr>
<th>Category of food</th>
<th>Total CLA</th>
<th>Percent of cis-9, cis-12 Isomer</th>
<th>Category of food</th>
<th>Total CLA</th>
<th>Percent of cis-9, cis-12 Isomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy Products</td>
<td></td>
<td></td>
<td>Meat (fresh)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homogenized Milk</td>
<td>5.5</td>
<td>92</td>
<td>Fresh Ground Beef</td>
<td>4.3</td>
<td>85</td>
</tr>
<tr>
<td>Butter</td>
<td>4.7</td>
<td>88</td>
<td>Beef Round</td>
<td>2.9</td>
<td>79</td>
</tr>
<tr>
<td>Sour Cream</td>
<td>4.6</td>
<td>90</td>
<td>Veal</td>
<td>2.7</td>
<td>84</td>
</tr>
<tr>
<td>Plain Yogurt</td>
<td>4.8</td>
<td>84</td>
<td>Lamb</td>
<td>5.6</td>
<td>92</td>
</tr>
<tr>
<td>Non-fat Yogurt</td>
<td>1.7</td>
<td>83</td>
<td>Pork</td>
<td>0.6</td>
<td>82</td>
</tr>
<tr>
<td>Ice-cream</td>
<td>3.6</td>
<td>86</td>
<td>Poultry (fresh)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheddar Cheese</td>
<td>3.6</td>
<td>93</td>
<td>Chicken</td>
<td>0.9</td>
<td>84</td>
</tr>
<tr>
<td>Cottage Cheese</td>
<td>4.5</td>
<td>83</td>
<td>Vegetable Oils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mozzarella</td>
<td>4.9</td>
<td>95</td>
<td>Safflower</td>
<td>0.7</td>
<td>44</td>
</tr>
<tr>
<td>Seafood (fresh)</td>
<td></td>
<td></td>
<td>Sunflower</td>
<td>0.4</td>
<td>38</td>
</tr>
<tr>
<td>Salmon</td>
<td>0.3</td>
<td>—</td>
<td>Canola</td>
<td>0.5</td>
<td>44</td>
</tr>
<tr>
<td>Shrimp</td>
<td>0.6</td>
<td>—</td>
<td>Corn</td>
<td>0.2</td>
<td>39</td>
</tr>
</tbody>
</table>

### BRIEF DEPICTION

CLA, apart from milk fat, is also found naturally in animal tissues and other food sources i.e. meats, poultry, eggs and dairy products (cheeses and yogurt) that have undergone heat treatments (Table 1). Though, vegetable fats are poor sources of CLA, yet, it is produced from linoleic acid in safflower and sunflower oil after special treatment of these oils. There are evidences that human milk contains CLA. The cis-9, trans-11 isomer of CLA, also known as rumenic acid (RA), is produced in the rumen via microbial metabolism of linoleic and linolenic acids. Another CLA isomer of ruminant tissues is trans-10, cis-12 CLA.

Most of the commercial CLA preparations mainly contain cis-9, trans-11 CLA and trans-10, cis-12 CLA along with smaller amounts of other isomers (trans-9, cis-11; cis-10, cis-12; trans-9, trans-11; trans-10, trans-12; and isomers with conjugated double bonds at positions 8, 10 and 11, 13 (Table 2). The different isomers produce different biological effects.

### MICROBIAL-MEDIATED BIOSYNTHESIS OF CLA IN RUMEN

CLA is synthesized as an intermediate via biohydrogenation of LA to stearic acid in the rumen by *Butyrivibrio fibrisolvens* and other ruminal bacteria (Kritchevsky, 2000; Kim et al., 2000; Fukuda et al., 2005; 2006). It may also be formed in the mammary gland by the endogenous conversion of t-11 C18:1 (transvaccenic acid, TVA), another intermediate of rumen biohydrogenation of LA or linolenic acid, by the desaturase enzyme (Corl et al., 2001; Griinari and Bauman, 1999) (Fig. 2). LA biohydrogenation starts with isomerization, where double bond at carbon-12 position is transferred to carbon-11 forming c-9, t-11 CLA, followed by rapid hydrogenation of cis-9 bond leaving behind TVA. The enzyme for conjugation of cis-9, cis-12 double bonds is linoleic acid isomerase (EC 5.3.1.5) bound to bacterial cell membrane (Griinari and Bauman, 1999) that requires a cis-9, cis-12 diene system and a free carboxyl group as substrate (Kepler et al., 1970). Similarly, LNA in the beginning is isomerized at cis-12 position to form c-9, t-11, c-15 C18:3 that is reduced at both the cis bonds for producing TVA.

Following this, a product-precursor relationship between TVA and CLA was observed with increasing concentrations of LA in the diet of a sheep; however, biohydrogenation of LA derived from triglyceride followed a different pathway (Noble et al., 1974). With a low fiber diet, a change occurred in the transoctadecenoic acid profile of milk and being trans-10 isomer predominant in fat (Griinari et al., 1998). This led to propose another pathway for the ruminal synthesis of t-10, c-12 CLA (Fig. 3) involving bacterial c-9, t-10 isomerase with the formation of a t-10, c-12 double bond as the first step (Griinari and Bauman, 1999). The cis-12, t-11 isomerase from *B. fibrisolvens* hydrogenate t-10, c-12 octadecadienoic acid (Kepler et al., 1966), thus producing t-10 octadecenoic acid. More than 50% of the LA is converted to t-10, c-12 isomer of CLA and only 10% is converted to t-10 octadecenoic acid by Propionibacterium (Verhulst et al., 1987). *Megapharma edenii* YJ-4 have also been found to produce t-10, c-12 isomer of CLA in the rumen (Kim et al., 2000). The t-10, c-12 isomer was formed from LA but not from either of the LNA as was the case with c-9, t-11 isomer of CLA. Mosley et al., (2002) have shown that oleic acid also forms various trans C18:1 including TVA during its biohydrogenation to stearic acid. Moreover, oils and seeds of peanuts, rapeseed, palm, canola, sunflower etc. contain higher proportion of oleic acid that may enhance the milk fat CLA from ruminants.

### SYNTHESIS OF CLA IN NON-RUMINANTS

Palquist and Santora (1999) have shown desaturation TVA to CLA in adipose tissue when pure TVA was fed to mice, even though the site of fat synthesis in non-ruminants is the liver. Banni et al., (2001) also demonstrated that feeding rats with increasing amounts of transvaccenic acid an in vitro system led to the formation of a variety of trans C18:1 isomers including CLA.

![FIGURE 2. Pathway for CLA synthesis in the rumen and small intestine of cow (Bell and Kennelly, 2000).](image-url)
 TABLE 2. Different types of milk fat CLA isomers (Bauman et al., 2000).

<table>
<thead>
<tr>
<th>cis/ transisomers of CLA</th>
<th>Percent of total CLA</th>
<th>cis/ transisomers of CLA</th>
<th>Percent of total CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>9, 11</td>
<td>76.5</td>
<td>11, 13</td>
<td>0.4</td>
</tr>
<tr>
<td>7, 9</td>
<td>6.7</td>
<td>8, 10</td>
<td>0.3</td>
</tr>
<tr>
<td>10, 12</td>
<td>1.1</td>
<td>cis, cis</td>
<td>4.8</td>
</tr>
<tr>
<td>12, 14</td>
<td>0.8</td>
<td>trans, trans</td>
<td>9.4</td>
</tr>
</tbody>
</table>

of TVA resulted in a progressive increase of tissue CLA. Corl et al., (2003) showed that conversion of dietary TVA to CLA is a dose-dependent accumulation of CLA in the mammary fat-pad of rats suggesting that endogenous synthesis of CLA occur using TVA as precursor. In lactating mice fed dietary supplementation of TVA, Loor et al., (2002) showed an increased CLA in blood, plasma, milk, tissue lipids and carcass of the pups suckling these mothers. This might have been related to increased activity of desaturase in the mammary tissue, suggesting adipose tissue to be the major site for the bioconversion of CLA from TVA.

Synthesis of CLA from TVA has been shown to occur in humans too and several species of bacteria derived from the human intestine can synthesize CLA (Coakley et al., 2003; Alonso et al., 2003). However, the amount of CLA synthesized endogenously or from intestinal bacteria is not estimated in humans or non-ruminants. Given the fact that humans or other non-ruminants do not have an appreciable amount of bacteria in their digestive system indicates that endogenous synthesis could be the major source. Very little or no CLA in chickens (Raes et al., 2002; Yang et al., 2002) and swine (Chin et al., 1993) was detected.

Low concentrations of CLA in human blood and tissues may be accounted for via two possible pathways (Cawood et al., 1983; Harrison et al., 1985) i.e. (i) in vivo from free radical-mediated oxidation of LA; and (ii) may be derived from dietary sources. Blood CLA have been increased in human by feeding CLA-rich diets. Huang et al., (1994) reported an increase in plasma CLA form 19% to 27% after a four weeks feeding of cheddar cheese, however, no appreciable changes in linoleic and arachidonic acids, cholesterol or phospholipid levels were observed. Such findings may be of significance in varying CLA content in biological fluids by altering specific dietary foods and fatty acids as sources of CLA and, thereby, protecting against cancer and other dreaded diseases.

MICROORGANISMS INVOLVED IN CLA SYNTHESIS

CLA formation in rumen has been mainly associated with bacterial activity; however, CLA content of protozoa is much higher than bacteria, suggesting that protozoa may be responsible for a high proportion of CLA (Devillard et al., 2004). Among 15 isomers of CLA, ruminal bacteria only produce significant amounts of the cis-9, trans-11 and trans-10, cis-12 isomers (Griinari and Bauman 1999). The ruminal bacterium Butyribrio fibrisolvens has been used as a model for cis-9, trans-11 CLA production (Kim et al., 2000; Fukuda et al., 2005; 2006), but this bacterium does not produce the trans-10, cis-12 isomer (Kepler and Tove 1967; Kim et al., 2000). Microbial CLA production, in addition to rumen microflora, has also been reported in Propionibacteria used as dairy starter cultures (Jiang et al., 1998), strains of the intestinal flora of rats (Chin et al., 1994), and some lactic cultures (Lin et al., 1999). Jiang et al., (1998) reported the formation of CLA from linoleic acid by Propionibacterium freudenreichii. Lin et al., (1999) and Ogawa et al., (2001; 2005) reported the production of CLA from free linoleic acid by Lactobacillus acidophilus. Kishino et al., (2002) found that Lactobacillus plantarum formed high levels of CLA from free linoleic acid upon extended incubation. Bifidobacteria also produce CLA, mainly the cis-9, trans-11 isomer (Coakley et al., 2003). Several strains of probiotic Lactobacilli and Bifidobacteria are capable of converting linoleic acid to CLA (Alonso et al., 2003; Ogawa et al., 2005; Yadav et al., 2007a).

BIOLOGICAL ATTRIBUTES OF CLA

Anticarcinogenic activities: The recent interest in CLA began with the isolation from hamburger meat as an anticarcinogenic factor. CLA has been repeatedly shown to have anticarcinogenic effects in animal models for stomach neoplasia (Ha et al., 1990), mammary tumors (Briggs et al., 1996), and skin papillomas (Belury et al., 1996). CLA is effective in reducing the size and metastasis of transplanted human breast cancer cells and prostate cancer cells in severely compromised immunodeficient mice (Cesano et al., 1998). This property of CLA is in sharp contrast to the pro-cancer activity associated with feeding linoleic acid (Scimeca, 1999). Also, CLA was reported to inhibit the in-vitro proliferation of human malignant melanoma, colorectal and breast cancer cells (Shultz et al., 1992). CLA-enriched butterfat was reported to alter mammary gland morphogenesis and to reduce the risk of cancer in rats (Banni et al., 1999). A study suggested that CLA might act by antioxidant mechanisms (Ha et al., 1990), others suggested inhibition of nucleotide synthesis (Shultz et al., 1992) or inhibiting both DNA-adduct formation (Zu and Schut, 1992) and carcinogen activation, as opposed to direct interaction with the pro-carcinogen, scavenging of electrophiles or selective phase I detoxification pathways (Liew et al., 1995). However, there might be more than one mechanism by which CLA influences carcinogenesis (Pariza et al., 2000).

Fat reducing activity: In general, fatty acids with trans- double bonds have a negative impact on lipid metabolism and depress the amount of milk fat (Wonsil et al., 1994). Dietary CLA has been shown to increase the level of total saturated fatty acids (mainly C14:0, C16:0 and C18:0) whereas monounsaturated fatty acids (mainly C18:1, n-9) are decreased due to regulation of stearoyl-CoA desaturase activity by t-10, c- 12 CLA isomer (Lee et al., 1998). Chouinard et al., (1999) reported that CLA infusion into abomasum of cows had no effect on milk protein, but reduced the content and yield of milk fat. Dietary CLA was shown to decrease subcutaneous fat depot and increase lean body mass in pigs fed a cereal based diet containing CLA compared to pigs fed control diet (Dugan et al., 1997). Studies conducted in dairy cows demonstrated that abomasal infusion of CLA decreased milk fat yield. Those effects may be due to CLA isomers containing a t-10 double bond (Baumgard et al., 2000). In another study, t-10, c-12 CLA was shown to result in a reduction in milk fat ranging from 25% with abomasal infusion of 3.5 g/d to 50% with 14.0 g/d (Baumgard et al., 2001). CLA was shown to
reduce body fat mass in animals. In a study conducted in obese humans, CLA was shown to reduce body fat mass and that no additional effect on body fat mass was achieved with doses >3.4 g CLA/d (Blankson et al., 2000).

**Antitherogenic activity of CLA:** While considerable research has focused on a potential anticarcinogenic effect of CLA, there are few studies indicating that CLA may also reduce the risk of cardiovascular diseases in animal models (Lee et al., 1994; Nikolosi et al., 1997). A study conducted in rabbits showed that dietary CLA resulted in a marked decline in the levels of total plasma cholesterol, triacylglycerol, and the ratio of LDL to HDL cholesterol (Lee et al., 1994). Similar results on cholesterol metabolism were reported in hamsters fed CLA, who had lower levels of total plasma cholesterol, non-HDL cholesterol, and triacylglycerol compared to control-fed hamsters. Recently, a study conducted in hamsters showed that a mixture of CLA influenced body weight gain and plasma lipids (Gavino et al., 2000). In this study, the three experimental diets fed to the hamsters consisted of the mild atherogenic diet plus CLA mixture at 10g/kg diet (CLA group), c-9, t-11 CLA at 2g/kg diet (c-9, t-11 group) or linoleic acid at 2g/kg diet (LA group). The CLA mixture was reported to decrease the levels of plasma triacylglycerol, total cholesterol and non-HDL cholesterol significantly, but not the c-9, t-11 CLA group (Gavino et al., 2000). Munday et al., (1999) reported that CLA actually did not reduce the incidence of atherosclerosis, but increased the incidence of fatty acid streaks in mice compared to control-fed mice. The mice fed CLA had significantly lower concentration of serum triacylglycerol and a significantly higher ratio of serum HDL-cholesterol: total cholesterol (Munday et al., 1999). The addition of CLA to the atherogenic diet actually was shown to increase the development of aortic fatty streaks. It is possible that dietary CLA has different influences on the fatty acid and cholesterol metabolism in different animal species. More research is necessary to elucidate the effects of dietary CLA on lipid metabolism and atherogenesis in animal models and eventually human beings.

**CLA and diabetes:** Feeding of CLA to rats prone to developing diabetes normalized glucose tolerance and improved hyperinsulinemia as effectively as currently used medications (Houseknecht et al., 1998). The study was short-term and needs to be replicated and extended before the results can be applied to human health. Recently, a significant production of CLA was shown to increase energy expenditures by animals through increased energy intake. CLA has been demonstrated to induce a relative decrease in body fat and an increase in lean body mass in several species of growing animals including mice, rats, hamsters, and pigs (Chin et al., 1994; DeLany and West, 2000; Gavino et al., 2000). When mice were pair-fed high fat diets with or without CLA for 12 weeks, body fat was significantly reduced in the animals fed CLA compared to the controls (DeLany et al., 1999). CLA reduced body fat within two weeks, and increased carcass protein content without affecting the animals’ food or energy intake. CLA favorably influenced body composition in another investigation in which mice were pair fed either a high fat or low fat diet with or without CLA (1%) for six weeks, where CLA also reduced body fat independent of dietary fat intake (West et al., 1998). Recent findings in mice and hamsters indicate that the trans-10, cis-12 CLA isomer is largely, if not totally, responsible for CLA’s effect on body composition (Park et al., 1997). However, there is little evidence regarding CLA’s effect on body composition in humans.

**Effect of CLA on energy intake:** Numerous feeding trials have shown that rats, mice and chickens fed CLA enhanced diets tended to consume less feed for equivalent or greater weight gain (MacDonald, 2000). The reduced intakes of food associated with the intake of CLA are not considered to be significant enough to account for the observed reduction in fat deposition (Wang and Jones, 2004). Along with reducing energy intake, CLA has been shown to increase energy expenditures by animals through increased metabolic rate (West et al., 2000). This increase in activity is in part responsible for the decreased adipose deposition observed in CLA-treated animals (Wang and Jones, 2004). Increased energy expenditure has been linked to CLA’s effects on uncoupling protein 2 (UCP2), a major uncoupling protein of white adipose tissue.

**PURPORTED MECHANISM OF ACTION**

The mechanism(s) of actions of CLA are not clearly understood. To reach a better understanding, it is necessary to determine the specific, possibly varying, effects of the different isomers. For example, the CLA-associated body composition changes observed in animals appear to be associated mainly with the trans-10, cis-12 CLA isomer (DeLany and West, 2000; Gavino et al., 2000). In mouse tissue culture, the trans-10, cis-12 CLA isomer was found to reduce lipoprotein lipase activity and concentrations of...
isomers, alone or in combination, will become clearer. These materials, the effects and mechanisms of actions of these enzymes to produce the major isomers are being cloned in bacteria, and it is expected that with the availability of larger amounts of anti-atherogenic effects of CLA reported in animal studies. The account, in part, for the anti-carcinogenic, lipid-lowering and 1998). Activation of PPAR-gamma and/or PPAR-alpha may modulate eicosanoid activity as well as the activity of such cytokines as tumor necrosis factor-alpha (MacDonald, 2000). It is also speculated that activation of peroxisome proliferator-activated receptor-gamma (PPAR-gamma) may play some role in the putative anti-diabetic activity of CLA (Houseknecht et al., 1998). Activation of PPAR-gamma and/or PPAR-alpha may account, in part, for the anti-carcinogenic, lipid-lowering and anti-atherogenic effects of CLA reported in animal studies. The enzymes to produce the major isomers are being cloned in bacteria, and it is expected that with the availability of larger amounts of these materials, the effects and mechanisms of actions of these isomers, alone or in combination, will become clearer.

TABLE 3. Dietary factors which affect CLA in milk fat (Bauman et al., 2001).

<table>
<thead>
<tr>
<th>Dietary Factor</th>
<th>Effect on CLA Content of Milk Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipid Substrate</strong></td>
<td></td>
</tr>
<tr>
<td>Unsaturated vs saturated fat</td>
<td>Increased by addition of unsaturated fat</td>
</tr>
<tr>
<td>Type of plant oil</td>
<td>Greatest with oils high in C18:2</td>
</tr>
<tr>
<td>Level of plant oil</td>
<td>Dose dependent increase</td>
</tr>
<tr>
<td>Ca salts of plant oils</td>
<td>Increased as with free oils</td>
</tr>
<tr>
<td>Fat in animal byproducts</td>
<td>Minimal effect</td>
</tr>
<tr>
<td><strong>High oil plant feeds</strong></td>
<td></td>
</tr>
<tr>
<td>high oil corn</td>
<td>Minimal effect</td>
</tr>
<tr>
<td>soybeans</td>
<td>Heat processing will increase</td>
</tr>
<tr>
<td>Rapseed vs soybean</td>
<td>Similar effect</td>
</tr>
<tr>
<td><strong>Modifiers of Biohydrogenation</strong></td>
<td></td>
</tr>
<tr>
<td>Forage:concentrate ratio</td>
<td>Increased with high ratio</td>
</tr>
<tr>
<td>Non structural carbohydrate level</td>
<td>Minor effect</td>
</tr>
<tr>
<td>Restricted feeding</td>
<td>Increased with restricted</td>
</tr>
<tr>
<td>Fish oils</td>
<td>Greater increase than with plant oils</td>
</tr>
<tr>
<td>Monensin - ionophore</td>
<td>Variable effect</td>
</tr>
<tr>
<td>Dietary buffers</td>
<td>Little effect</td>
</tr>
<tr>
<td><strong>Combination</strong></td>
<td></td>
</tr>
<tr>
<td>Pasture vs conserved forages</td>
<td>Higher on pasture</td>
</tr>
<tr>
<td>Growth stage of forage</td>
<td>Increased with less mature forage</td>
</tr>
</tbody>
</table>

FACTORS AFFECTING CLA CONTENT IN MILK
The CLA content in milk fat can be affected by a cow’s diet, breed, age, non-nutritive feed additives, such as ionophores, and by the use of synthetic mixtures of CLA supplements (Table 3). Among these factors, the diet is known to strongly influence the CLA content of milk and includes feedstuffs such as pasture, conserved forages, plant seed oils, cereal grains, marine oils and feeds, and animal fat.

A number of studies have shown the positive effects of pasture-based diets on the CLA content of milk fat. Dhiman et al. (1999) reported that cows grazing pasture had 500% higher CLA content in milk fat compared to cows fed a diet containing 50% conserved forage (hay and silages) and 50% grain. About 48 to 56% of the total fatty acids (FA) in fresh forages consist of C18:3 (Bauchart et al., 1984). Fresh grass supplies C18:3 FA as a substrate for ruminal biohydrogenation. Besides this, the high concentrations of soluble fiber and fermentable sugars present in fresh grass may create an environment in the rumen without lowering the ruminal pH that is favorable to the growth of the microbes responsible for CLA and TVA production. Cows supplemented with 0, 6, or 12 kg/d of grain on pasture had 2.21, 1.43, and 0.89% CLA in milk fat, respectively (Dhiman et al., 1999). Forage maturity and method of preservation also seem to be important factors influencing the CLA content of milk. Cows fed immature forages have higher levels of CLA in milk than cows fed mature forage. Cows fed grass silage cut at early heading, flowering, and second cutting had 1.14, 0.48, and 0.81% CLA in milk fat, respectively (Chouinard et al., 1998). The high C18:3 content of immature grass and its low fiber content compared to mature grass probably interact to increase the production of CLA and TVA.

Feeding plant seed oils, such as sunflower, soybean, peanut, canola, and linseed also increases CLA content in milk (Kelly et al., 1998; Dhiman et al., 1999; Loor and Herbein, 2003), as these oils are rich in C18:2 and C18:3 FA. There are a number of other research reports suggesting that feeding processed soybeans, canola, or flax seeds to dairy cows was more effective at increasing milk CLA content than feeding unprocessed seeds (Stanton et al., 1997; Chilliard et al., 2000; Ward et al., 2002; 2003). Besides, the feeding of fish oil has been shown to enhance the CLA contents of milk fat, but reduced total fat content of milk (Offer et al., 1999). The inclusion of marine feeds, such as fish meal or sea algae, into dairy cow diets has also been shown to enhance the CLA content of milk (Dhiman et al., 1999; 2000; Abu-Ghazaleh et al., 2001. In some studies, fish oil/fish meal was more effective at increasing milk CLA content than feeding unprocessed seeds (Stanton et al., 1997; Chilliard et al., 2000; Ward et al., 2002; 2003). Besides, the feeding of fish oil has been shown to enhance the CLA contents of milk fat, but reduced total fat content of milk (Offer et al., 1999). The inclusion of marine feeds, such as fish meal or sea algae, into dairy cow diets has also been shown to enhance the CLA content of milk (Dhiman et al., 1999; 2000; Abu-Ghazaleh et al., 2001. In some studies, fish oil/fish meal was more effective at enhancing the CLA content of milk than adding similar amounts of soybean oil or combinations of fish oil and soybean oil through extruded soybeans or soybean meal (Ramaseswamy et al., 2001; Abu-Ghazaleh et al., 2001; 2002; Whitelock et al., 2002). Fish or marine oils are usually rich in long chain PUFA. It has been shown that the CLA:TV ratio is lower in milk fat from cows fed fish oil compared to milk.
fat from cows that are fed plant oils (Chilliard et al., 2000). These findings suggest that a large amount of TVA is produced in the rumen of cows fed fish oil and may exceed the desaturation capacity of desaturase in the mammary gland, (Harfoot and Hazelwood, 1988) resulting in high levels of TVA in milk fat. It is also possible that certain fatty acids (especially PUFA) from fish oil may inhibit the desaturase activity in the mammary gland. However, the reduction of milk fat percentage is a common problem when feeding fish oil to lactating dairy cows and can influence the total CLA yield (Donovan et al., 2000). Feeding fish oil to dairy cows results in the production of t-C18:1 FA in the rumen. There is a positive correlation between the concentrations of t-C18:1 FA and milk fat depression (Grinari and Bauman, 1999). Specifically, the t-10 C18:1 isomer has been shown to decrease milk fat content and milk fat depression (Grinari and Bauman, 1999). Supplementing dairy cattle diets with animal fat has the potential to increase the CLA content in milk. Animal fat may sometimes be a source of TVA and CLA that could ultimately become sources of CLA in the mammary gland. In general, fat of ruminant origin is high in C18:1 and C16:0 FA (Jones et al., 2000).

Cow management systems also influence the CLA content of milk (Riel, 1963; Jahreis et al., 1999; Mackle et al., 1999; Lock and Garnsworthy, 2003). Jahreis et al., (1999) collected milk samples over a period of one year from three farms with different management systems: 1) conventional farming with indoor feeding using preserved forages; 2) conventional farming with grazing during the summer season; 3) ecological farming with no use of chemical fertilizers to produce forages and grazing during the summer season. The CLA content was 0.34, 0.61, and 0.80% of fat in milk from cows fed indoors, grazed during summer, and cows grazed in ecological farming conditions, respectively. Reasons for these results could be due to differences in vegetation or forage quality among the three systems. Therefore, most of the time, differences in CLA content of milk from cows under different management systems are actually due to the differences in feedstuffs produced under different management styles. Dairy cow's breed can also influence the CLA content of milk. For e.g., Montbeliard cows displays a tendency to have higher CLA in milk fat compared to Holstein-Friesian or Normande cows grazing on pasture (Lawless et al., 1999). Holstein-Friesian cows have higher CLA content in milk compared to Jerseys fed diets containing conserved forages and grains (Dhiman et al., 2002). The average difference in CLA content of milk fat among Brown Swiss, Holstein-Friesian, and Jersey breeds is 15 to 20% when fed similar diets. Brown Swiss cows have inherently higher CLA in milk fat, followed by the Holstein-Friesian and Jersey breeds. Preliminary work by Medrano et al., (1999) shows that there are differences between Brown Swiss, Holstein-Friesian, and Jersey breeds with respect to the activity of the mammary enzyme stearoyl Co-A desaturase, that oxidizes C16:0 and C18:0 to C16:1 and C18:1 and is involved in CLA production. Further understanding of the activity of the desaturase enzyme may offer an explanation as to why there are breed differences in milk fatty acid composition, including CLA.

Moreover, the cow's age can also influence the CLA content of milk. For e.g., older cows (>7 lactations) had higher CLA in milk than younger cows (1–3 lactations) (Lal and Narayanan, 1984). Age differences in milk fat CLA content could be due to differences in desaturase enzyme activities and/or fatty acid metabolism and synthesis between older and younger cattle. Further research is needed to understand the mechanisms involved in differences in CLA production with age of the cow. The CLA content in milk varies from cow to cow, even when the same diet is fed, and these differences could be simply due to the differences in desaturase enzyme activities in the mammary gland, age of animals, disease conditions, differences in ruminal metabolism, or other unknown factors. It has also been reported that feeding ionophores inhibits the growth of gram-positive bacteria, which are involved in ruminal biohydrogenation (Fellner et al., 1997). The variability in results due to the addition of ionophores could be related to dose used or a decrease or a modification in the population of bacteria responsible for biohydrogenation.

**APPROACHES FOR ENHANCING THE CONCENTRATION OF CLA IN MILK**

Research has demonstrated various means of enriching the CLA content of animals and their products. Researchers have been using dietary modification to increase the natural production of CLA in bovine milk fat. A study showed that the concentration of CLA in the milk could be enhanced by the addition of sunflower oil and linseed oil (high in linolenic acid) in ruminant diets (Kelly et al., 1998). Another study conducted in goat showed that 2% or 4% canola oil in the diet increased the levels of CLA 2- and 3-fold, respectively, in the milk compared to control (Mir et al., 1999). Pasture feeding has been found to result in a much higher milk fat CLA concentration than that achieved with typical total mixed rations (TMR) based on conserved forage and grain. Dhiman, et al., (1999) reported a higher milk CLA concentration with pasture feeding compared to TMR feeding. Similar enrichment of CLA has often been achieved when the TMR is supplemented with unsaturated fat from oilseeds. Kelly et al., (1998) supplemented the basal diet with 55g/kg dry matter of peanut oil, sunflower oil, or linseed oil. CLA concentrations were 13.3, 24.4, and 16.7 mg/g milk fat, respectively. The increase in CLA levels observed with the sunflower oil diet treatment represented levels approximately 500% greater than those typically seen in traditional diets. The level of CLA obtained using supplemental fat varies to a large extent depending on the ruminal conditions. Grinari, et al., (1998) showed that high concentrate diets could alter the products of rumen biohydrogenation of polyunsaturated fatty acids resulting in an increase in the proportion of trans-10 isomers. Bell and Kennelly (2000) carried out a feeding trial to manipulate the animal's diet in a way that would increase the CLA content. In their study, cows were fed a control diet; Low fat diet; High fat diet A; and High fat diet B. The total CLA yield (g/day) was 5.1, 5.4, 28.5 and 45.8, respectively; while e-9, t-11 CLA (g/day) was 0.49, 0.56, 3.70 and 5.63, respectively. Overall, the study showed that milk fat can be modified to give a more favorable composition. Furthermore, it demonstrated the feasibility of producing CLA enriched milk using modifications to the diet of the cow.
FUTURE PROSPECTS

Almost all of the research on CLA to date has been conducted in *in vitro* and experimental animal studies. Only recently have human clinical trials been initiated. Furthermore, most studies have used synthetically prepared mixtures of CLA isomers. Much remains to be learned about the underlying mechanisms by which CLA exerts its diverse physiological effects. New evidence is beginning to emerge that CLA found in foods such as dairy foods may be beneficial to health and that different forms of CLA exert unique health effects, including tumor shrinkage, the promotion of youthful metabolic function, the lowering of body fat, and the lessening of arteriosclerosis. Yet, how CLA accomplishes all that it does is still not known. The mechanisms of CLA’s actions may involve nothing less than the regulation of gene expression. The unique mechanisms by which CLA can improve immune function and help protect us from free radicals and age-related degeneration make it a valuable addition to any life extension program. Although it is likely that CLA concentrations could be improved by manipulation of the nutritional regimes of the animals, by food processing technology or by altering food habits, certain borders of the existing evidence must be documented before any recommendations can be made.

CONCLUSION

CLA, found naturally in foods such as dairy foods and meat from ruminant animals, has an extensive array of promising health benefits. Support for these preliminary benefits comes from *in vitro* and experimental animal investigations. Little or no data exist regarding CLA’s health protective effects in humans. Likewise, the mechanism(s) by which CLA exerts its effects remains to be determined. New scientific findings indicate that CLA’s diverse physiological roles may be explained in part by the unique biological effects of different CLA isomers. Although several intriguing questions remain, the potential health benefits of CLA may provide yet another reason to consume CLA-rich foods as part of an overall healthful diet and may unlock the entrance for CLA-enriched dairy foods.

LITERATURE CITED


isomerizes linoleic acid to conjugated linoleic acid without hydrogenation, and its utilization as a probiotic for animals. *Journal of Applied Microbiology* **100**, 787–794.


Conjugated Linoleic Acid


