Conjugated Linoleic Acids in the Dairy Cow: Biological Activities and Applications

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INTRODUCTION

There has been an increasing recognition over the past several years that dietary lipids can play important roles in metabolism and signaling in addition to their classical role as a source of energy (e.g. Jump and Clarke, 1999; Chong and Marx, 2001). One example of a family of lipids that has been implicated in modulating such divergent processes as cancer development, lipid metabolism, bone mineralization and others are conjugated linoleic acids, or CLA.

The interest in CLA really began with an attempt by Pariza and coworkers to identify carcinogenic compounds in cooked beef (reviewed by Pariza, 1999). Ironically, this project instead identified a compound with very potent anti-cancer properties that was finally identified as cis-9, trans-11 CLA. Parodi (1977) had identified this compound several years prior as a component of cow’s milk, and it is now clear that ruminant fat depots are the most abundant natural source of this fatty acid. Subsequent studies performed by Ip and coworkers (1991; 1999) elegantly demonstrated that CLA is a potent anticarcinogen in mouse models of mammary cancer and others have demonstrated these anticarcinogenic properties in many other models (reviewed by Pariza, 1999).

CLA are actually a family of related fatty acid forms, or isomers, each with apparently divergent biological activities. The structural differences lie in the arrangement of the two carbon-carbon double bonds within the molecule, each isomer having a unique combination of position (9, 10, 11, 12, etc.) and geometry (cis- or trans-; Figure 1). There are two isomers of CLA that have received the greatest attention in scientific investigations, cis-9, trans-11 CLA and trans-10, cis-12 CLA, each formed by the isomerization of the cis-12 or cis-9 double-bonds of linoleic acid, respectively (Figure 1).

Figure 1. Structure of linoleic acid and two common CLA isomers. Arrows indicate the position of carbon-carbon double bonds.

linoleic acid (cis-9, cis-12) cis-9, trans-11 CLA trans-10, cis-12 CLA
The cis-9, trans-11 isomer is implicated in the anti-cancer properties of CLA while the trans-10, cis-12 isomer is generally associated with effects on lipid metabolism in models of lactation as well as adipose and liver metabolism. Both of these fatty acids naturally occur in ruminant lipids due to their production in the microbial process of biohydrogenation of linoleic acid in the rumen. While cis-9, trans-11 CLA is the predominant CLA isomer in the milk and body fat of ruminants, trans-10, cis-12 CLA is but one of more than a dozen other isomers that occur in trace amounts in ruminant fat (Sehat et al., 1998; Fritsche et al., 2000).

**CLA ENRICHMENT OF MILK FAT**

Rumen microbes are responsible for the alteration of many dietary components; indeed, without their fermentation of plant matter, the ruminant would not survive. One alteration that is of paramount interest in regard to understanding CLA is the process of biohydrogenation of polyunsaturated fatty acids (PUFA), or the reduction of PUFA to saturated fatty acids (SFA). Intermediates in the pathways of biohydrogenation include CLA isomers, as well as related fatty acids that contribute to the CLA content of ruminant fat. It is ironic that the same process that is responsible for the relatively high saturated fat content of ruminant-derived foods, a characteristic of fat generally considered to be unhealthy, is also responsible for the formation of CLA, a fatty acid that may have health benefits.

Early work characterizing the process of rumen biohydrogenation established that cis-9, trans-11 CLA was an intermediate in the biohydrogenation of linoleic acid to stearic acid (Dawson and Kemp, 1970; Keeney, 1970; Harfoot 1978). The cis-9, trans-11 isomer of CLA is the most abundant in ruminant tissues and food products derived from ruminants due in large part to its potential to be synthesized endogenously from another rumen biohydrogenation intermediate, trans-11 18:1 (Bauman et al., 2003). This process is carried out by the enzyme stearoyl CoA desaturase (SCD) that introduces a cis-9 double bond in the trans-11 18:1 intermediate, forming cis-9, trans-11 CLA (Figure 2).

![Pathways diagram](image)

**Figure 2.** Pathways for ruminal biohydrogenation and endogenous synthesis of cis-9, trans-11 CLA. Pathways for biohydrogenation of linolenic and linoleic acids yielding trans-11 18:1 are shown in the rumen box and endogenous synthesis by stearoyl CoA desaturase is shown in the tissues box. Figure adapted from Bauman et al. (2003).
Estimates of the contribution of endogenous synthesis range from 60% and 90% of the CLA in milk fat (Griinari et al., 2000; Corl et al., 2001; Kay et al., 2002). Even though SCD converts a portion of the cow’s trans-11 18:1 to CLA in the mammary gland, the amount of trans-11 18:1 in milk fat is generally about 3-fold greater than the amount of CLA. This is important because animals (including humans) consuming cow’s milk also have the SCD enzyme used to endogenously synthesize CLA. In a particularly notable cancer study, Ip et al. (1999) demonstrated that CLA-enriched butter produced by feeding cows to promote CLA synthesis is perhaps even more effective against cancer than purified cis-9, trans-11 CLA. This was postulated to be due to the presence of trans-11 18:1 in the lipid that can be converted to cis-9, trans-11 CLA in tissues (Ip et al., 1999). It has since been demonstrated that this conversion of trans-11 18:1 contributes significantly to the cancer-fighting properties of natural CLA-enriched milk fat (Corl et al., 2003).

Dietary factors that affect the CLA content of milk fat include addition of biohydrogenation substrates such as linoleic and linolenic acids, and modification of the rumen environment to promote incomplete biohydrogenation (see reviews by Griinari and Bauman, 1999; Bauman et al., 2001). Most attempts to elevate milk fat CLA content result in variable degrees of success depending on the individual; while one animal may respond very well, another may have little to no response. In an attempt to further characterize the nature of this variation, we conducted a study involving 3 groups of animals (n=10/group); one was fed a standard TMR, one fed a diet designed to enhance milk fat CLA, and one group was switched between these diets at 3 week intervals (Peterson et al., 2002b). Our diet designed to elevate milk fat CLA content employed full-fat extruded soybeans and led to a significant increase in milk fat content of cis-9, trans-11 CLA. Interestingly, when the responses of animals in the alternating diet group were considered individually, it was noted that animals varied 2- to 3-fold in their response, and that the hierarchy among animals remained largely constant across dietary shifts (see figure 3). The data from this study indicated that while diet has a major influence on the milk fat content of CLA, individual animal differences also have a substantial effect. This animal-specific effect likely involves the SCD enzyme that is critical to the endogenous synthesis of CLA from trans-11 18:1. Because of the importance of this enzyme in the synthesis of cis-9, trans-11 CLA, current research both in the US and worldwide is focused on identifying variation in the SCD gene that may be used for animal selection.

![Figure 3](image)

**Figure 3.** Temporal pattern in the individual hierarchy in milk fat CLA for cows alternated between a standard diet (periods 1 and 3) and a diet designed to elevate milk fat CLA (periods 2 and 4). The duration of each period was 1 weeks. Figure from Peterson et al. (2003b).
CLA AND MILK FAT DEPRESSION

Milk fat content and composition can be impacted by many factors, and one example is the low-fat milk syndrome, also referred to as milk fat depression (MFD). In this situation, cows fed diets high in readily fermentable carbohydrates and low in effective fiber, or diets containing supplements of polyunsaturated oils of either plant or fish origin exhibit a dramatic and specific reduction in the secretion of milk fat. These diets can reduce milk fat by up to 50% without any effect on other aspects of lactation, and this reduction is quickly rescued when the dietary treatment is removed (reviewed by Bauman and Griinari, 2001; Bauman et al., 2003). Boussingault was the first to document MFD in an 1845 report of a reduction in milk fat secretion in response to feeding cows a diet containing beets (cited by Van Soest, 1994). Again in 1926, when Golding and coworkers (1926) were attempting to manipulate the fat-soluble vitamin content of milk, they observed a precipitous decline in milk fat secretion when cows were fed a diet containing cod liver oil. Ever since these observations, investigators have sought to determine the mechanism behind this phenomenon with little success—until recently.

Early attempts at enhancing the content of cis-9, trans-11 CLA in milk fat included simply supplying chemically synthesized CLA isomers postruminally to lactating dairy cows. It was quickly observed that these treatments led to a precipitous decline in milk fat yield (Loor and Herbein, 1998; Chouinard et al., 1999a; 1999b) which is also the hallmark of the 150 year-old MFD phenomenon. This activity was subsequently attributed to the trans-10, cis-12 CLA isomer, and we have established a dose-response relationship demonstrating the extreme potency of this compound in inhibiting milk fat synthesis (Figure 4; Baumgard et al., 2000; 2001; Peterson et al., 2002a).

\[ y = 0.24x^2 - 6.99x \]
\[ R^2 = 0.99 \]

Figure 4. Relationship between the decrease in milk fat yield and dose of trans-10, cis-12 CLA abomasally infused into lactating dairy cows. All values represent the mean (n=4) decrease after 5 days of infusion at the indicated dose. Figure from Peterson et al. (2002a).

Based in part on these observations, Bauman and Griinari (2001) proposed that MFD diets cause alterations in the biohydrogenation pathways leading to the production of alternative fatty acid intermediates such as trans-10, cis-12 CLA. These fatty acids can then be absorbed in small quantities, and act to inhibit lipid synthesis in the mammary gland. Subsequent studies revealed that diet-induced MFD led to increases in the abundance of trans-10, cis-12 CLA in milk fat.
that were correlated with reductions in the activity of genes involved in lipid synthesis in the mammary gland (Peterson et al., 2003). Additionally, the reductions in gene expression observed during diet-induced MFD were very similar to those observed during MFD induced by CLA administration (Baumgard et al., 2002). These data support the biohydrogenation theory as a unifying explanation for diet-induced milk fat depression.

Our understanding of the anti-lipogenic properties of trans-10, cis-12 CLA has also provided a potentially valuable management tool. Fat is the primary energy component in milk, and therefore comprises the greatest metabolic burden on a lactating animal. The strategic inhibition of milk fat synthesis can be used to relieve metabolic strain during times of nutrient insufficiency such as the onset of lactation when intake lags behind nutrient demand, or when adequate nutrients are not available as can occur at times in a pasture-based system. Additionally, CLA can be used to alter milk composition to better match market demands such as in areas where milk fat quotas are employed.

CONCLUSIONS

Conjugated linoleic acids are potent compounds that naturally occur in ruminant fats such as milk fat. The most abundant CLA isomer in milk fat is cis-9, trans-11 and has been shown to possess potent anti-cancer activities. Also important is endogenous synthesis of this fatty acid from trans-11 18:1 via the SCD enzyme both in terms of CLA enrichment of milk fat as well as in terms of the cancer fighting effects in the consumer. It is likely that the animal-to-animal variation in milk fat CLA content is due to intrinsic factors such as SCD. The trans-10, cis-12 isomer of CLA is produced in the rumen under certain dietary conditions and is a potent inhibitor of milk fat synthesis. In addition to being implicated in the condition known as milk fat depression, this CLA isomer has potential for managing milk fat synthesis to improve animal health in response to metabolic challenges, or to match production markets.

REFERENCES


Baumgard, L. H., E. Matitashvili, B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002. trans-10, cis-12 CLA decreases lipogenic rates and


