Conjugated Linoleic Acid (CLA): A Review

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Abstract

The object of this review is to create an overview of Conjugated Linoleic Acid (CLA), from the presence of these molecules in products of animal origin to the benefits brought to the health of human beings who ingest these products. We will examine the literature to describe the CLA synthesis process in the rumen and in tissues and the method by which CLA becomes incorporated into consumer products of animal origin. We will also investigate the physiological functions and cellular metabolism of CLA, including the effect of CLA on oncogenesis, atherogenesis, lipid metabolism, bone growth and metabolism, immune response and diabetes and insulin resistance. We will also examine factors influencing CLA content in meat such as dietary factors, pastures and conserved forages, feed of plant oils and seeds and production of meat in pasture versus feedlots. We conclude that it is possible to increase the concentration of CLA in milk and in meat through the manipulation of diet and dietary factors in ruminants and non-ruminants.

Keywords*:* Biosynthesis of CLA, CLA in health human, CLA in animal products

Introduction

Conjugated linoleic acid (CLA) is a collective term describing a mixture of positional and geometrical isomers of linoleic acid (LA, C18:2), which involve a double bond at positions 8 and 10, 9 and 11, 10 and 12 or 11 and 13. Each of these positional conjugated diene isomers can occur in a *cis-trans*, *trans-cis*, *cis-cis*or *trans-trans* geometrical configuration. 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. In the rumen, dietary lipids are rapidly hydrolyzed, and the resulting unsaturated free fatty acids can undergo biohydrogenation via the rumen microorganisms. As a result, ruminant animals absorb mainly saturated fatty acids, and foods made from ruminants contain these same fatty acids, regardless of the fatty acid composition of the ruminant's diet. However, when biohydrogenation is incomplete, CLA can escape the rumen and be absorbed through the gastrointestinal tract, thereby providing the peripheral tissues with various isomers of CLA.

The close relationship between *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA in milk fat is also consistent with a precursor-product relationship. Based on this observation and the kinetics of rumen biohydrogenation required to produce *trans*-11 C18:1, we proposed that a portion of the CLA found in ruminant fat was of endogenous origin. We hypothesized that endogenous *cis*-9, *trans*-11 CLA could originate from the desaturation of *trans*-11 C18:1 by Delta-9-desaturase, and we examined this in a series of studies. In the first experiment, we supplied substrate for the reaction by abomasally infusing *trans*-11 C18:1.

Research conducted over the past ten years suggests that there may be many health benefits leading from the consumption of CLA.

Conjugated linoleic acid has been found to have an anti-carcinogenic effect in many cell culture and animal models. It also has positive effects on cell growth, improves the function of the immune system, is anti-atherogenic and may improve glucose tolerance.

The object of this review is to create an overview of the literature on CLA generated since its synthesis, touching on its origins in certain animal products, as well as the benefits brought to the health of human beings.

Synthesis in the Rumen

Reiser (1951) noticed that the body fats of ruminants possessed less LNA (Alpha Linolenic Acid) than horses on the same high LNA diet and suspected that the rumen was responsible for this difference (Fig 1). He incubated LNA in rumen fluid and demonstrated the formation of *trans*FA (Fat Acid) in the rumen (Reiser, 1951). Utilizing rumen contents from fistulated sheep grazing pasture, Shorland*et al*. (1955) confirmed the existence of *trans* FA resulting from rumen biohydrogenation. Shorland*et al*. (1957) also showed that conjugated dienoic acids accumulated when LA was incubated with rumen contents, but not when LNA was incubated with it. Subsequent work (Wilde and Dawson, 1966) confirmed the formation of an array of Conjugated Fat Acids (CFAs) with 18 varying degrees of saturation and positional isomerization, including conjugated dienes. In a classic experiment, Kepler and Tove (1967) showed the production of *c*-9, *t*-11 CLA from LA (Linoleic Acid) using the bacteria *B. fibrisolvens*.

When the same bacteria were incubated with LNA as the substrate, *c*-9, *t*-11, *c*-15 was produced, of which 18:3 was later found to be hydrogenated to TVA (Harfoot and Hazlewood, 1988). No *c*-9, *t*-11 CLA was formed from LNA. The biohydrogenation of LA and LNA occurs in a similar manner. The first reaction in LA biohydrogenation is the isomerization where the double bond at carbon-12 position is transferred to the carbon-11 position, forming *c*-9, *t*-11 CLA. This is followed by the rapid hydrogenation of the *cis*-9 bond, resulting in TVA. Both of these steps are carried out by group A bacteria, while the last step of biohydrogenation of oleic to stearic acid is carried out by group B bacteria (Harfoot and Hazlewood, 1988). The enzyme responsible for the conjugation of *cis*-9, *cis*-12 double bonds was identified as linoleic acid isomerase (EC 5.3.1.5). It is a particulate enzyme bound to the bacterial cell membrane (Griinari and Bauman, 1999) and demonstrates an absolute substrate requirement for a *cis*-9, *cis*-12 diene system and a free carboxyl group (Kepler*et al*., 1970), as found in both LA and LNA. Similarly, LNA is first isomerized at the *cis*-12 position to form *c*-9, *t*-11, *c*-15 C18:3, which is then reduced at both the *cis*bonds to produce TVA. The final step is similar to that of LA.

TVA is the common intermediate during the biohydrogenation of both LA and LNA (Harfoot and Hazlewood, 1988). Its reduction appears to be rate limiting in the complete biohydrogenation of unsaturated C fatty acids, resulting in the accumulation of TVA in the rumen (Keeny, 1970). This is the predominant pathway of rumen biohydrogenation of LA and LNA. Consequently, a product precursor relationship between TVA and CLA was observed both "*in vitro*" and"*in vivo*" (sheep), with increasing concentrations of LA in the diet (Noble et al., 1974). Noble *et al*. (1974) also observed that biohydrogenation of LA derived from triglyceride followed a different pathway from that presented of LA from free acid. With a low fiber diet, however, a change in the *trans*octadecenoic acid profile of milk occurred, and *trans*-10 octadecenoic acid became the predominant *trans*octadecenoic acid in milk fat (Griinari*et al*., 1998). This led Griinari and Bauman (1999) to propose another pathway for the ruminal synthesis of *t*-10, *c*-12 CLA involving bacterial *c*-9, *t*-10 isomerase with the formation of a *t*-10, *c*-12 double bond as the first step in the process. The *c*-12, *t*-11 isomerase from *B. fibrisolvens*can hydrogenate *t*-10, *c*-12 octadecadienoic acid (Kepler*et al*., 1966), thus producing *t*-10 octadecenoic acid. It has been shown that more than 50% of the LA was converted into the *t*-10, *c*-12 isomer of CLA and only 10% was converted into *t*-10 octadecenoic acid by anaerobic *Propionibacterium* isolated from mouse cecum (Verhulst*et al*., 1987). Another rumen bacteria *Megasphaeraelsdenii*YJ-4 has also been shown to produce the *t*-10, *c*-12 isomer of CLA (Kim *et al*., 2000). The *t*-10, *c*-12 isomer was formed from LA but not from LNA as was the case with the *c*-9, *t*-11 isomer of CLA. It is not clear whether the *t*-10 octadecenoic acid is desaturated at the *cis*-12 position to produce the *t*-10, *c*-12 isomer in the rumen or in some other tissues endogenously. Recently, Mosley *et al*. (2002) have shown *in vitro* that oleic acid also forms several *trans*C FAs, including TVA, during its biohydrogenation to stearic acid. This finding may have implications for the synthesis of CLA, if the endogenous pathway turns out to be entirely different from that observed in vitro. Moreover, for the high yielding dairy cows of the west of Unites States, where cows eat rapeseed, palm, canola, high-oleic sunflower, are kept largely in confinement and are fed a total mixed rations(TMR) diet containing a high proportion of oleic acid, one may containing 50% conserved forage and 50% concentrate.

TVAs from dairy cows and possibly other ruminants and from seeds and oils, which are readily available and less expensive, have greater implications for enhancing milk fat CLA. Pathways for the synthesis of other isomers of CLA have not been elucidated in detail. Rumen pH has an important role in maintaining a viable rumen environment suitable for *B. fibrisolvens,* which is involved in the biohydrogenation of LA and LNA. It has been shown that ruminal pH at 6.0 or above has a positive effect on TVA and CLA contents in rumen cultures (Troegeler-Meynadir*et al*., 2003). This becomes important in considering the diets of high yielding dairy and beef animals, where large amounts of grain are included in the diet and thus decrease the rumen pH below 6.0. Other than its positive effects on *B. fibrisolvens,* how rumen pH affects overall biohydrogenation of unsaturated FA of 18, 20 or 22 carbons in the rumen in relation to CLA and TVA has not been investigated in detail. It has been shown that CLA levels can be increased by supplementing TMR diets with feed sources such as fish oil or marine algae (*Schizochytrium sp*.), which are rich in 20 or 22-carbon FA (Donovan *et al*., 2000), neither of which actually yields CLA or TVA during biohydrogenation in the rumen. The mechanism by which supplementation of fish oil or marine algae increases CLA and TVA concentration in milk fat is not clear. It has been proposed that the longer chain polyunsaturated FAs from fish oil inhibit the complete biohydrogenation of C in the rumen by inhibiting the 18:2 growth of bacteria responsible for hydrogenating TVA or by inhibiting the bacterial hydrogenases (Griinari and Bauman, 1999). This would lead to an increased release of TVA from the rumen. Further research is needed to elucidate the pathway for rumen biohydrogenation of longer chain oly-unsaturated FA from fish oils and other FA sources of marine origin to clearly define the mechanisms involved in enhancing CLA and TVA contents in food products from ruminants.

Tissue Synthesis of Cla

A close linear relationship between milk fat *trans*octadecenoic acids and conjugated dienoic fatty acids was first observed in Canadian butter samples based on differential infrared spectroscopy (Bartlett and Chapman, 1961). Subsequent work showed that it was the *trans*-11 C18:1 isomer that was linearly related to *cis*-9, *trans*-11 CLA concentrations in milk fat, and this relationship was observed across a wide range of diets (Griinari and Bauman, 1999). This relationship is widely taken as evidence for these two fatty acids being intermediates in ruminalbiohydrogenation.

The close relationship between *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA in milk fat is also consistent with a precursor-product relationship. Based on this and on the kinetics of rumen biohydrogenation that would lead to *trans*-11 C18:1 being available for absorption (see previous section), we proposed that a portion of the CLA in ruminant fat was of endogenous origin (Griinari et al., 1997). We hypothesized that endogenous *cis*-9, *trans*-11 CLA would originate from the desaturation of *trans*-11 C18:1 by Delta-9-desaturase, and we examined this in a series of studies. In the first experiment we supplied substrate for the reaction by abomasally infusing *trans*-11 C18:1 (12.5 g/d) (Corl et al., 1998). At the end of the 3-d infusion period, the content of CLA in milk fat had increased by over 40%, indicating that lactating cows have the ability to endogenously synthesize CLA. To quantify the relative importance of desaturase in CLA production, we abomasally infused sterculic acid, a very potent and specific inhibitor of Delta-9-desaturase (Corl et al., 1999). The results demonstrated that sterculic acid caused a dramatic reduction in milk fat content of *cis*-9, *trans*-11 CLA.

When the lack of complete desaturase inhibition is considered, it is clear that endogenous synthesis via Delta-9-desaturase represents the predominant source of CLA in milk fat. We predict that endogenous synthesis of *cis*-9, *trans*-11 CLA will also be the major source of CLA in the body fat of ruminants.

The desaturase system is a multienzyme complex that includes NADH-cytochrome b5 reductase, cytochrome b5, acyl-CoA synthase, and the terminal Delta-9-desaturase. The Delta-9-desaturase reaction introduces a *cis*-double bond between carbons 9 and 10 of fatty acids. Stearoyl-CoA and palmitoyl-CoA are the major substrates for Delta-9-desaturase, and the fatty acid products of this reaction are important components of phospholipids and triglycerides, which are particularly important for the maintenance of membrane fluidity. However, a wide range of saturated and unsaturated acyl CoAs can serve as substrates, including *trans*-11 octadecenoic acid (Pollard et al., 1980). In addition to *cis*-9, *trans*-11 CLA, the presence of other *cis*-9, *trans*-n octadecadienoic acids in milk fat also supports a role for an active Delta9-desaturase. Recently, Yurawecz et al. (1998) identified a new CLA isomer, *trans*-7, *cis*-9 octadecadienoic acid, and Ulberth and Henninger (1994) identified the *cis*-9, *trans*-13 octadecadienoic acid in milk fat.

There are inter-species differences in the tissue distribution of Delta-9-desaturase. For rodents, concentrations of mRNA and enzyme activity are greatest in the liver (Tocher et al., 1998).

In contrast, growing sheep and cattle have substantially higher Delta-9-desaturase levels in adipose tissue, as indicated by mRNA abundance and enzyme activity (Page et al., 1997). Thus, adipose tissue seems to be a major site of endogenous synthesis of *cis*-9, *trans*-11 CLA in growing ruminants. The mammary gland is the apparent site of endogenous synthesis of *cis*-9, *trans*-11 CLA for lactating ruminants, based on the activity of Delta-9-desaturase (Kinsella, 1972).

In vivo results are also consistent with the lactating mammary gland being of primary importance in the endogenous synthesis of *cis*-9, *trans*-11 CLA during lactation. Bickerstaffe and Johnson (1972) demonstrated that intravenous infusion of sterculic acid resulted in a marked decrease in the oleic acid: stearic acid ratio in milk fat but only minimal differences in plasma fatty acid composition in lactating goats. Because circulating sterculic acid would inhibit Delta-9-desaturase in all organs, the authors concluded that the mammary gland must be the major site of desaturation for fatty acids found in milk fat. Studies of Delta-9-desaturase have predominantly involved the hepatic enzyme in rats. Results demonstrate that mRNA expression and enzyme activity are responsive to changes in diets, hormonal balance, and physiological state (Tocher et al., 1998). Similar studies with Delta-9-desaturase in ruminants are limited. Martin et al. (1999) characterized the ontogeny of gene expression for the enzyme in adipose tissue of growing cattle. Ward et al. (1998) determined tissue-specific changes in the mRNA abundance of Delta-9-desaturase in sheep at different physiological states and observed a decrease in mRNA abundance in adipose tissue and an increase in mammary tissue with the onset of lactation. Ward et al. (1998) also demonstrated that insulin regulated Delta-9-desaturase gene expression in sheep adipose tissue explants.

Cla Isomers

Conjugated linoleic acid is a collective term for a series of conjugated dienoic positional and geometrical isomers of linoleic acid (C18:2). Conjugated linoleic acid isomers are found naturally in foods, especially those of ruminant origin (Chin et al., 1992). In ruminants, CLA is synthesized by ruminal bacteria using C18:2 or C18:3 as the precursor (Kepler et al., 1966). Conjugated linoleic acid isomers can also be synthesized in the laboratory from C18:2 or from sources high in C18:2, such as sunflower, safflower, soybean, or corn oils, by a reaction involving alkaline water isomerization51 and isomerization in propylene glycol (Sehat et al., 1998).

Throughout the rest of the text, the *cis*double bond will be abbreviated as *c* forces and the *trans*double bond as *t*. The *cis*-9, *trans*-11 isomer is the principle dietary form of CLA exhibiting biological activity and accounts for 73 to 94% of total CLA in milk, dairy products, meat, and processed meat products of ruminant origin (Chin et al., 1993). In recent years, biological activities have been proposed for other CLA isomers, especially *trans*-10, *cis*-12 C18:2 (Park et al., 1999) (*t*10, *c*12 CLA). The structures of *c*9, *t*11 CLA, *t*10, *c*12 CLA, and C18:2 have been described. A total of 17 natural CLA isomers have been detected in milk, dairy products, beef, human milk, and human adipose tissue using silver ion-high performance liquid chromatography and gas chromatography-electron ionization mass spectrometry.1,52,56-61 Identified CLA isomers are: *t*12, *t*14; *t*11, *t*13; *t*10, *t*12; *t*9, *t*11; *t*8, *t*10; *t*7, *t*9; t7, *c*9; t6, *t*8; *c*12, *t*14; *t*11, *c*13; *c*11, *t*13; *c*10, *t*12; *c*9, *t*11; *c*8, *t*10; *c*7, *t*9; *c*9, *c*11; and *c*11, *c*13. Bauman et al. (2000) observed that butter contained *c*9, *t*11 (76.5%) and *c*7, *t*9 (6.7%) isomers. Sehat et al. (1998) identified the distribution of CLA isomers in cheese fat: *c*9, *t*11 (78 to 84%); *t*7, *c*9 plus *t*8, *c*10 (8 to 13%); *t*11, *c*13 (1 to 2%); *c*12, *t*14 (<1%); with a total *trans*/*trans* isomer level of 5 to 9%. Recently, Fritsche et al. (2000) identified the distribution of CLA isomers in beef samples and found that *c*9,*t*11 was the predominant isomer (72%), followed by the *t*7, *c*9 isomer (7.0%). Typical synthetic CLA isomer mixtures consist of *c*9, *t*11 (40.8 to 41.1%); *t*10, *c*12 (43.5 to 44.9%); *t*9, and *t*11/*t*10, *t*12 (4.6to 10.0%) isomers. Fritsche et al. (2000) reported on a different synthetic CLA isomer mixture that contained *c*8, *t*10 (14%); *c*9, *t*11 (30%); *t*10, *c*12 (31%); and *c*11, *t*13 (24%). Most of the aforementioned CLA isomers are present in foods in very minute amounts and are of little biological importance or have not been studied in detail. Therefore, the ensuing discussion will focus on the two predominant forms of CLA, namely the *c*9, *t*11 and *t*10, *c*12 isomers.

Physiological Functions and Metabolism of Cla

Scientific interest in CLA was confined largely to rumen microbiologists who studied the *cis*-9, *trans*-11 CLA isomer as an intermediate in the biohydrogenation of linoleic acid. This changed when Ha *et al*. (1987) reported that CLA produced by base-catalyzed isomerization of linoleic acid was an effective inhibitor of benzopyrene-initiated mouse epidermal neoplasia. Since then, CLA has gained considerable attention as a nutrient that exerts the following beneficial physiological effects in experimental animals and human beings:

(i) inhibition of carcinogenesis; (ii) prevention of cholesterol-induced atherosclerosis; (iii) reduction of body fat accumulation; (iv) enhancement of the immune response; (v) ability to promote growth; (vi) improvement of diabetes and (vii) improvement of bone metabolism.

Anti-oncogenic effect of CLA

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 dieneunsaturations (Ha et al., 1987). Dietary studies with rat mammary tumor models have established CLA as a potent anticarcinogen. In a seminal study, Ip et al. (1991) fed CLA to 37-day-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 and 0.5% produced dose-dependent inhibition of tumor incidence. In another study (Ip et al. 1994b), short-term feeding of CLA from weaning (21 days of age) to time of carcinogen administration (50 days) also resulted in suppressed tumor production when either DMBA or methylnitrosourea (MNU) were used as carcinogens. 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 oncogenic cells (Shultz et al., 1992). In contrast, LA had no inhibitory effects on growth of the cell lines.

The 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 (Ip et al., 1991), pro-oxidant cytotoxicity (Schonberg and Krokan, 1995), inhibition of nucleotide synthesis (Shultz et al., 1992), reduction of proliferative activity (Ip et al., 1994a) and inhibition of both DNA adduct formation (Zu and Schut, 1992) and carcinogen activation (Liew et al., 1995).

Antiatherogenic effect of CLA

Experimental animal studies indicate that CLA may have beneficial effects on the atherosclerotic process (Deckere et al., 1999). When rabbits were fed an atherogenic diet containing 0.5g CLA/ day for 22 weeks, marked reductions were observed in the total plasma and LDL cholesterol, the LDL/HDL cholesterol ratio, and in triglyceride levels after 12 weeks (Lee et al., 1994). Examination of the aortas revealed less atherosclerosis in rabbits fed CLA than in control animals (Lee et al., 1994). An investigation by Nicolosi et al. (1997) supports the above observations. In this study, hamsters were fed no CLA (control), CLA between 0.1% and 2.0% of energy of diet, or 1.1% linoleic acid for 11 weeks (Nicolosi et al., 1997). Compared to the control animals, the CLA-fed hamsters exhibited lower levels of plasma total cholesterol, non-HDL cholesterol, and triglycerides. CLA had no effect on HDL cholesterol levels. Measurement of the aortic fatty streak areas, an initial event in early atherosclerosis, revealed less build up in the CLA-fed hamsters.

The above studies used synthetic mixtures of CLA primarily of the cis-9, trans-11 and trans-10, cis-12 isomers. In a recent study in hamsters, these CLA isomers were found to differ greatly in their effects on blood lipid levels (Deckere et al., 1999). In this study, animals fed either the CLA mix or the trans-10, cis-12 isomer for 8 weeks exhibited lower levels of LDL and HDL cholesterol, increased very low density lipoprotein (VLDL) triglyceride levels, and decreased epididymal fat pad weights than in the control animals (Deckere et al., 1999). In contrast, the natural isomer, cis-9, trans-11 CLA present in the dairy products and meat of ruminants, had little or no effect on lipid metabolism in the hamsters (Deckere et al., 1999).

Effects of CLA on lipid metabolism

Conjugated linoleic acid has been shown to decrease body fat while not affecting total body mass. Pigs fed CLA develop less body fat and show improved feed conversion efficiency (Dugan *et al*. 1997). CLA reduces the carcass fat content in mice (West *et al*. 1998) and reduces the back fat thickness in pigs (Cook *et al*. 1998).
Dietary CLA supplements increase lean tissue deposition and decreases fat deposition in pigs (Ostrowska*et al*. 1999).

The mechanism by which CLA leads to a decrease in fat deposition is unknown. Conjugated linoleic acid seems to cause a loss of appetite in human subjects. The activity of carnitinepalmitoyltransferase, the rate-limiting enzyme for fatty acid oxidation, also increases (Park *et al*. 1997). Furthermore, in a related *in vitro* experiment (Park *et al*. 1997), it was observed that adding CLA to the media of 3T3-L1 adipocytes (at levels found in the blood of rats fed 0.5% CLA) significantly reduces the activity of lipoprotein lipase (LPL), an enzyme involved in the uptake of fatty acids into adipose tissues from the blood, and reduces intracellular concentrations of triglyceride and glycerol.

CLA treatment also enhances the release of fatty acids from these cells into the media. The latter observation was interpreted as indicating that CLA may enhance lipolysis. Therefore, the decrease in body fat associated with CLA may be partially a result of reduced fat deposition and increased lipolysis in adipocytes, possibly coupled with increased fatty acid oxidation in both skeletal muscle cells (the principal site of fatty acid oxidation) and adipocytes (the principal site of fat storage). Pariza*et al*. (2001) reported a model for the effects of CLA on adipocytes where the effects of CLA on lipid metabolism are mediated by the *trans*-10, *cis*-12 CLA isoform. Park *et al*. (1999) reported that changes in the body composition were observed in mice fed a diet supplemented with *trans*-10, *cis*-12 CLA. *Trans*-10, *cis*-12 CLA resulted in a decrease in LPL activity, and a decrease in the levels of intracellular triglyceride and glycerol in 3T3-L1 adipocytes. However, *cis*-9, *trans*-11 CLA did not cause these effects. The data shown indicate that *cis*-9, *trans*-11 CLA is active in enhancing bodyweight gain and also appears to enhance feed efficiency in weanling mice. However, *cis*-9, *trans*-11 CLA has no effect on body fat levels. In contrast, *trans*-10, *cis*-12 CLA reduces body fat levels relative to the control but do not enhance either body growth or feed efficiency. It is thought that *trans*-10, *cis*-12 CLA reduces the uptake of lipids by adipocytes, and that *cis*-9, *trans*-11 CLA is active in inhibiting carcinogenesis. These effects of *trans*-10, *cis*-12 CLA appear to be related to the observation that abomasal infusion of *trans*-10, *cis*-12 CLA results in an immediate decrease in milk fat synthesis. Therefore, a role for *trans*-10, *cis*-12 CLA as a specific inhibitor of milk fat synthesis has been proposed (Griinari*et al*. 1998). Dairy breed variations in milk fat CLA content may be related to differences in Delta-9-desaturase activity (DePeters*et al*., 1995).

The various CLA isomers have different effects on metabolites. Sebedio*et al*. (2001) demonstrated that *cis*-9, *trans*-11 CLA is converted into C18:3 and C20:3 conjugated fatty acids. *Trans*-10, *cis*-12 CLA is converted mainly into delta 06, 10 and 12, whereas only smaller quantities of the conjugated C20:3 delta 8, 12 and 14 have been detected. Only a small quantity of conjugated C20:4, formed from *trans*-10, *cis*-12 CLA, has been found in membrane phospholipid. Furthermore, *trans*-10, *cis*-12 CLA can be converted into a conjugated C16:2 which are found only in liver lipid from animals fed *trans*-10, *cis*-12 CLA. This may suggest that *trans*-10,*cis*-12 and *cis*-9, *trans*-11 CLA are metabolized differently through the peroxisomal b-oxidation pathway. In addition to the beneficial health effects already described, there is evidence suggesting that CLA may exhibit important physiological functions, such as antidiabetic properties, immune modulation and improved bone metabolism, although the detailed mechanisms are not clear. The main dietary source of CLA is ruminant products, which are the best products available for humans to obtain a significant intake of CLA in natural foods.

Effect of CLA on bone growth and metabolism

Bone metabolism is orchestrated by three types of cells, and their activities are affected by several local growth factors such as insulin-like growth factor (IGF-1), prostaglandin E2 (PGE2), and the cytokines interleukin-1 and tumor necrosis factor. Dr. Bruce Watkins at the Center for Enhancing Foods to Protect Health, PurdueUniversity, described the effects of CLA on some biomarkers of bone metabolism in rats. For example, when supplied at a level of 1% w/w of the diet, CLA isomers depressed "*in vivo"* synthesis of PGE_2 and serum IGF-1 and the rate of bone formation in male rats (Li et al., 1999). At a dietary level of 0.5% w/w, CLA isomers maintained bone formation rates in growing male rats given a diet rich in omega-6 fatty acids. CLA isomers increased collagen synthesis in primary cultures of growth plate chondrocytes in a dose-dependent manner. Effects of CLA on bone metabolism in rats are influenced by the type and amount of CLA isomers as well as the ratio of omega-6 to omega-3 fatty acids in the diet. Studies from the University of Manitoba reported that CLA did not affect bone formation and resorption in a rat model of metabolic bone disease. Metabolic bone disease is characterized by elevated parathyroid hormone levels and increased rates of bone turnover. In their study, 52 weanling male Han:SPRD-cy rats were randomized to identical diets supplemented with or without CLA (1% of dietary fat) for eight weeks. CLA did not affect bone mass, but it decreased the release of PGE2 in kidney and attenuated parathyroid hormone levels by 60%.

CLA modulation of immune response

Considerable evidence supports the idea that aging is associated with a decline in the immune response in mammals (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). 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 mammals. 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). In addition, dietary CLA enhanced the phytohemagglutinin response and alleviated the catabolic effect of immune stimulation in rats (Cook et al., 1993). 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 is a potent immunostimulator in mammals. The potential of CLA to prevent catabolic losses without affecting the generation of adaptive immunity could improve growth and development.

Effect of CLA on diabetes and insulin resistance

CLA may have therapeutic potential in managing type 2 diabetes. Type 2 diabetes mellitus is characterized by a variety of metabolic derangements, particularly hyperglycemia, insulin resistance and relative (rather than absolute) insulin deficiency (ECDCDM, 2003). CLA has been shown to improve oral glucose tolerance and delay the development of diabetes in rat models. Dr. Carla Taylor at the University of Manitoba presented data showing that dietary CLA improved oral glucose tolerance and lowered serum insulin levels in the fa/faZucker rat, a model of obesity and insulin resistance. In her study, weanling male fa/faZucker and lean rats were fed a 1.5% w/w CLA mixture or a control diet without CLA for eight weeks.

The CLA-fed rats had lower serum glucose and insulin concentrations during an oral glucose tolerance test, smaller adipocytes, smaller pancreatic cells, decreased liver weight and decreased fatty liver tissue than control rats. The consumption of CLA delayed the onset of hyperglycemia and diabetes in the diabetic fatty Zucker rat. In a human study, CLA decreased fasting blood glucose concentrations in patients with type 2 diabetes. The 21 subjects in this pilot study were not taking medications for blood glucose control and were randomized to consume 6.0 g CLA or safflower oil daily for eight weeks. By the end of the 8-week intervention, 9 out of 11 (81%) subjects on CLA supplementation experienced decreases in fasting plasma glucose compared with two out of 10 (20%) subjects not taking CLA (Belury&Banni, 2003). Despite these positive preliminary findings, it is reported that there is little evidence of any improvement in the metabolic status of humans after CLA supplementation (Risérus et al., 2003).

Cla Content in Meat

Although there is a vast amount of literature available about the CLA content of milk, the number of research trials focusing on factors affecting the CLA content of meat is limited.

Dietary factors affecting CLA in meat

Pastures and conserved forages

In the case of dairy cattle, feeding procedures such as grazing animals on pasture, feeding them with fresh forages, or increasing the amount of forage in the diet will elevate the percentage of CLA as a proportion of total FA in meat from ruminants. Grazing beef steers on pasture or increasing the amount of silage in the diet increased the *c*9, *t*11 CLA content in fat by 29 to 45% compared to control (McGuire et al., 1998). The increase in beef CLA content varies with the quality and quantity of forage in the animal's diet. Beef from steers raised on green pasture had 200 to 500% more *c*9,*t*11 CLA as a proportion of fat compared to steers fed an 87% corn grain-based feedlot diet (Rule et al., 2002). This study also reported that the percentage of *c*9, *t*11 isomer of CLA was higher in intramuscular fat of range cattle compared with that of steers fed a high-grain diet under feedlot conditions. The increase in *c*9, *t*11 CLA content in beef is not as dramatic as the increase seen in milk from cows grazed on pasture. This difference is probably due to differences in CLA production in the rumen or differences in the endogenous synthesis of CLA in intramuscular fat of beef cattle fed high-forage diets.

Feed plant oils and seeds

Supplementing beef cattle diets with C18:2 or C18:3- rich plant oils has yielded varied results as far as increasing the CLA content of beef. Feeding 4 to 6% of diet dry matter (DM) as soybean oil to beef cattle fed high grain diets either marginally increased or did not increase the *c*9, *t*11 CLA content of beef (Griswold et al., 2003). There was a small increase in the *c*9, *t*11 CLA content of fat from beef muscle when steers were fed 3 to 6% sunflower oil compared to beef from cattle fed no oil (Mir et al., 2003). In another study by the same authors, feeding 6% sunflower oil to cattle from Wagyu, Limousin x Wagyu, and Limousin breeds increased CLA content to 1.25% of fat in beef muscle compared to 0.28% in cattle fed 0% sunflower oil (Mir et al., 2002). Feeding linseed oil at 6% of diet DM increased the *c*9, *t*11 CLA content to 0.80% of fat compared to 0.32% of total FA in muscle from control cattle (Enser et al., 1999). The increase seen in CLA content of beef muscle when including free oils in the animal's diet is small, with linseed oil or sunflower oil showing more promising effects than soybean oil.

Feeding processed plant oil seeds has also resulted in marginal or no increase in the CLA content of beef. Replacing normal corn with high-oil corn or feeding cracked safflower seeds as a source of oil to beef cattle did not increase CLA content in beef muscle (Bottger et al., 2000). However, in one study, feeding whole sunflower seeds at 5% of diet DM increased the CLA to 0.73% of fat compared to 0.31% CLA in subcutaneous fat in the control group (Garcia et al., 2003). Feeding full-fat extruded soybeans at 12.7 and 25.6% of diet DM to beef cattle fed high grain diets marginally increased the CLA and TVA contents of beef muscle compared to the control (Madron et al., 2002). Some researchers have attempted to increase the CLA content of lamb by manipulating the diet. Feeding up to 6% safflower oil, 6% sunflower oil, or adding 5% additional fat through cracked safflower seeds resulted in a two-fold increase in the CLA content of muscle (Kott et al., 2003).

This is only a small increase when compared to the observed increase in CLA content as a proportion of total fatty acids when animals are raised on forages and pasture. However, it is important to realize that total body fat content is decreased when animals are grazed on pasture or fed high forage diets; therefore, actual CLA yields may actually be higher when supplementing oil to grain-based diets compared to grazing animals on pasture. Interestingly, the CLA content was increased from 1.0 to 1.6% of fat in muscle when lambs were fed whole linseeds, but there was no increase reported when fish oil was added to the diet (Wachira et al., 2002). Feeding feed sources rich in C18:2 or C18:3 to dairy cows results in a 3- to 4-fold increase in the *c*9, *t*11 CLA content in milk fat, (Fritsche et al., 1999) but only marginal increases in beef fat. It is very possible that the mechanisms and routes of CLA synthesis (ruminal and endogenous) are different in the mammary gland and adipose tissue. Additional factors regulating the synthesis of CLA in the rumen, muscle, and mammary gland are poorly understood. Further research is also needed to demonstrate synthesis of *c*9, *t*11 CLA in the adipose tissue from TVA in ruminants.

Finishing beef cattle are typically fed diets containing 85 to 92% grain (NRC, 19960, whereas a typical diet for a high-producing dairy cow consists of only 50 to 60% grain. The lower proportion of *c*9, *t*11 CLA in beef fat compared to milk fat in animals fed diets rich in C18:2 or C18:3 probably relates to the effects of the traditional high-grain, low-fiber diets fed to finishing cattle in the United States. It seems likely that the acidic ruminal pH often occurring in finishing beef cattle alters the microbial population involved in lipid biohydrogenation, and therefore influences the ruminal synthesis of CLA isomers.

Research suggests that high-grain diets resulting in low ruminal pH lead to shifts in the ruminal environment that favor the production of the *t*10, *c*12 CLA isomer and TVA in the rumen, (Kalscheur et al., 1997) thereby resulting in higher concentrations of these FAs in the beef muscles (Dhiman et al., 1999). In muscle, the substrate TVA is present; however, it may not be converted to *c*9, *t*11 CLA. The *t*10, *c*12 isomer of CLA has been shown to inhibit the activity and gene expression of the Delta-9-desaturase enzyme (Bretillon et al., 1999), which would result in a reduction in the endogenous synthesis of *c*9, *t*11 CLA. However, this seems an unlikely explanation for the observed lack of increase in *c*9, *t*11 CLA in beef fat because steers finished on pasture or high forage diets show increased percentages of both *c*9, *t*11 and *t*10, *c*12 isomers of CLA as a proportion of total FA (Poulson et al., 2001).The abundance of mRNA and the enzyme activity of *Delta*-9-desaturase are also affected by hormone levels, physiological state, insulin levels, and other activating and inhibiting factors (Tocher et al., 1998). A decline in insulin resulted in decreased *Delta*-9-desaturase gene expression in adipose tissue (Ward et al., 1998). It seems likely that the method with the greatest potential to increase the total CLA yield of beef is to supplement the high-grain diet with oils, such as soybean oil, linseed oil, or sunflower oil, although results have not always been positive.

Grazing animals on pasture substantially increases CLA as a proportion of total fatty acids, but total fat content in the final product is reduced. Therefore, any increase in the CLA content of beef should be evaluated based on total CLA available in the edible fat rather than concentrations in raw meat.

Production of meat in pasture or feedlot

Many factors affect ruminant carcass and meat quality and all of them can be divided into two categories: endogenous factors directly linked with the animal (breed, age, sex, etc.) and exogenous factors (diet, weather, slaughtering procedures etc.) indicated by the generic expression 'environmental'. Among the environmental factors, feeding plays an important role in the determination of quality.

Beef meat has in recent years gained a reputation for being less healthy and has often wrongly been identified as having a high fat and high saturated fatty acid concentration. In fact, lean beef meat is very low in fat (2-3 %). Fat, especially animal fat, has been the subject of much interest and debate due to their implications for human health and association with disease risks when consumed in excess (Ender et al., 1997). Fat is not only a concentrated source of energy for the body; the fat in meat provides flavors, aroma and texture. Fat is a carrier of the soluble vitamins A, D, E and K and the essential fatty acids, important in growth and in the maintenance of many body functions (Valsta et al., 2005). Dietary fats are capable of acting on the composition, organization and functions of membranes.

Today, it is known that not only the amount but also the structure of the fatty acids plays a major role in maintaining health. In Germany, it is recommended that human beings should increase their intake of *n*-3 fatty acids and decrease the *n*-6/*n*-3 ratio in the diet to levels 5:1. Animal experiments and clinical intervention studies indicate that a lower ratio of *n-6/n-3* fatty acids in dietary fat is desirable for reducing the risk of many chronic diseases (Simopoulus, 2002). Consumer interest in the nutritional aspects of health has increased in the last few years. This has led to an increased interest in animal production practices, particularly in the nutrient composition of the diet, which can affect the carcass and meat quality, as well as the fatty acid profile of meat (Dannenberger et al., 2004; Hollo et al., 2005; Nuernberg et al., 2005).

Grass feeding has been reported to affect several meat quality characteristics of beef, in particular color, flavor and fatty acid composition compared to concentrate diet systems (Knowles et al., 2004; Hollo et al., 2004; Nuernberg et al., 2005). A pasture-based feeding system, including fresh and conserved forages and also occasional dietary supplements, leads to improved nutritional quality of meat coming from cattle to consumers (Knowles et al., 2004). Pasture feeding and diets containing a proportionally high level of linolenic acid in fat, such as fresh grass and grass silage, resulted in increased deposition of *n*-3 fatty acids in the muscles (Dannenberger et al., 2004; Nuernberg et al., 2005).

The results of this study (Dannenberger, et al., 2006) demonstrated that different diets, in particular longer periods of pasture feeding, affect the carcass and meat quality of German Holstein and German Simmental bulls. The meat color investigations of muscle showed that the beef produced by pasture feeding is darker. Beef produced by pasture feeding produced meat with enhanced nutritional quality for the consumer, resulting from a higher accumulation of beneficial fatty acids (*n*-3 fatty acids) and a lower concentration of saturated fatty acids and *n*-6 fatty acids, compared with beef meat from a concentrate feeding system. However, pasture feeding led to a lower intramuscular fat content and a tougher meat for both breeds compared with the concentrate-fed bulls. Further research is needed to identify factors that increase the intramuscular fat content of pasture-fed bulls and consequently improve the meat quality, with regard to toughness and tenderness, and therefore to ensure consumer acceptability.

Increasing the Concentration of Cla In Milk

With regard to the potential benefits of CLA for human health, a number of researchers began looking at possible ways of increasing the CLA concentration in bovine milk fat. There appears to be two practical approaches to achieving this goal. The first approach is to use dietary modification in an attempt to increase the natural production of CLA in the cow. The second approach is to feed the synthetic mixture of CLA isomers, protected in some way from the microbial biohydrogenation in the rumen. Both approaches will be discussed below.

Manipulation of the diet

The concentration of CLA in bovine milk fat can vary quite substantially depending on the feeding strategy adopted.

For instance, pasture feeding has been found to result in a much higher milk fat CLA concentration than that achieved with typical TMR based on conserved forage and grain (White, et al., 2001). Dhiman et al. (1999) reported the CLA concentration of milk to be 22.1mg/g fat with pasture feeding compared to 3.8mg/g fat with TMR feeding. Kelly et al. (1998) supplemented the basal diet with 53g/kg DM of peanut oil (high oleic acid), sunflower oil (high linoleic acid), or linseed oil (high linolenic acid). 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 treatment represented levels approximately 500% greater than those typically seen in traditional diets.

Chouinard et al. (1998) fed diets supplemented with 4% DM of calcium salts of fatty acids from canola oil, soybean oil, or linseed oil. The resulting milk CLA concentrations were 13.0, 22.0, 19.0 mg/g fat for canola oil, soybean oil, and linseed oil respectively, and 3.5mg/g fat for control. Soybean oil, which is high in linoleic acid, was most effective at increasing the CLA level. It appears that the availability of the oils to the rumen microbes is an important determinant of subsequent CLA production. Chouinard et al (2001) showed that processing soybeans, especially by extrusion, increased milk CLA above that obtained by feeding ground soybeans. The extrusion process ruptures the seed, likely making the oil more available for rumen biohydrogenation. The amount of CLA, and the type of CLA isomers, produced as a result of feeding supplemental fat varies to a large extent depending on the ruminal conditions. A study at CornellUniversity using supplemental fat found that the CLA levels in milk were halved when the forage:concentrate ratio of the diet was changed from 50:50 to 20:80 (Kelly and Bauman, 1996).

Furthermore, Griinari, 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 18:1 and trans-10, *cis*-12 CLA isomers. Dietary fish oil supplementation has also been found to increase the concentration of CLA in bovine milk from 0.2-0.6% in control diets to 1.5-2.7% in supplemented diets (Chilliard, et al., 2001). This was somewhat surprising as fish oils are generally high in fatty acids of 20 or more carbons but low in 18 carbon polyunsaturated fatty acids. It is thought that the supplemental fish oils interfere with the biohydrogenation of 18:2 and 18:3 from the basal diet, specifically inhibiting the conversion of *trans*-11 18:1 to 18:0. As discussed already, *trans*-11 18:1 can be desaturated to *cis*-9, *trans*-11 CLA in the mammary gland. Fish oil supplementation has been shown to increase ruminal production of *trans*-octadecanoic acids (Wonsil, et al., 1994). In addition, studies looking at the effect of fish oil supplementation on milk CLA values reported that the increase in CLA was almost exclusively in the *cis*-9, *trans*-11 isomer (Offer et al., 1999). Feeding fish oil in combination with a source of 18:2 or 18:3 would therefore be expected to increase the level of milk CLA much more than would be achieved with 18:2 or 18:3 alone. This hypothesis was tested by Abu-Ghazaleh et al (2002) who fed diets containing 0.5% fish oil, 2.5% soybean oil, or a combination of 0.5% fish oil and 2% soybean oil. They reported levels of *cis*-9, *trans*-11 CLA (g/100g fatty acids) of 0.33, 0.47, 0.79, and 1.39 for control, fish oil, soybean oil, and the combination, respectively. Butter from milk containing these higher than average levels of CLA and other polyunsaturated fatty acids is softer but the flavor characteristics of both the milk and butter appear to be unchanged by altering the milk fatty acid profile (Ramaswamy, et al., 2001).

We have carried out a series of feeding studies to determine if we could manipulate the animal's diet in a way that would increase the CLA content more than had previously been achieved (Bell and Kennelly, 2000). Details of the diets used in these studies cannot be given at this time for reasons of patent confidentiality. Generally, feeding the CTL diet resulted in milk fat with a *cis*-9, *trans*-11 CLA concentration of approximately 0.4 to 0.6%, similar to that typically reported for whole milk. Cows fed CLA-producing diets produced milk fat with as much as 5% *cis*-9, *trans*-11 CLA, approximately 10 times greater than the CTL diet. Although the yield of fat was lower in TRT, the yield of CLA was still approximately nine times greater than the yield of CLA for the CTL treatment. Cows fed the TRT diet also had significantly higher levels of *trans*18:1 fatty acids in their milk. In the past decade there has been an accumulation of evidence that suggests that *trans*fatty acids may contribute to the development of coronary heart disease (CHD). Investigations found that *trans*fatty acids increased blood cholesterol levels, which are believed to be an important risk factor for CHD. This was supported by strong epidemiological evidence. A study reported by Willett et al. (1993), which followed more than 80,000 people for 8 years, found an association between high intakes of *trans*fatty acids and coronary heart disease. This created the impetus for plans to make labeling of *trans*fatty acids on food packaging mandatory. However, the study reported by Willett et al (1993) showed that the association between *trans*fatty acids and CHD was specific for *trans* fatty acids from industrial hydrogenated fats, whereas *trans* fatty acids of animal origin were not correlated with CHD.

Approximately 80 to 90% of the *trans*fatty acid content in our diet comes from partially hydrogenated vegetable oils like those found in baked foods, certain types of margarine, and foods that are deep fried. The composition of *trans*isomers from these sources is different from *trans* fatty acids of ruminant origin, which may provide a rationale for the differences seen in the epidemiological associations. The primary *trans*fatty acids in bovine milk are 18:1 *trans*-11 and CLA, whereas partially hydrogenated vegetable oils are characterized by a range of *trans* fatty acids such as 18:1 *trans*-8, *trans*-9, *trans*-10, *trans*-11, *trans*-12 and *trans*-13. As noted earlier, CLA has been found to inhibit cholesterol-induced atherosclerosis in rabbits and hamsters. Furthermore, there is evidence that 18:1 *trans*-11 can be desaturated to *cis*-9, *trans*-11 CLA in human tissues (Salminen et al. 1998).

Ruminant fat has been associated with an elevation in blood cholesterol because of its high content of saturated fatty acids, which are believed to be hyper-cholesterolemic. In our study we found that the diets that increased CLA also resulted in a decrease in saturated fatty acids. The TRT milk compared to CTL had approximately 30% lower 14:0 and 45% lower 16:0 FAs. There was also an increase in 18:1 *cis*-9 levels. This represents a positive change as 14:0 and 16:0 in particular have been associated with increases in blood cholesterol. On the other hand, 18:1 *cis*-9 is thought to have a cholesterol lowering effect. Overall, this study showed that milk fat can be modified to give a more favorable fatty acid composition. Furthermore, the study demonstrated the feasibility of producing CLA enriched milk using modifications to the diet of the cow.

Dietary Factors Affecting Cla Content in Foods from Non-Ruminants

As mentioned above, foods from non-ruminants do not contain any appreciable amounts of CLA isomers under traditional feeding. In some cases it was not even detected (Yang *et al*., 2002). However, several researchers have shown a considerable enhancement in CLA content of egg yolk, broiler meat, or pork through dietary supplementation of CLA (Szymczyk and Pisulweski, 2003). Egg yolk contents of *c*-9, *t*-11 and *t*-10 *c*-12 isomers of CLA have been shown to increase linearly with an increasing concentration of CLA isomers in the layer's diet (Cherian*et al*., 2002). Jones *et al*. (2000) found that incorporation of CLA in the egg yolk was highest on day 24 and day 36, whereas Chamruspollert and Sell (1999) observed it after 11 days. High concentration of CLA at 0.82, 5.82, and 11.2% of the total FA in egg yolk lipids occurred when laying hens were fed a diet with 0.5, 2.5, or 5.0% CLA, respectively (Chamruspollert and Sell, 1999), which is even higher than the concentration observed in milk or meat fat from ruminants. However, such a high dose of CLA in the diet may have other implications for the growth, production, and reproduction of birds. The transfer efficiency of *c*-9, *t*-11 isomer was higher than that of *t*-10, *c*-12 isomers with an overall CLA incorporation into egg yolk of 7.95g CLA/100 g of total FA (Schafer *et al*., 2001). Similarly, feeding CLA to broiler chickens at 0.5, 1.0, or 1.5% of the diet resulted in substantial incorporation of CLA isomers into their tissue lipids (Szymczyk*et al*., 2001), thus providing another potential CLA-rich source of meat for humans. Sirri*et al*. (2003) have also observed an increased deposition of CLA in muscles with an increasing CLA level in the diet of broiler chickens.

Concentration of CLA isomers in ham fat was higher for pigs fed CLA regardless of the level of supplementation in the diet (Corino*et al*., 2003). The back fat, liver, and muscle lipids showed an increased concentration of CLA isomers in pigs fed 2.0% CLA in the diet, with the highest concentration of 5.65% of total FA in the back fat followed by liver lipids at 2.41% and muscle lipids at 1.47% (Teschendorf*et al*., 2002). Feeding pigs with increasing levels of *trans*CFA resulted in a linear increase in both TVA and CLA levels in backfat as well as in neutral lipids and phospholipids of *M. Longissimusdorsii*, with back fat having higher concentration of CLA than TVA(Glaser *et al*., 2002). Moreover, rate of bioconversion of TVA to CLA in pig adipose tissue was not limited up to 25 g total *trans* C FA including 3.3 g of TVA per kg feed. Similarly, both the*c*-9, *t*-11 and *t*-10, *c*-12 isomers of CLA were increased in belly and longissimus fat depots of pigs fed CLA (Gatlin *et al*., 2002).

Transfer efficiency of dietary CLA isomers was 41 to 52% for the backfat and 55 to 69% for when pregnant and lactating sows were fed diets with supplemental CLA (Bee, 2000). Ostrowska*et al*. (2003), however, concluded that although feeding pigs diets supplemented with CLA increases lipid CLA, the resultant change in the FA profile in pig fat could potentially outweigh the benefits of increasing CLA. Since TVA is converted into CLA in humans and other monogastric animals (Loor*et al*. 2002), it should be possible to enhance the CLA concentration in human or other monogastric milk by supplementing diets with TVA. Loor*et al*. (2002) have also shown that dietary TVA increases the CLA content in tissues of lactating mice and suckling pups. Although no substantial amount of CLA isomers is present in milk or meat from non-ruminants under traditional feeding (Chin *et al*., 1993), these studies have shown that CLA content in foods from non-ruminants can be enhanced substantially through incorporation of CLA into the diet.

Whether the mature milk with highest values for *c*-9, *t*-11 isomer supplementation of CLA in the diet of non-ruminants has other negative effects in the economics and overall production and reproduction performance of animals has not been investigated in detail.

Conclusions

CLA is a predominant polyunsaturated fat acid (PUFA) in products originating from ruminant animals whose beneficial effects on the health of human beings are evidenced in diverse research. However it is necessary to carry out more studies to elucidate the as yet unknown mechanisms involved in this process so that all the potential benefits can be fully exploited.

The concentrations of CLA in products of animal ruminants such as meat and milk can be modified through the manipulation of the animals' diet. In ruminant animals, the incorporation of CLA into the meat or milk products does not occur without the action of the rumen microorganisms.

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