Biosynthesis of conjugated linoleic acid in ruminants[1]

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Abstract

Food products from ruminants are the major dietary source of conjugated linoleic acids (CLA) for humans. The uniqueness of CLA in ruminant fat relates to the biohydrogenation of dietary unsaturated fatty acids by rumen bacteria. The CLA are intermediates in the biohydrogenation, and a portion escape the rumen and are incorporated into milk fat and body fat. In addition, the animal itself synthesizes *cis*-9, *trans*-11 CLA from *trans*-11 octadecenoic acid, another intermediate in ruminal biohydrogenation that is absorbed. This involves Δ^9-desaturase, which is present in mammary tissue (lactation) and adipose tissue (growth). Investigations to alter the content of CLA have typically involved lactating cows (milk fat); fewer data from growing cattle (body fat) are available. Dietary factors that alter the content of CLA because of effects on the rumen biohydrogenation processes include unsaturated fatty acid substrates and altered rumen environment. The *cis*-9, *trans*-11 CLA isomer is the major isomer found in ruminant fat; this isomer typically represents 80 to 90% of the total CLA in milk fat, but its proportion in beef fat is less. Under certain dietary conditions the proportion of the *trans*-10, *cis*-12 CLA isomer increases. Thus, dietary factors also alter the direction of the biohydrogenation pathways in the rumen. The CLA possess anticarcinogenic effects, which relates to the *cis*-9, *trans*-11 CLA isomer, as evident from results with mammary tumors in a rat model. Lipid accretion and nutrient partitioning are also altered by CLA in several species. Recent work demonstrates that this relates primarily to the *trans*-10, *cis*-12 CLA isomer, as evident by effects on milk fat synthesis in lactating cows and body fat accretion in growing mice. Overall, consideration of functional foods containing CLA represents an exciting area of potential importance in producing food products derived from ruminants.

Key Words: Conjugated Linoleic Acid, Fats, Stearyl-CoA Desaturase, Milk Products, Beef

Introduction

Animal products contribute significantly to the total nutrients in our food supply (NRC, 1988). They provide a nearly ideal pattern of amino acids and account for over 60% of the total protein intake in the United States. In addition, they are a primary source of many vitamins and minerals, including vitamin B_{12}, vitamin B_6, riboflavin, niacin, zinc, phosphorus, and calcium. The goal of increasing the efficiency of animal production has been, and continues to be, an important consideration in producing animal-derived food products. There is also increasing recognition that foods can be contributing factors in the prevention and development of some disease conditions. As a result, additional focus has been given to designing foods with enhanced components that have beneficial effects on human health (NRC, 1988).

The term *functional foods* is increasingly used as a generic description for the beneficial effects of ingested foods that go beyond their traditional nutritive value (Milner, 1999). A report by the National Academy of Sciences defined functional foods as "any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains" (NRC, 1994). This concept is gaining awareness and acceptance by consumers, particularly as it relates to the value of certain fruits and vegetables. Food products derived from animals are also known to contain microcomponents that have positive effects on human health and disease prevention beyond those associated with traditional nutritive values (Allen, 1993; Knekt et al., 1996; Parodi, 1997; Molkentin, 1999). Conjugated linoleic acids (**CLA**) represent one of these microcomponents in animal products. In the following sections we will provide additional background and review the biology of CLA in ruminants.

Background

Food products derived from ruminant animals are the major source of CLA in human diets (Chin et al., 1992; Fritsche and Steinhart, 1998; McGuire and McGuire, 2000). The discovery of a "functional food" role for CLA occurred over a decade ago when Pariza and coworkers found that ground beef contained an anticarcinogen factor that consisted of a series of conjugated dienoic isomers of linoleic acid (Pariza et al., 1979; Pariza and Hargraves, 1985; Ha et al., 1987). Subsequent work has found that dietary CLA are able to reduce the incidence of tumors in animal models for mammary, forestomach, colon, and skin tumorigenesis (see reviews by Belury, 1995; Scimeca et al., 1995; and Banni and Martin, 1998). The uniqueness of these effects was recognized in the National Academy of Science report *Carcinogens and Anticarcinogens in the Human Diet,* which stated that "conjugated linoleic acid (CLA) is the only fatty acid shown unequivocally to inhibit carcinogenesis in experimental animals" (NRC, 1996).

Recently, the range of positive health effects associated with CLA in experimental models has been extended to include reduction in body fat accretion and altered nutrient

partitioning, antidiabetic effects, reduction in the development of atherosclerosis, enhanced bone mineralization, and modulation of the immune system (Belury, 1995; Banni and Martin, 1998; Houseknecht et al., 1998). The companion article in this symposium by McGuire and McGuire (2000) reviews the biological aspects of CLA related to human health. Obviously, CLA are fascinating compounds of interest for animal scientists, human nutritionists, and the medical community.

Conjugated linoleic acids represent a mixture of positional and geometric isomers of octadecadienoic acid with conjugated double bonds. The presence of fatty acids with conjugated double bonds was first demonstrated in food products derived from ruminants by Booth et al. (1935) working with milk fat from cows turned out to spring pasture. Subsequently, Parodi (1977) demonstrated these primarily represented conjugated *cis*-9*, trans*-11 octadecadienoic acid. Theoretically, a number of CLA isomers are possible that differ in the positions of the double bond pairs (e.g., 7–9, 8–10, 9–11, 10–12, and so forth). Additional differences can exist in the configuration of the double bond so that *cis-trans*, *trans-cis*, *cis-cis,* or *trans-trans* configurations are all possible. Industrial processes to produce CLA from linoleic acid result in products that contain significant quantities of many isomers of CLA, although this varies with process conditions (Banni and Martin, 1998; Sehat et al., 1999). Recently, innovative analysis applications have been used to quantify the isomers of CLA. These have included combining analytical methods (e.g., gas chromatography, silver ion high performance liquid chromatography, Fourier-transform infrared spectroscopy, and electron ionization mass spectrometry) (Banni and Martin, 1998; Sehat et al., 1998) and sequential application of an analytical method (e.g., two to six high performance liquid chromatography columns in series; Sehat et al., 1999). This has resulted in an improved ability to separate and quantify trace concentrations of different isomers of CLA.

The major isomer of CLA in milk fat is *cis*-9, *trans*-11, and it represents 80 to 90% of the total CLA (Parodi, 1977; Chin et al., 1992; Sehat et al., 1998). Recent studies have demonstrated that the *cis*-9, *trans*-11 isomer reduces mammary tumor incidence in rats when added to the diet or consumed as a natural component of butter (Ip et al., 1999). Rumenic acid has been proposed as the common name for this specific CLA isomer (Kramer et al., 1998a). Under certain dietary conditions, such as high-concentrate, low-fiber diets, the profile of CLA can be altered so that the concentration of the *trans*-10, *cis*-12 isomer increases in milk fat (Griinari et al., 1999).

Structures of the *cis*-9, *trans*-11, and *trans*-10, *cis*-12 isomers of CLA are presented in Figure 1. The *cis*-9, *trans*-11 isomer is also the predominant isomer in meat from ruminants but constitutes less of the total (Chin et al., 1992; Shantha et al., 1994). The lower proportion of *cis*-9, *trans*-11 isomer in meat fat as compared to milk fat probably relates to effects of traditional high-concentrate, low-fiber diets fed to finishing cattle in the United States, as will be discussed later. Consistent with this idea, the *cis*-9, *trans*-11 isomer was > 90% of the total CLA in subcutaneous and intramuscular fat of German Simmental cattle fed corn-silage-based diets with a moderate level of grain supplement (Fritsche and Fritsche, 1998). Recently, investigations using the more elaborate analyses cited above have revealed that trace concentrations of many additional isomers of CLA are also present in milk fat, and we would expect the same for body fat from growing ruminants. For example, analysis of commercial cheese products demonstrated that *cis*-9, *trans*-11 was the predominant isomer (78 to 84%), but additional isomers of CLA were identified that resolved into seven *trans-trans* (5 to 9%), three *cis/trans* (*cis-trans* or *trans-cis*) (10 to 13%), and five *cis, cis* (< 1%) isomers (Sehat et al., 1998).

A number of investigations have examined the effect of manufacturing and storage practices on the concentration of CLA in food products derived from ruminants. In general, results demonstrate that processing and storage have minimal effects, indicating that CLA are relatively stable (Shantha et al., 1995; Banni and Martin, 1998). Thus, the content of CLA in foods is, in large part, dependent on the concentration of CLA in raw products. Concentrations of CLA are generally expressed in relation to total fat, and dairy products and meat from ruminants typically have concentrations in the range of 3 to 7 mg/g of fat (Chin et al., 1992; Lin et al., 1995; Banni and Martin, 1998). However, the concentration of CLA can vary widely. Riel (1963) was the first to demonstrate this when he surveyed milk from Canadian creameries and demonstrated an eightfold variation in the content of CLA of milk fat. More recent comparisons of milk from dairy herds in the northeastern United States (Kelly and Bauman, 1996) indicate similar variation. Fewer studies have examined this for growing ruminants. However, concentrations of CLA in the fat from meat cuts of cattle raised in Australia and Germany are approximately two- to threefold greater than those found in U.S. cattle (Fogerty et al., 1988; Shantha et al., 1994; 1997; Fritsche and Steinhart, 1998). These differences are largely related to diet, and this will be addressed later. However, studies with lactating dairy cows have demonstrated that even in herds in which all cows are managed similarly and fed the same diet, there is still a threefold variation in the milk fat content of CLA (Jiang et al., 1996; Kelly et al., 1998a,b).

Biosynthesis of CLA

The CLA found in milk and meat fat of ruminants originate from two sources (Griinari and Bauman, 1999). One source is CLA formed during ruminal biohydrogenation of linoleic acid. The second source is CLA synthesized by the animal's tissues from *trans*-11 $C_{18:1}$, another intermediate in the biohydrogenation of unsaturated fatty acids. Thus, the uniqueness of CLA in food products derived from ruminants relates to the incomplete biohydrogenation of dietary unsaturated fatty acids in the rumen. Ironically, rumen biohydrogenation of dietary lipids is responsible for the high levels of saturated fatty acids in fat of ruminants, a feature considered undesirable for some aspects of human health, as well as for

ruminant fat containing CLA, fatty acids with many putative beneficial effects on human health.

Rumen Biohydrogenation

The lipid composition of forages consists largely of glycolipids and phospholipids, and the major fatty acids are the unsaturated fatty acids linolenic ($C_{18:3}$) and linoleic ($C_{18:2}$) acid. In contrast, the lipid composition of seed oils used in concentrate feedstuffs is predominantly triglycerides containing linoleic and oleic acid (*cis*-9 $C_{18:1}$) as the predominant fatty acids. When consumed by ruminant animals, dietary lipids undergo two important transformations in the rumen (Dawson and Kemp, 1970; Keeney, 1970; Dawson et al., 1977). The initial transformation is hydrolysis of the ester linkages catalyzed by microbial lipases. This step is a prerequisite for the second transformation: biohydrogenation of the unsaturated fatty acids.

Bacteria are largely responsible for biohydrogenation of unsaturated fatty acids in the rumen; protozoa seem to be of only minor importance (Harfoot and Hazlewood, 1988). For a number of years, the only bacterium known to be capable of biohydrogenation was *Butyrivibrio fibrisolvens* (Kepler et al., 1966). However, as research efforts expanded a diverse range of rumen bacteria have been isolated that have the capacity to biohydrogenate unsaturated fatty acids (see review by Harfoot and Hazlewood [1988]). Biohydrogenation of unsaturated fatty acids involves several biochemical steps. Investigations with pure cultures suggest that no single species of rumen bacteria catalyzes the complete biohydrogenation sequence. Kemp and Lander (1984) divided bacteria into two groups based on the reactions and end products of biohydrogenation. Group A bacteria were able to hydrogenate linoleic acid and α-linolenic acid, *trans*-11 $C_{18:1}$ being their major end product. Group B bacteria utilized *trans*-11 $C_{18:1}$ as one of the main substrates with stearic acid being the end product. A listing of the bacteria species in Groups A and B is provided in the review by Harfoot and Hazlewood (1988).

The biohydrogenation sequence of linoleic acid is presented in Figure 2. Isomerization of the *cis*-12 double bond represents the initial step during biohydrogenation of fatty acids containing a *cis*-9, *cis*-12 double bond system. The isomerase reaction is unusual because it has no cofactor requirement and occurs in the middle of a long hydrocarbon chain remote from any activating functional groups. Linoleate isomerase (EC 5.2.1.5) is the enzyme responsible for forming conjugated double bonds from the *cis*-9, *cis*-12 double bond structure of linoleic as well as α- and γ-linolenic acids. It has been partially purified, and its kinetic properties have been characterized in a limited number of bacterial species (Kepler and Tove, 1967; Kepler et al., 1970; Yokoyama and Davis, 1971; Kemp et al., 1984). The enzyme is bound to the bacterial cell membrane and demonstrates an absolute substrate requirement for a *cis*-9, *cis*-12 diene system and a free carboxyl group.

The second reaction is a reduction in which *cis*-9, *trans*-11 CLA is converted to *trans*-11 $C_{18:1}$ (Figure 2). In vitro studies using labeled linoleic acid cultured with rumen contents demonstrated that isomerization of the *cis*-12 double bond was followed by rapid conversion of *cis*-9, *trans*-11 CLA to *trans*-11 octadecenoic acid. Hydrogenation of the *trans*-11 monoene occurred less rapidly, and therefore it increased in concentration (Tanaka and Shigeno, 1976; Singh and Hawke, 1979). Similar results were obtained in time course studies of linoleic acid biohydrogenation (Harfoot et al., 1973b; Kellens et al., 1986). Therefore, *trans*-11 $C_{18:1}$ reduction seems to be rate-limiting in the biohydrogenation sequence of unsaturated C_{18} fatty acids. As a consequence, this penultimate biohydrogenation intermediate accumulates in the rumen (Keeney, 1970) and is, therefore, more available for absorption (Figure 2).

Similar to biohydrogenation of linoleic acid, biohydrogenation of linolenic acid begins with an isomerization followed by a sequence of reductions and terminates with the formation of stearic acid. The predominant $C_{18:3}$ fatty acid in feedstuffs is α-linolenic acid (*cis*-9, *cis*-12, *cis*-15 octadecatrienoic acid). Rumen biohydrogenation of α-linolenic acid produces *cis*-9, *trans*-11, *cis*-15 conjugated octadecatrienoic acid as the predominant initial isomerization product, and this is followed by reduction of the *cis*-double bonds. As a consequence, *trans*-11 octadecenoic acid is a common intermediate in the biohydrogenation of both α-linolenic acid and linoleic acid. In addition, biohydrogenation of γ-linolenic acid, *cis*-6, *cis*-9, *cis*-12 octadecatrienoic acid, also results in formation of *trans*-11 $C_{18:1}$ (see reviews by Harfoot and Hazelwood [1988] and Griinari and Bauman [1999]).

Decreased rumen pH often results in bacterial population shifts and consequent changes in the pattern of fermentation end products (Van Soest, 1994). Leat et al. (1977) provided evidence showing that changes in rumen bacteria populations are associated with modifications in the biohydrogenation pathways consistent with the altered *trans*-octadecenoic acid profile found in ruminal digesta and tissue lipids. In addition, Griinari et. al. (1998) demonstrated that an altered rumen environment induced by feeding high-concentrate, low-fiber diets is associated with a change in the *trans*-octadecenoic acid profile of milk fat. During this situation, *trans*-10 octadecenoic acid replaced *trans*-11 $C_{18:1}$ as the predominant *trans* $C_{18:1}$ isomer in milk fat. Putative pathways for the production of *trans*-10 octadecenoic acid have been proposed (Griinari and Bauman, 1999), and these involve a specific *cis*-9, *trans*-10 isomerase in rumen bacteria with the formation of *trans*-10, *cis*-12 conjugated double bond structure as the first intermediate. Further evidence in support of a specific bacterial *cis*-9, *trans*-10 isomerase is provided by observations that low-fiber diets increase the proportion of *trans*-10, *cis*-12 CLA isomer in milk fat (Griinari et al., 1999). *Trans*-10, *cis*-12 CLA has also been observed as one of the three major isomers of CLA in rumen digesta obtained from continuous flow-through fermenters (Fellner et al., 1997).

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 demonstrated that it was the *trans*-11 $C_{18:1}$ 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 (Jiang et al., 1996; Jahreis et al., 1997; Precht and Molkentin, 1997; Griinari and Bauman, 1999). This relationship has been generally attributed to a common source for these two fatty acids as intermediates in ruminal biohydrogenation.

The close relationship between *trans*-11 $C_{18:1}$ and *cis*-9, *trans*-11 CLA in milk fat is also consistent with a precursor-product relationship. Based on this and the kinetics of rumen biohydrogenation that would lead to *trans*-11 $C_{18:1}$ 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 endogenous *cis*-9, *trans*-11 CLA would originate from the desaturation of *trans*-11 $C_{18:1}$ by Δ^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 $C_{18:1}$ (12.5 g/d) (Corl et al., 1998). At the end of the 3-d infusion period, content of CLA in milk fat had increased 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, specific inhibitor of Δ^9-desaturase (Corl et al., 1999). Results demonstrated that sterculic acid caused a dramatic reduction in milk fat content of *cis*-9, *trans*-11 CLA (Figure 3). When the lack of complete desaturase inhibition is considered, it is clear that endogenous synthesis via Δ^9-desaturase represents the predominant source of CLA in milk fat. We predict endogenous synthesis of *cis*-9, *trans*-11 CLA will also be the major source of CLA in body fat of ruminants.

The desaturase system is a multienzyme complex that includes NADH-cytochrome b_5 reductase, cytochrome b_5, acyl-CoA synthase, and the terminal Δ^9-desaturase (Figure 4). The Δ^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 Δ^9-desaturase, and the fatty acid products of this reaction are important components of phospholipids and triglycerides, particularly for maintenance of membrane fluidity. However, a wide range of saturated and unsaturated acyl CoA can serve as substrates, including *trans*-11 octadecenoic acid (Enoch et al., 1976; Mahfouz et al., 1980, 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 Δ^9-desaturase. Recently, Yurawecz et al. (1998) identified a new CLA isomer, *trans*-7, *cis*-9 octadecadienoic acid, and Ulberth and Henninger (1994) identified *cis*-9, *trans*-13 octadecadienoic acid in milk fat.

There are species differences in the tissue distribution of Δ^9-desaturase. For rodents, concentrations of mRNA and enzyme activity are greatest in liver (see reviews by Ntambi [1995] and Tocher et al. [1998]). In contrast, growing sheep and cattle have substantially greater Δ^9-desaturase in adipose tissue, as indicated by mRNA abundance and enzyme activity (Wahle, 1974; St. John et al., 1991; Chang et al., 1992; Cameron et al., 1994; 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 Δ^9-desaturase (Bickerstaffe and Annison, 1970; Kinsella, 1972). In vivo results are also consistent with the lactating mammary gland being of primary importance in 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 Δ^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.

Investigations of Δ^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 (see reviews by Ntambi [1995] and Tocher et al. [1998]). Similar studies with Δ^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 mRNA abundance of Δ^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 Δ^9-desaturase gene expression in sheep adipose tissue explants.

Factors Affecting CLA Content of Ruminant Fats

The content of CLA in fat from ruminant-derived food products will be dependent on the ruminal production of both CLA and *trans*-11 $C_{18:1}$ and the tissue activity of Δ^9-desaturase. Most studies examining the content of CLA in ruminant lipids have used lactating dairy cows. However, we anticipate that factors affecting the CLA in milk fat would similarly affect the content of CLA in body lipids of growing and lactating ruminants.

Dietary Factors

The substantial variation in content of CLA in milk fat between herds discussed earlier suggests that diet has a major influence. Many dietary factors are known to affect CLA in milk fat; these are presented in Table 1, where they are

grouped into categories relative to the potential mechanism by which they may act. The first category includes dietary factors that provide lipid substrate for the production of CLA or *trans*-11 $C_{18:1}$ in the rumen. The second grouping consists of dietary factors that alter the rumen environment, thereby affecting the bacteria involved in rumen biohydrogenation. The third group includes dietary factors that may involve a combination of lipid substrate and modification of the rumen population of bacteria.

Dietary addition of plant oils results in substantial increases in milk fat concentration of CLA (Table 1). Plant oils have included sunflower, soybean, corn, canola, linseed, and peanut. However, feeding grain and silage harvested from high-oil varieties of corn has minimal effect on the concentration of CLA in milk fat (Table 1). In general, plant oils high in linoleic acid give the greatest response (Kelly et al., 1998a), and there is a clear dose-dependent increase in milk fat content of CLA (Table 1). Harfoot et al. (1973a) reported that high levels of linoleic acid irreversibly inhibit the hydrogenation of *trans*-11 octadecenoic acid, and this would result in additional substrate for endogenous synthesis of *cis*-9, *trans*-11 CLA (Figure 2). In vitro rumen culture studies have also demonstrated that increased levels of linoleic acid result in an unusual pattern of biohydrogenation whereby *trans*-$C_{18:1}$ acid, rather than stearate, is the major end product (Polan et al., 1964; Harfoot et al., 1973a). This suggests effects on the "Group B" bacteria involved in biohydrogenation (Harfoot and Hazlewood, 1988), and the mechanism has been attributed to linoleic acid acting as a competitive inhibitor for the biohydrogenation of the monoenoic acid (Polan et al., 1964).

Plant oils are not normally included in ruminant diets because they produce inhibitory effects on rumen microbial growth (Jenkins, 1993). A method to minimize this effect is to feed Ca salts of the fatty acids so that most of the fatty acids bypass the rumen and only a portion are biohydrogenated (Table 1). Another method is to feed full-fat seeds. However, studies have demonstrated that feeding raw seeds has no effect on the milk fat concentration of CLA (Table 1), suggesting that the polyunsaturated fatty acids in the intact seeds are relatively unavailable to the rumen bacteria. In contrast, substantial increases in milk fat concentration of CLA occur when the diet supplement contains full-fat seeds that have been processed. These investigations have included rapeseeds, soybeans, and cottonseeds, and the processed seeds have been ground, roasted, micronized, flaked, and extruded (Table 1).

Increases in milk fat concentration of CLA are also observed with dietary addition of fish oils or fish meal (Table 1). Furthermore, fish oils seem to produce a larger increase in milk fat CLA than an equal amount of plant oils (Chouinard et al., 1998a). Although the rumen biohydrogenation of the long-chain polyunsaturated fatty acids in fish oil is not well understood (Harfoot and Hazelwood, 1988), neither CLA nor *trans*-11 octadecenoic acid seem to be intermediates. It may be that the inhibitory effect of fish oil on ruminal biohydrogenation of *trans*-octadecadienoic acid is similar to the inhibitory effect of high levels of linoleic acid cited earlier. Consistent with this, Chilliard et al. (1999) demonstrated that the feeding of fish oil results in increased ruminal production of *trans*-11 octadecenoic acid. The inhibitory effect could involve inhibition of the growth or a specific inhibition of the reductases of bacteria that reduce octadecenoic acid.

Changes in the rumen bacterial population are often the result of decreased pH, as indicated by increased propionate production (Van Soest, 1994). Associated with these changes, a high-concentrate, low-fiber diet increases the rumen production and milk fat content of *trans*-octadecenoic acids (Kalscheur et al., 1997; Griinari et al., 1998). Consistent with this hypothesis, addition of buffer to a low-fiber diet increased rumen pH and decreased production of *trans*-octadecenoic acids (Kalscheur et al., 1997). However, it is difficult to distinguish between the amount of lipid substrate and rumen pH effects on biohydrogenation. For example, decreasing the dietary forage:concentrate ratio from 50:50 to 20:80 has resulted in both an increase (Chouinard et al., 1998b) and a decrease (Griinari et al., 1998) in the concentration of CLA in milk fat. To illustrate this, Griinari et. al (1998) fed a diet that resulted in lowered rumen pH while maintaining a constant dietary lipid content. Results demonstrated that total *trans* fatty acid production was unchanged, but the profile of *trans* fatty acids was altered such that *trans*-10 $C_{18:1}$ became the predominant *trans*-$C_{18:1}$ isomer. This shift in *trans*-$C_{18:1}$ profile was also observed when a low-fiber, high-oil diet was fed; a decrease in milk fat content of CLA was associated with a decrease in the proportion of *trans*-11 $C_{18:1}$ and an increase in the percentage of *trans*-10 $C_{18:1}$ (Griinari et al., 1999).

The effects of pasture on milk fat concentration of CLA have been described in a number of studies (Table 1). Generally, pasture feeding increases milk fat content of CLA compared to feeding either a total mixed ration with a similar lipid content or conserved forages. The lipids in pasture forages consist mainly of glycolipids and phospholipids, which are only 2% of the dietary dry matter (Van Soest, 1994). In vitro studies with rumen cultures suggest that glycolipids are hydrolyzed and hydrogenated similarly to triglycerides (Dawson et al., 1974, 1977; Singh and Hawke, 1979). Forage maturity also seems to be an important factor affecting milk fat content of CLA (Table 1). Diets containing forage at the early growth stage resulted in increased milk fat CLA compared to diets that included late-growth or second-cutting forage (Chouinard et al., 1998b). However, forage lipid content and composition seems only to partly explain observed differences in milk fat content of CLA. Synergistic effects between lipid substrate and other pasture components may also alter rumen biohydrogenation.

Alterations in feed intake have had variable effects on milk fat content of CLA. Restricting feed intake by approximately 30% resulted in milk fat concentration of CLA being increased in one study (Jiang et al., 1996) and decreased in another (Stanton et al., 1997). Timmen and Patton (1988) more severely restricted feed intake and observed that the concentration of CLA in milk fat more than doubled. Alterations in feed intake would obviously affect substrate supply and change the rumen environment. Both of these factors

would contribute to a change in the ruminal biohydrogenation process. In addition, underfeeding would increase the supply of CLA and *trans*-11 $C_{18:1}$ from mobilized body fat stores, and the magnitude of this increase would relate to the extent of the negative energy balance.

Ionophores inhibit the growth of Gram-positive bacteria. Several of the Gram-positive bacteria are involved in rumen biohydrogenation, including *Butyrivibrio fibrisolvens*. Using a continuous flow-through rumen fermentor, Fellner et al. (1997) observed that addition of ionophores inhibited linoleic acid biohydrogenation, resulting in decreased stearic acid and increased monounsaturated $C_{18:1}$ concentrations in ruminal contents. However, including ionophores in dairy cattle diets have given variable results on milk fat concentration of CLA. Sauer et al. (1998) reported an increase, whereas both Dhiman et al. (1996) and Chouinard et al. (1998b) observed no effect on milk fat concentration of CLA in cows receiving monensin. Differences may relate to ruminal adaptations in which ionophore-resistant species replace ionophore-sensitive bacteria responsible for ruminal biohydrogenation. In addition, variable levels of dietary polyunsaturated fatty acids may explain differences, although these data were not reported. In this case, bacterial populations involved in biohydrogenation may be altered, but substrate supply may be inadequate to allow the change in biohydrogenation to be expressed.

CLA Supplements and Lipid Metabolism

The milk fat and body fat content of CLA can also be increased by dietary supplements of CLA (Table 1). To date studies have involved lactating dairy cows and in initial investigations the supplement has been abomasally infused as an experimental means to by-pass rumen fermentation processes (Loor and Herbein, 1998; Chouinard et al., 1999a,b). However, technologies exist to protect supplements from alterations by rumen bacteria (Doreau et al. 1997), and this was successfully done for CLA in a recent study with lactating dairy cows (Giesy et al., 1999). Investigations with lactating cows have established that dietary supplements of CLA result in dose-related increases in the concentration of CLA in milk fat. Supplements have contained a number of CLA isomers, primarily consisting of *cis/trans* 8,10, *cis/trans* 9,11, *cis/trans* 10,12, and *cis/trans* 11,13, and results demonstrated that all isomers of CLA were transferred to milk fat (Chouinard et al., 1999a; 1999b).

Administration of supplements of CLA to lactating dairy cows also caused a dramatic reduction in the content and yield of milk fat (Loor and Herbein, 1998; Chouinard et al., 1999a,b; Giesy et al., 1999). This is illustrated in Figure 5, which demonstrates that abomasal infusion of a supplement that contained approximately 60% CLA caused a greater than 50% reduction in milk fat content across all doses. Effects are specific for milk fat; little or no changes occurred in yield of milk or other milk components. Earlier studies with lactating rats fed a supplement of CLA gave no indication of a reduction in milk fat secretion as indicated by growth rates of the nursing pups (Chin et al., 1994). However, in more recent work dietary supplements of CLA resulted in decreased milk fat content of nursing women (Masters et al., 1999) and lactating sows (R. J. Harrell and D. E. Bauman, unpublished observations).

Supplements of CLA have also been shown to markedly alter lipid metabolism in several species of growing animals, but in this case it involves adipose tissue. Body composition changes, specifically reduced fat, have been reported in mice (Park et al., 1997; DeLany et al., 1999) and pigs (Dugan et al., 1997; Ostrowska et al., 1999). Generally, the dose of CLA was .5 to 2.0% of the diet and most studies measured changes in whole-body fat percentage or fat content of specific organs. However, Ostrowska et al. (1999) determined that rates of fat accretion in growing pigs were decreased linearly with increasing dietary levels of CLA; a 31% reduction in lipid accretion was observed at the greatest dose of CLA (Figure 6).

A number of dietary situations, such as high-concentrate, low-fiber diets or increasing intake of plant oils, cause a reduction in milk fat secretion in dairy cows. This is generally referred to as milk fat depression (**MFD**), and several theories have been proposed and subsequently proven inadequate to explain the mechanism(s) (Davis and Brown, 1970; Erdman 1996). However, one theory of current interest is that the mechanism may involve an inhibition of milk fat synthesis by specific fatty acid intermediates produced in the ruminal biohydrogenation of polyunsaturated fatty acids. This idea was proposed almost 30 yr ago (Davis and Brown, 1970), and it has been supported by observations of MFD when lactating cows received abomasal infusions of partially hydrogenated vegetable oils (Selner and Schultz, 1980; Erdman, 1996). Across a number of studies the reduction in milk fat percentage was closely related to an increase in the *trans*-$C_{18:1}$ fatty acids in milk fat (Erdman 1996; Griinari et al., 1998). However, detailed analysis of the *trans* fatty acid isomers revealed the reduction in milk fat was specifically related to an increase in *trans*-10 $C_{18:1}$, and we proposed that dietary-induced MFD was caused by *trans*-10 $C_{18:1}$ or related metabolites (Griinari et al., 1998). More recent studies have shown that increases in the milk fat content of *trans*-10, *cis*-12 CLA also closely parallel the dietary-induced MFD (Griinari et al., 1999).

Based on the above observations with dietary-induced MFD, we hypothesized that the *trans*-10, *cis*-12 isomer was responsible for the reduction in milk fat observed with dietary supplements of CLA. We obtained relatively pure isomers and examined the effects of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA independent of each other. Effects were specific for milk fat, and after 4 d of abomasal infusion of *trans*-10, *cis*-12 CLA the milk fat percentage and yield were reduced 42 and 44%, respectively. In contrast, infusion of a similar amount of *cis*-9, *trans*-11 CLA had no effect on milk fat (Baumgard et al., 2000). The amount of CLA infused represented approximately .05% of the daily dry matter intake. Based on other studies in which various isomer enrichments of CLA were infused, *cis/trans* 8,10 may also be able to inhibit milk fat synthesis (Chouinard et al., 1999b). It is apparent that isomers of CLA or their metabolites containing

a double bond at the 10 position have inhibitory effects on milk fat synthesis. A recent study by Park et al. (1999) demonstrated that dietary addition of *trans*-10, *cis*-12 CLA caused a reduction in body fat in growing mice, whereas *cis*-9, *trans*-11 CLA had little or no effect. Thus, the same CLA isomer that caused a reduction in milk fat synthesis may also be the CLA isomer that caused reductions in body fat in different species of growing animals.

Mechanisms by which CLA inhibit milk fat synthesis have not been clearly delineated. Milk fat contains fatty acids derived from de novo synthesis by the mammary gland ($C_{4:0}$ to $C_{14:0}$ plus a portion of $C_{16:0}$) and from mammary uptake of preformed fatty acids (a portion of $C_{16:0}$ and all longer chain fatty acids). On a molar basis, approximately 80% of the reduction in milk yield of fatty acids was accounted for by the reduction in de novo synthesized fatty acids when cows received a mixture of CLA (Chouinard et al., 1999a) or the specific *trans*-10, *cis*-12 CLA isomer (Baumgard et al., 2000). An additional change in milk fat composition was a reduction in the fatty acids arising from Δ^9-desaturase activity. The ratios of $C_{14:1}$ to $C_{14:0}$, $C_{16:1}$ to $C_{16:0,}$ and *cis*-9 $C_{18:1}$ to $C_{18:0}$ were all decreased when cows received CLA. These ratios represent a proxy for Δ^9-desaturase activity, so it is evident that CLA, in particular the *trans*-10, *cis*-12 CLA isomer, decreases Δ^9-desaturase activity. Thus, in dairy cows, changes in the milk fatty acid composition suggest that CLA causes an attenuation of the pathways of de novo lipogenesis and a reduction in Δ^9-desaturase capacity. In lactating women and sows, the mechanism whereby CLA reduce milk fat secretion may also involve lipoprotein lipase and the use of preformed fatty acids, because this process is the major source of milk fat in these species. Therefore, the mechanism by which CLA reduce milk fat synthesis may be multifaceted, and it could even involve fatty acid esterification and triglyceride synthesis.

The mechanisms whereby CLA cause reduced body fat accretion in growing animals are also not clearly established. Effects could involve reduced de novo synthesis, reduced use of preformed fatty acids, increased rates of lipolysis, or some combination of these. There has been some support for each of these based on work with rodents and measurements involving enzyme activities, mRNA abundance, or cell culture results. However, there has been no consensus on the mechanism for effects of CLA on lipid metabolism, so perhaps the mechanism is multifaceted. Consistent with this, West et al. (1998) recently reported that carcass fat was reduced in CLA-treated mice fed either high-fat or high-carbohydrate diets. Lipid accretion in adipose tissue for animals fed the former diet would primarily involve the utilization of preformed fatty acids, whereas fat accretion for animals consuming the latter diet would be dependent on de novo fatty acid synthesis.

The *trans*-10, *cis*-12 isomer in supplements of CLA that is responsible for reduced milk fat synthesis in lactating animals and reduced body fat accretion in growing animals is also naturally produced by rumen bacteria under certain dietary situations. In the case of lactating cows these dietary situations are known to be associated with MFD, and thus a role in lipid metabolism for the *trans*-10, *cis*-12 CLA isomer in supplements of CLA is consistent with its probable involvement in diet-induced reductions in milk fat synthesis by the mammary gland. However, body fat accretion is generally increased when MFD occurs in lactating cows, which seems inconsistent with the concept that the *trans*-10, *cis*-12 CLA isomer causes a reduced body fat accretion. Likewise, there are some paradoxes in the possible role of *trans*-10, *cis*-12 CLA in the reduction of body fat accretion in growing animals. Dietary conditions causing an increase in *trans*-10, *cis*-12 CLA are a high-grain, low-fiber diet such as that fed to cattle during the finishing period, yet the finishing period is when cattle typically have the greatest rate of body fat accretion and intramuscular fat deposition. Perhaps the above paradoxes relate to quantity of the *trans*-10, *cis*-12 CLA isomer. Consistent with this, the level of *trans*-10, *cis*-12 in fat from beef cattle in the finishing period was 1 mg/g of lipid (Dhiman et al., 1999b) and 17 mg/g of lipid in growing pigs fed a supplement of CLA at 1% of dietary dry matter (Kramer et al., 1998b). In terms of exogenous CLA, variation in CLA supplements makes comparisons difficult, but the amount of CLA required to inhibit milk fat synthesis in lactating cows seems to be substantially less than the amount required to reduce body fat synthesis in growing animals (Ostrowska et al., 1999; Baumgard et al., 2000). Developing an understanding of the mechanisms whereby CLA are able to elicit their diverse range of biological effects should clarify some of these issues and represents an exciting opportunity.

Implications

Food products from ruminants contain conjugated linoleic acids (CLA), which are fatty acids that have beneficial health effects as shown in research with animal models. The biosynthesis of CLA and dietary factors that cause variation in the content of CLA in ruminant fat have been identified. Thus, the opportunity exists to substantially increase the concentration of CLA in food products. There are many isomers of CLA in fat of ruminants, but *cis*-9, *trans*-11 is predominant. Research with animal models has demonstrated that this specific isomer has anticarcinogenic properties, and mammary tumors are reduced when butter containing a high concentration of CLA is fed. Under certain dietary conditions *trans*-10, *cis*-12 CLA increases in the rumen, and this isomer causes reduced milk fat synthesis (lactation) and body fat accretion (growth). Thus, consideration of functional foods containing CLA represents an exciting research area of potential importance in the production of food products derived from ruminants.

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Notes

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Table 1. Summary of dietary factors that affect concentrations of conjugated linoleic acids (CLA) in milk fat[a]

Dietary factor	Content of CLA in milk fat	Reference[b]
Lipid substrate		
Unsaturated vs saturated fat	Increased by addition of unsaturated fat	M
Plant oils		
Type of plant oil	Increased with oils high in unsaturated fatty acids	B , G, K, N
Level of plant oil	Dose-dependent increase	B, E, G
Ca salts of plant oils	Increased	K
High-oil plant seeds		
Raw seeds	No effect	G, K
Processed seeds	Increased	J, K, P, V
High-oil corn grain and silage	Minimal effect	C, K
Animal fat by-products	Minimal effect	K
Modifiers of rumen environment		
Forage:concentrate ratio	Variable effect	D, L, M
Nonstructural carbohydrate level	Minor effect	L, Y
Restricted feeding	Variable effect	A, D, J
Fish oils/fish meal	Increased	C, K, S
Marine algae	Increased	W
Ionophores	Variable effect	C, L, R
Dietary buffers	Little effect with sufficient fiber	L
Combination		
Pasture	Higher than on conserved forages	A, C, F, H, I, O
Growth stage of forage	Increased with less mature forage	L
CLA supplement	Dose-dependent increase	Q, T, U, X

[a]Adapted from Griinari and Bauman (1999).

[b]Symbols are as follows: A = Timmen and Patton, 1988; B = Tesfa et al., 1991; C = Dhiman et al., 1996; D = Jiang et al., 1996; E = McGuire et al., 1996; F = Zegarska et al., 1996; G = Dhiman et al., 1997; H = Jahreis et al., 1997; I = Precht and Molkentin, 1997; J = Stanton et al., 1997; K = Chouinard et al., 1998a; L = Chouinard et al., 1998b; M = Griinari et al., 1998; N = Kelly et al., 1998a; O = Kelly et al., 1998b; P = Lawless et al., 1998; Q = Loor and Herbein, 1998; R = Sauer et al., 1998; S = Chilliard et al., 1999; T = Chouinard et al., 1999a; U = Chouinard et al., 1999b; V = Dhiman et al., 1999a; W = Franklin et al. 1999; X = Giesy et al., 1999; and Y = Solomon et al., 2000.

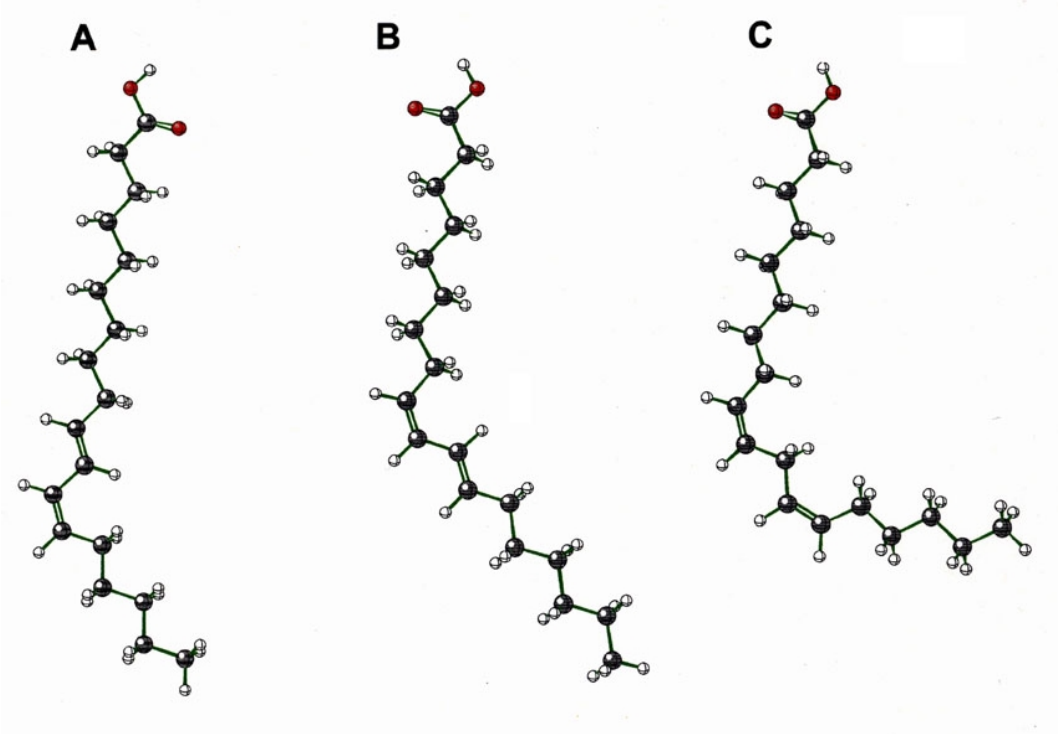

Figure 1. Chemical structure of conjugated linoleic acid isomers and linoleic acid. Fatty acids are *trans*-10, *cis*-12 octadecadienoic acid (A), *cis*-9, *trans*-11 octadecadienoic acid (B) and *cis*-9, *cis*-12 octadecadienoic acid (linoleic acid) (C).

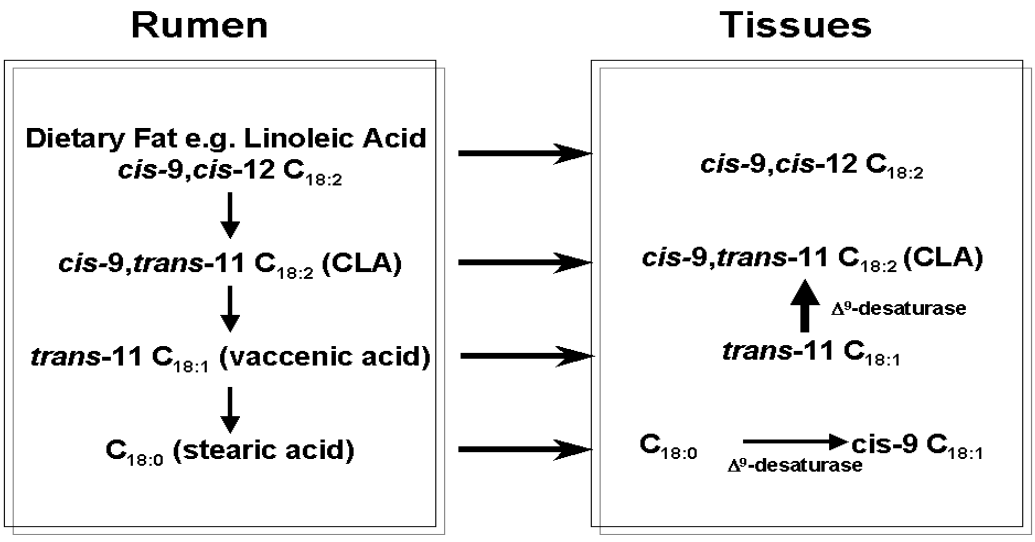

Figure 2. Role of rumen biohydrogenation and tissue Δ^9-desaturase in the production of *cis*-9, *trans*-11 conjugated linoleic acid in ruminant fat.

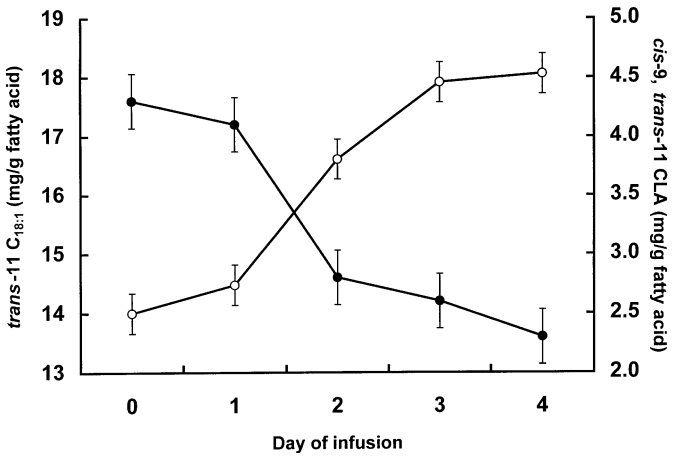

Figure 3. Effect of abomasal infusion of sterculic acid on milk fat content of *cis*-9, *trans*-11 conjugated linoleic acid (closed circles) and *trans*-11 $C_{18:1}$ (open circles). Sterculic acid is a specific inhibitor of Δ^9-desaturase; abomasal infusions delivered 5 g/d of sterculic acid and bars about each data point represent SEM. Adapted from Griinari et al. (2000).

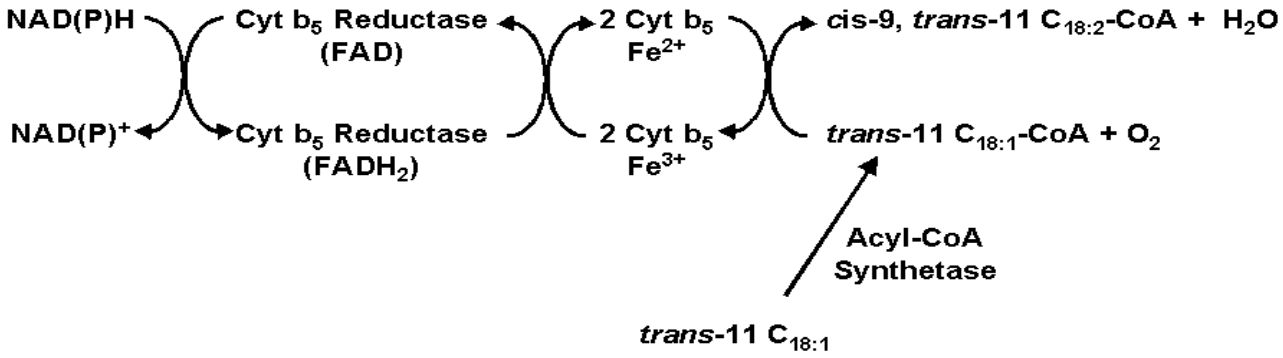

Figure 4. Putative biochemical pathways for the Δ^9-desaturase system involved in the endogenous synthesis of *cis*-9, *trans*-11 conjugated linoleic acid. Adapted from Ntambi (1995).

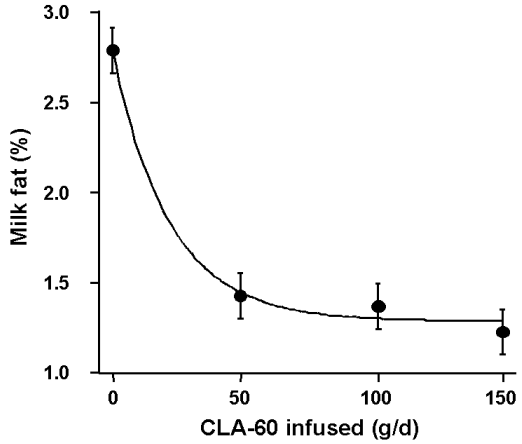

Figure 5. Effect of abomasal infusion of conjugated linoleic acid (CLA) supplement on milk fat synthesis by lactating cows. Predominant isomers of CLA in the supplement were *cis/trans* 8,10 (15%), *cis/trans* 9,11 (24%), *cis/trans* 10,12 (35%) and *cis/trans* 11,13 (17%). Note: *cis/trans* indicates the double bonds could be *cis-trans* or *trans-cis.* Adapted from Chouinard et al. (1999a).

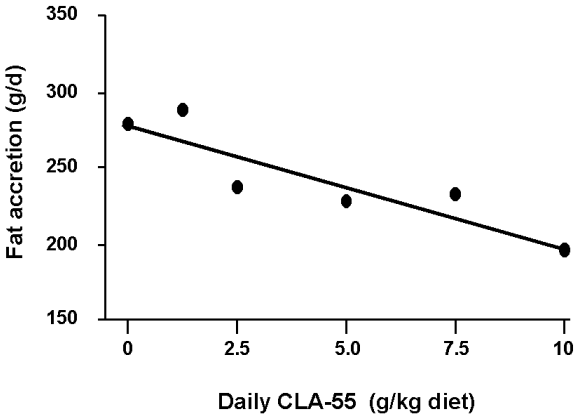

Figure 6. Effect of dietary supplement of conjugated linoleic acids (CLA) on rates of fat accretion in growing pigs. Supplementation was for 8 wk (initial body weight = 56 kg) and predominant isomers of CLA in the supplement were *cis/trans* 8,10 (14%), *cis/trans* 9,11 (25%), *cis/trans* 10,12 (30%), and *cis/trans* 11,13 (18%). Note: *cis/trans* indicates the double bonds could be *cis-trans* or *trans-cis.* Adapted from Ostrowska et al. (1999).