Conjugated Linoleic Acid Enriched Milk: A Designer Milk with Potential

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Take Home Messages

- Conjugated linoleic acid (CLA) is a naturally occurring component of ruminant milk fat with potent cancer-fighting properties.
- The concentration of CLA in bovine milk is strongly influenced by diet. Manipulation of the animal's diet can result in a 10 fold increase in the concentration of CLA in milk.
- Consumption of CLA enriched milk could provide considerable benefits for human health.
- As consumers are becoming more and more conscious of the relationship between food and health, conjugated linoleic acid may prove to be a "designer milk" with substantial potential.
- The successful introduction of CLA enriched milk, and milk products, is dependent on our finding an interested processing partner who is prepared to work with us to bring these products to market.

Introduction

Consumers have been encouraged for years to reduce dietary fat intake. This has resulted in a steady increase in the consumption of low fat milk. However, bovine milk fat contains many substances that may be beneficial to health. Conjugated linoleic acid (CLA) is one such component that has been shown in recent years to have numerous potential benefits for human health, including potent cancer-fighting properties. This is especially interesting considering that most naturally occurring anti-carcinogens are of plant origin. Since CLA is a product of ruminant animals, bovine milk and milk products are among the richest dietary sources. The objective of this paper is to provide an overview of conjugated linoleic acid as it relates to milk production, and to illustrate the feasibility and potential of producing CLA enriched milk.

▪ Formation of CLA in the Cow

Conjugated linoleic acid is formed as an intermediate product in the ruminal digestion of dietary fat. Forages and grains fed to dairy cows are characterized by a high percentage of poly-unsaturated fatty acids such as linoleic acid (C18:2) and linolenic acid (C18:3). Certain rumen bacteria are capable of converting linoleic acid to conjugated linoleic acid in a process known as *biohydrogenation* (Figure 1).

The biohydrogenation process adds hydrogen atoms to unsaturated fatty acids. The process results in poly-unsaturated fatty acids like C18:2 and C18:3 (18 carbon fatty acids with two and three double bonds respectively) and mono-unsaturated fatty acids like C18:1 (one double bond) becoming saturated (no double bonds). The higher melting point of these saturated fatty acids compared to their more unsaturated precursors is one of the reasons that butter is solid at room temperature, and said to be “high in saturates”.

Conjugated linoleic acid is produced from linoleic acid in the first step of the biohydrogenation process. The two double bonds in linoleic acid shift from their normal position at the 9^{th} and 12^{th} carbon to the 8/10, 9/11, 10/12, or 11/13 positions. Each of these double bonds can be in a *cis* or *trans* configuration, giving a range of possible CLA types, or isomers. The term conjugated linoleic acid actually refers to this whole group of 18 carbon conjugated fatty acids. The cis-9, trans-11 is the most common isomer in ruminant products and accounts for more than 90% of the CLA in milk. The CLA formed in the rumen undergoes rapid hydrogenation, first to C18:1-trans and finally C18:0. Some of the fatty acids at each stage in the process inevitably escape the rumen and become incorporated into milk and meat. Linolenic acid (C18:3) goes through a similar process producing C18:1 trans-11 and 18:0, but does not appear to produce CLA as an intermediate product. The biohydrogenation of linoleic and linolenic acid to C18:1 trans-11 appears to occur quickly and several studies have suggested that there may be little accumulation of CLA in the rumen. Although it is accepted that CLA is formed in the rumen, there is good evidence that much of the cis-9, trans-11 CLA found in bovine milk is actually synthesized within the mammary gland from C18:1 trans-11 (Griinari and Bauman, 1999). This is possible through the action of stearoyl-CoA desaturase (SCD), an enzyme capable of adding a cis-9 double bond to C18:1 trans-11 to give cis-9, trans-11 CLA. Regardless of the origin of CLA, manipulation of the biohydrogenation process remains the key to increasing CLA in milk by dietary means, either by increasing rumen production of CLA, or by increasing the rumen production of C18:1 trans-11.

Milk and meat from ruminants therefore contains more CLA than that of non-ruminants (Table 1). The amount of CLA found in whole milk is generally about 4.5 to 5.5 mg/g fat (approximately 0.45 to 0.55%), although variation of as much as 2.5 to 18 mg/g fat has been reported. Breed, age of the dairy cow, and stage of lactation may influence the milk CLA content to some degree but the effect of these parameters has not been well characterized. The CLA content of meat and dairy products is altered little by processing, storage, or cooking and hence, the concentration in food depends primarily on the concentration in the raw material.

That CLA is produced in the rumen during the biohydrogenation process has been known for a long time. The unexpected effects of these fatty acids on health have only been discovered in more recent years.

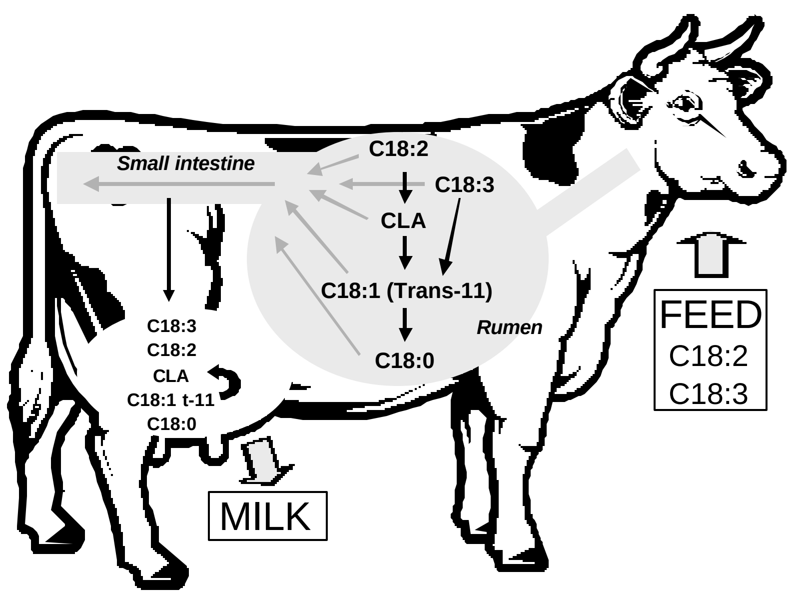

Figure 1. Formation of CLA in the cow

Table 1. CLA content of various foods

Foodstuff	Total CLA content (mg/g fat)
Dairy products	
Homogenized milk	5.5
Butter fat	4.7
Mozzarella cheese	4.9
Plain yogurt	4.8
Ice cream	3.6
Meats	
Ground beef	4.3
Lamb	5.6
Pork	0.6
Chicken	0.9
Salmon	0.3
Ground turkey	2.5

Source: Chin et al., 1992

▪ Potential Health Benefits of CLA

Most substances in nature that demonstrate anti-carcinogenic (cancer-fighting) activity are of plant origin and are only present at trace levels (Wattenberg, 1992). In contrast, CLA is found almost exclusively in animal products. It has been shown to be one of the most potent of all naturally occurring anti-carcinogens. The origins of research in this area can be traced to studies from the laboratory of Michael Pariza at the University of Wisconsin. While studying the effects of temperature and time on mutagen formation in pan-fried hamburger they obtained evidence for mutagenic inhibitory activity in the uncooked and fried hamburger (Pariza et al. 1979). Pariza and Hargreaves (1985) subsequently partially purified the mutagenesis inhibitor from fried ground beef and showed that it was capable of inhibiting the initiation of chemically induced mouse epidermal tumors. This was the first study to show that ground beef contained an anti-carcinogen that was effective in an intact animal. That cooked beef contained a substance that could inhibit tumor growth was intriguing since it was well known that the cooking of protein-rich foods could produce a range of mutagens and carcinogens. The next stage of the research was to elucidate the identity of the unknown anti-carcinogen. Pariza and Hargreaves (1985) had earlier noted that the anticarcinogen was a very nonpolar molecule. Subsequent work from Pariza's laboratory showed that it was actually a mixture of four isomeric derivatives of linoleic acid, each containing a conjugated double-bond system (Ha, et al. 1987). The anticarcinogenic mixture was henceforth designated as conjugated linoleic acid or CLA. To prove that the anticarcinogenic effects were indeed due to CLA they tested a synthetically prepared mixture of the CLA isomers on the mouse tumor model. The CLA-treated mice developed only about half as many papillomas and exhibited a lower tumor incidence compared with the control mice (Ha, et al. 1987). This initial work started a cascade of research on CLA.

Conjugated linoleic acid has since been shown to be effective in experimental animal models of mouse skin carcinogenesis, mouse forestomach tumorigenesis, and rat mammary tumorigenesis (Belury, 1995). It was effective *in vitro* with breast tumor cells, malignant melanoma, colorectal cancer cells, leukemia, prostate carcinoma and ovarian carcinoma (Scimeca, 1999). It seems to act in a dose dependent manner as demonstrated *in vitro* with breast cancer cells (Shultz, et al 1992), and *in vivo* with chemically induced mammary tumors in rats (Ip et al. 1994). Feeding as little as 0.05g CLA/100g of diet caused a reduction in the number of mammary tumors (Ip, et al 1994). Ip et al (1999) evaluated the effect of CLA enriched butter on mammary tumors in rats. The butter contained 4.1% CLA, 92% of which was the cis-9, trans-11 isomer. CLA enrichment was achieved by including sunflower oil in the diet of 20 dairy cows at 5.3% of dry matter. Milk was collected from nine of these cows that were producing the highest levels of CLA in their milk. They showed that CLA enriched butter was able to inhibit rat mammary tumor yield by 53%. This study clearly showed that the predominant isomer in ruminant products, the cis-9, trans-11 isomer, was anti-carcinogenic.

Other research reports indicate that CLA may have other physiological effects in addition to the cancer-fighting properties. Some of these effects include a role in reducing atherosclerosis (Lee et al. 1994, Nicolosi et al. 1997) and a benefit for diabetes treatment (Houseknecht et al. 1998). Isomers of CLA have also been shown to reduce body fat and increase body protein in growing animals (Park et al. 1997); counteract immune induced muscle wasting in poultry (Cook et al. 1993); and have benefits in bone formation (Watkins et al. 1999).

Most of the studies carried out so far have used a mixture of CLA isomers prepared in the laboratory. This mixture is composed mainly of the cis-9, trans-11 and trans-10, cis-12 isomers but has a range of other isomers as well. As research continues, the specific physiological effects of each of the isomers will likely be better defined. However, as shown above, there is good evidence that the predominant isomer in milk fat, the cis-9, trans-11, possesses potent anti-carcinogenic activity.

▪ Increasing the Concentration of CLA in Milk

In view of the potential benefits of CLA for human health, a number of researchers began looking at possible ways of increasing the concentration of CLA in bovine milk fat. There appears to be two practical approaches to achieve 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 rumen environment. 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 total mixed rations (TMR) based on conserved forage and grain. Dhiman, et al. (1999) reported a milk CLA concentration of 22.1mg/g fat with pasture feeding compared to 3.8mg/g fat with TMR feeding. The exact reasons for the effect of pasture on CLA levels is not certain. Similar enrichment of CLA has often been achieved when the TMR is supplemented with unsaturated fat from oilseeds. Kelly et al. (1998) supplemented the basal diet with 53g/kg dry matter (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. The level of CLA obtained using supplemental fat varies to a large extent depending on the ruminal conditions. A study at Cornell University 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 isomers.

Using 28 lactating Holstein cows located at the Dairy Research and Technology Centre, we carried out a feeding trial 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). All cows were first fed a control (CTL) diet for eight days (covariate period). The cows were then divided into four groups and each group was fed one of four diets for a 15 day treatment period: (1) Control (CTL); (2) Low fat diet (LF); (3) High fat diet A (HFA); (4) High fat diet B (HFB). (Details of the diets can not be given at this time for reasons of patent confidentiality). Milk yield was recorded daily. Milk was sampled on the last two days of the covariate and treatment periods and analyzed for percentage of lactose, protein and fat, and fatty acid composition.

Milk yield and percentage and yield of protein were unaffected by treatment (Table 2). Yield of lactose was not significantly different among treatments and lactose percentage was in the normal range, although the CTL and HFA tended to have a lower lactose percentage compared to LF and HFB. Both percentage and yield of fat were significantly affected by treatment. Both the low fat (LF) and high fat (HFA and HFB) treatments produced milk with a lower milk fat percentage than CTL, the greater reduction being seen for the high fat diets. This is not surprising as a reduction in milk fat is often observed when supplemental fat is added to the dairy ration.

The CTL diet, representative of diets fed in Alberta, resulted in milk fat with a cis-9, trans-11 CLA concentration of 0.49%, similar to that typically reported for whole milk (Table 3). Cows fed the HFB diet produced milk fat with 5.63% cis-9, trans-11 CLA, approximately 12 times greater than the CTL diet. Although the yield of fat was lower in the HFB treatment, the yield of CLA was still approximately nine times greater than the yield of CLA for the CTL treatment (Table 3).

The HFA and HFB diets also resulted in a significant increase in the amount of trans fatty acids (especially C18:1 trans-11) in the 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 where not correlated with CHD.

Approximately 80 to 90% of the trans fatty acids in our diet comes from partially hydrogenated vegetable oils like those found in baked foods, certain types of margarine, and foods that are deep fat fried. The composition of trans isomers from these sources is different from trans fatty acids of ruminant origin, which may provide rationale for the differences seen in the epidemiological associations. The primary trans fatty acids in bovine milk are C18:1 trans-11 and CLA, whereas partially hydrogenated vegetable oils are characterized by a range of trans fatty acids such as C18:1 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 C18:1 trans-11 can be desaturated to cis-9, trans-11 CLA in human tissues (Salminen et al. 1998). Convincing evidence that trans fatty acids from milk have an adverse effect on health is lacking.

Ruminant fat is associated with increasing blood cholesterol because of its high content of saturated fatty acids, which are believed to be more hyper-cholesterolemic than trans fatty acids. In our study we found that the diets that increased CLA also resulted in a decrease in saturated fatty acids. The HFA and HFB milk compared to CTL or LF had approximately 30% lower C14:0 and 45% lower C16:0. There was also an increase of approximately 50% in C18:1 cis-9 levels. This represents a positive change as C14:0 and C16:0 in particular have been associated with increases in blood cholesterol. On the other hand, C18:1 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 composition (Figure 2). Furthermore, it demonstrated the feasibility of producing CLA enriched milk using modifications to the diet of the cow. Work is underway to confirm these results using increased animal numbers per treatment.

Table 2. Influence of diet on the yield and composition of milk.

	CTL[3]	LF	HFA	HFB
Milk yield kg/day[1]	26.87[a]	27.58[a]	26.78[a]	27.82[a]
Fat %[2]	4.01[a]	3.57[b]	2.83[c]	2.95[c]
Fat yield kg/day	1.05[a]	0.97[a]	0.75[b]	0.81[b]
Protein %	3.33[a]	3.37[a]	3.11[a]	3.23[a]
Protein yield kg/day	0.87[a]	0.92[a]	0.82[a]	0.88[a]
Lactose %	4.3[ab]	4.54[a]	4.26[b]	4.50[ab]
Lactose yield kg/day	1.13[a]	1.24[a]	1.14[a]	1.24[a]

Within a row, values with different superscripts are significantly different ($P < 0.05$).
1 Average milk yield over the last eight days of the treatment period.
2 Values for fat, protein, and lactose represent average values for milk taken from the last two days of the treatment period.
3 CTL is Control; LF is Low fat diet; HFA is high fat diet A; HFB is high fat diet B.

Table 3. Fatty acid composition of milk fat from cows fed different diets.

Fatty acid[1]	CTL[2]	LF	HFA	HFB
C4:0	1.85[a]	1.73[ab]	1.84[a]	1.45[b]
C6:0	3.16[a]	2.99[a]	2.34[b]	2.00[b]
C8:0	2.16[a]	2.05[a]	1.36[b]	1.23[b]
C10:0	4.62[a]	4.58[a]	2.41[b]	2.25[b]
C11:0	0.70[a]	0.66[a]	0.29[b]	0.25[b]
C12:0	5.24[a]	5.23[a]	2.69[b]	2.63[b]
C14:0	15.04[a]	15.47[a]	10.05[b]	10.00[b]
C14:1	1.44[ab]	1.56[b]	0.93[c]	1.10[ac]
C15:0	1.84[a]	2.10[b]	1.07[c]	1.06[c]
C16:0	36.39[a]	35.19[a]	20.89[b]	20.37[b]
C16:1	2.04[a]	2.14[a]	1.22[b]	1.32[b]
C18:0	6.25[a]	5.68 [a]	9.92[b]	8.75[c]
C18:1 trans-11	1.52[a]	1.68[a]	10.55[b]	14.77[c]
C18:1 cis-9	12.65[a]	13.16[a]	20.41[b]	18.25[c]
C18:1 (n12)	0.77[a]	1.05[a]	2.08[b]	1.64[c]
C18:1 (n7)	0.67[a]	0.69[a]	0.95[b]	0.91[b]
C18:1 (n6)	0.45[a]	0.52[a]	3.05[b]	2.30[c]
C18:2	1.51[a]	1.62[a]	2.97[b]	2.82[b]
Cis-9, trans-11 CLA	0.49[a]	0.56[a]	3.70[b]	5.63[c]
Trans-10, cis-12 CLA	ND[a]	ND[a]	0.054[b]	0.054[b]
Trans/trans CLA	0.033[a]	0.046[a]	0.15[b]	0.17[b]
C18:3 n6	0.13[ab]	0.12[a]	0.16[b]	0.15[ab]
C18:3 n3	0.030[ab]	0.046[a]	0.015[bc]	0.066[c]
Other fatty acids	0.86[ab]	0.91[a]	0.84[ab]	0.81[b]
CLA yield g/day	5.1[a]	5.4[a]	28.5[b]	45.8[c]

Within a row, values with different superscripts are significantly different ($P < 0.05$).
1 All values presented as percentage of fatty acid methyl esters.
2 CTL is Control; LF is Low fat diet; HFA is high fat diet A; HFB is high fat diet B.
ND = Not detected

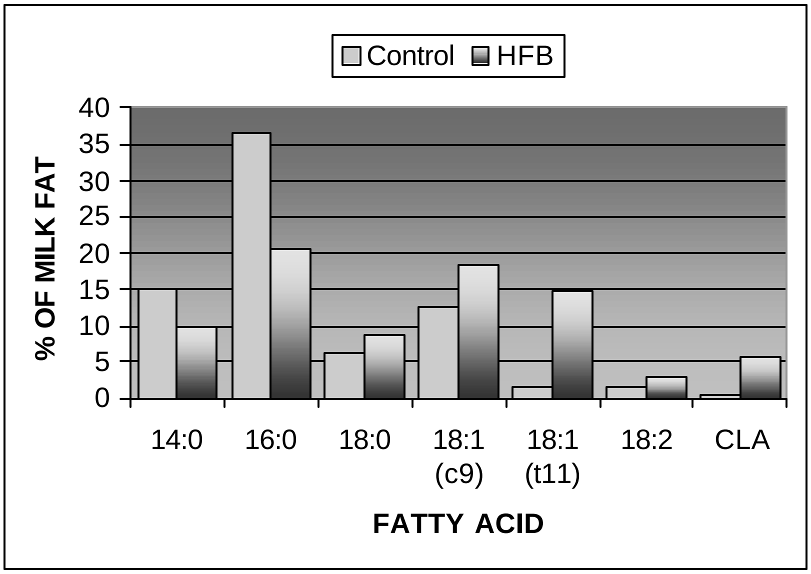

Figure 2. Summary of the main changes in fatty acid composition of milk from cows fed the control diet (CTL) and high fat diet B (HFB).

Synthetic CLA

Conjugated linoleic acid can be synthesized in the laboratory from vegetable oils like sunflower. As noted earlier, CLA produced in this way tends to contain a mixture of CLA isomers. This type of product is already available commercially for feeding to swine because of its ability to improve lean gain in the growing animal. Synthetic CLA could be used to increase the CLA concentration in bovine milk if protected in some way from the rumen environment. Methods available to achieve this include encapsulation of the fat in formaldehyde-treated casein or feeding the fat as a calcium salt, although the extent of protection obtained with these methods can be variable.

In view of the potential of synthetic CLA as a method of increasing the concentration of CLA in bovine milk, we recently carried out a study to evaluate the effect of this product on milk yield and composition. To achieve this we infused the CLA directly into the abomasum via a rumen cannula, thereby avoiding rumen biohydrogenation of the CLA isomers. Four rumen cannulated lactating Holstein cows received four treatments: (1) control, no fat infusion (CTL), (2) 150g/day of synthetic CLA (CLA), (3) 150g/day of safflower oil

(SAFF), and (4) 150g/day of tallow (TALL). Infusion was carried out for 20-22 hours per day for 11 day periods. The cows were rotated through the treatments over four periods so that each cow received each treatment once.

Infusion of CLA had dramatic effects on milk production and composition (Table 4). Milk yield dropped by 35 to 40% with CLA infusion compared to the other treatments. Percentage and yield of lactose and fat were also significantly lower with CLA infusion. Percentage of protein was significantly higher with CLA infusion although the yield of protein was lower compared to the other treatments. Analysis of fatty acid composition showed that the concentration of CLA isomers increased significantly as a result of CLA infusion (Table 5). The concentration of linoleic acid (C18:2) was significantly increased with infusion of safflower oil (76% linoleic acid). The fatty acid composition of milk from the tallow infusion was not significantly different from control. This is not surprising as tallow contains a variety of fatty acids similar to those normally found in bovine milk. Since the yield of milk fat was reduced with CLA infusion, the yield of all the fatty acids (except the CLA's) was significantly reduced with the CLA treatment (Table 6).

Most interesting was the effect of treatment on the somatic cell count (SCC). The SCC was approximately five to seven times greater as a result of CLA infusion compared to the other treatments, which had SCC values at levels considered normal for healthy cows. Somatic cell count is a count of white blood cells and sloughed off epithelial cells in milk. High somatic cell counts are generally indicative of an infection in the udder. However, we believe that infection was not the cause of the high SCC observed with CLA infusion for three main reasons. Firstly, the high SCC was observed in each cow but only during the period when that cow received CLA infusion. Milk from the period preceding or following the CLA period always had much lower counts. Secondly, there were no visible signs of mastitis during milking at any time in the course of the experiment. Thirdly, bacterial analysis of the milk showed counts of streptocuccus/enterococcus well within the normal range for raw milk and no signs of staphylococcus aureus, which are the common mastitis causing bacteria. We are convinced that the effects on milk production and composition observed with CLA infusion were the result of the effect of one or more of these CLA isomers on the physiology of the udder.

It is already well documented that CLA isomers (especially the trans-10 isomers) have a milk fat depressing effect (Griinari et al. 1997). Chouinard et al (1999) infused 0, 50, 100, or 150 g/day of CLA for five days and observed a reduction of 52 and 55% in the percentage and yield of milk fat. They also noted a small non-significant drop in milk production with increasing level of CLA infusion. In our study, infusion of 150 g CLA/day for 11 days also resulted in reduced fat content as well as other changes not previously noted with CLA infusion. We observed a somewhat surprisingly lower concentration of lactose with CLA infusion, which was counterbalanced by a higher concentration of sodium. Concentration of protein and chloride were also higher with CLA infusion. During the early stages of drying-off similar changes are seen in the mammary secretion as were observed with infusion of CLA. Although purely speculative, it is possible that infusion of these synthetic CLA isomers was initiating the dry-off mechanisms in the udder. More studies will be necessary to further explore these interesting effects of CLA on lactation. Overall, examination of the studies that have looked at the effects of synthetic CLA on milk production and composition would suggest that using synthetic CLA as a means of increasing CLA in bovine milk may have limited application.

Table 4. Yield and composition of milk from cows receiving abomasal infusion of CLA and other fatty acids.

	CTL[2]	TALL	SAFF	CLA
Milk yield (Kg/day)[1]	24.2[a]	23.0[a]	26.6[a]	15.0[b]
Lactose %	3.86[a]	3.86[a]	4.04[a]	3.36[b]
Lactose yield (kg/day)	0.94[a]	0.90[a]	1.07[a]	0.45[b]
Fat %	2.36[a]	2.46[a]	2.39[a]	1.66[b]
Fat yield (kg/day)	0.53[a]	0.54[a]	0.62[a]	0.21[b]
Protein %	3.04[a]	2.98[a]	3.14[a]	4.35[b]
Protein yield (kg/day)	0.70[ab]	0.65[a]	0.82[b]	0.55[a]

Within a row, values with different superscripts are significantly different ($P < 0.05$).
1 Average yield/day over the last two days of period.
2 CTL is control (no fat infusion); TALL is infusion of 150g/day beef tallow; SAFF is infusion of 150g/day of safflower oil; CLA is infusion of 150g/day of synthetic CLA.

Table 5. Fatty acid composition of milk fat from cows receiving abomasal infusion of CLA and other fatty acids.

Fatty acid[1]	CTL[2]	TALL	SAFF	CLA
C4-15	23.6[a]	22.3[a]	21.9[a]	18.2[b]
C16:0	32.3[a]	31.9[a]	29.9[a]	39.5[b]
C18:0	11.0[a]	11.0[a]	11.2[a]	13.5[a]
C18:1 cis-9	24.4[ab]	25.6[a]	22.8[b]	18.2[c]
C18:2	1.8[a]	2.1[a]	7.6[b]	2.3[a]
Cis-9, trans-11 CLA	0.59[a]	0.61[a]	0.58[a]	1.77[b]
Trans-10, cis-12 CLA	ND[a]	ND[a]	ND[a]	0.85[b]

Within a row, values with different superscripts are significantly different ($P < 0.05$).
1 Fatty acids expressed as percentage of fatty acid methyl esters.
2 CTL is control (no fat infusion); TALL is infusion of 150g/day beef tallow; SAFF is infusion of 150g/day of safflower oil; CLA is infusion of 150g/day of synthetic CLA.
ND = Not detected

Table 6. Yield of fatty acids in milk fat from cows receiving abomasal infusion of CLA and other fatty acids.

Fatty acid (g/day)	CTL[1]	TALL	SAFF	CLA
C4-15	124[a]	120[a]	136[a]	39[b]
C16:0	172[a]	176[a]	186[a]	81[b]
C18:0	58.5[a]	60.3[a]	70.5[a]	29.8[b]
C18:1 cis-9	130[a]	137[a]	142[a]	38.7[b]
C18:2	9.5[a]	10.9[a]	46.8[b]	4.7[c]
Cis-9, trans-11 CLA	3.08[a]	3.31[a]	3.59[a]	3.90[a]
Trans-10, cis-12 CLA	0[a]	0[a]	0[a]	1.86[b]

Within a row, values with different superscripts are significantly different ($P < 0.05$).
1 CTL is control (no fat infusion); TALL is infusion of 150g/day beef tallow; SAFF is infusion of 150g/day of safflower oil; CLA is infusion of 150g/day of synthetic CLA.

▪ CLA Enriched Milk – A New Product Opportunity?

The preceding sections illustrated the feasibility of producing CLA enriched milk. An important question is whether the degree of enrichment achieved will translate into any real benefit for the person consuming the milk. Intake of CLA in North America has been estimated at 52 to 137mg CLA/day (Ritzenenthaler et al 1998). Extrapolation from animal studies has suggested that the level of CLA intake necessary to produce anti-carcinogenic effects in humans may be about 3g per day. Using the CLA percentage achieved with the HFB diet (Table 3), one serving of whole milk (460mg CLA) and a sandwich with butter (365mg CLA) and cheddar cheese (721mg CLA) would provide 1546 mg (1.546g) CLA. This example illustrates how CLA enriched milk and milk products could supply dietary CLA at levels that may potentially benefit health, without the need for unrealistic changes to eating habits.

The concept of enhancing the levels of health promoting fatty acids in food is not new. A good example of this has been the introduction of eggs enriched in omega-3 fatty acids. This recognizes the trend among consumers towards an increased desire to make diet choices that promote good health. Of course, consumers could increase their CLA intake by taking synthetic CLA in pill form, which is already available in health food stores. The main difference between the CLA in these products and milk CLA is the broader range of isomers in the synthetically produced CLA. The relative value for human health of this range of CLA isomers compared to the CLA found in ruminant milk fat is uncertain. Nevertheless, CLA enriched milk produced through manipulation of the dairy ration has an advantage over this type of product in that it can be promoted as a "natural" source of CLA. It may also be easier for CLA enriched milk to gain acceptance since milk already has a wide distribution and consumers are well accustomed to seeing a broad variety of dairy products in the shops. The challenge will be in overcoming the existing public perception regarding milk fat and health.

A second challenge is the apparent reluctance of the Canadian processing sector to invest in new product development. The variety of dairy products available in the EU is much greater than in Canada. The long-term viability of the dairy industry depends on producing products to meet changing consumer demand. As indicated elsewhere in these proceedings, consumers are becoming more conscious of the health attributes of the food they consume. CLA enriched milk may be attractive to those who have abandoned milk and milk products, such as butter, due to concerns over the impact of milk fat on their health. However, the introduction of new products like CLA enriched milk does require significant investment in marketing and there are no guarantees that the product will attract sufficient consumer interest to be viable. The incentive for producers to feed the special supplement needed to enhance CLA levels would be the payment of a premium price for the milk.

■ Conclusion

That a compound exists in ruminant fat with such potent health promoting effects has been an unanticipated discovery. The ability to enhance the concentration of CLA through manipulation of the dairy ration demonstrates the feasibility of producing CLA enriched dairy products. As consumers become more and more conscious of the link between diet and health, milk designed to have enhanced levels of CLA may provide new market opportunities for milk and milk products such as butter and cheese.

■ References

Belbury, M.A. (1995) Conjugated dienoic linoleate: A polyunsaturated fatty acid with unique chemoprotective properties. Nutrition reviews, vol 53, no. 4:83-89.

Bell J.A. and Kennelly J.J. (2000) Producing conjugated linoleic acid enriched milk through practical dairy nutrition. Conference abstract: 8th World Congress on Clinical Nutrition, Bankok, Thailand. December 17-20, 2000.

Chin, S.F., Liu, W., Storkson, J.M., Ha, Y.L., Pariza, M.W. (1992) Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J. Food Comp. Anal. 5:185-197.

Chouinard, P.Y., Corneau, L., Barbano, D.M., Metzger, LE., Bauman, DE. (1999) Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. Journal of nutrition 129:1579-1584.

Chouinard, P.Y., Corneau, L., Bauman, D.E., Butler, W.R., Chilliard, Y., Drackley, J.k. (1998) Conjugated linoleic acid content of milk from cows fed different sources of dietary fat. J. Animal Sci. Vol. 76, suppl. 1/J. Dairy Sci. Vol. 81, suppl. 1/1998. (Abstract)

Cook, M.E., Miller, C.C., Park, Y., Pariza, M.W. (1993) Immune modulation by altered nutrient metabolisms: nutritional control of immune-induced growth depression, Poultry Science, 72: 1301-1305.

Dhiman, T.R., Anand, G.R., Satter, L.D., Pariza, M.W. (1999) Conjugated linoleic acid content of milk from cows fed different diets. Journal of Dairy Science 82:2146-2156.

Griinari, J.M., Chouinard, P.Y., Bauman, D.E. (1997) Trans fatty acid hypothesis of milk fat depression revised. Proceedings Cornell Nutr. Conf. Feed Manuf., Pages 208-216. 59th Cornell Nutrition Conference, Ithaca, New York, 1997.

Griinari, J.M., Dwyer, D.A., McGuire, M.A., Bauman, D.E., Palmquist, D.L., Nurmela, K.V.V. (1998) Trans-octadecanoic acids and milk fat depression in lactating dairy cows. J. Dairy Sci. 81:1251-1261.

Griinari. J.M., Bauman, D.E. (1999) Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk of ruminants. In: *Advances in Conjugated Linoleic Acid Research, Volume 1*, Eds. Yurawecz, M.P., Mossoba, M.M., Kramer, J.K.G., Pariza, MW., Nelson, G.J. AOCS press, Illinois, Chapter 13, pages 180-200.

Ha, Y.L., Grimm, N.K., Pariza, M.W. (1987) Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. Carcinogenesis. Vol. 8 no. 12:1881-1887.

Houseknecht, K.L., Vanden Heuvel, J.P., Moya-Camarena, S.Y., Portocarrerro, C.P., Peck, L.W., Nickel, K.P., Belury, M.A. (1998) Conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat. Biochem. Biophys. Res. Commun. 244: 678-682.

Ip, C., Banni, S., Angioni, E., Carta, G., McGinley, J., Thompson, H., Barbano, D., Bauman, D. (1999) Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. Journal of Nutrition 129:2135-2142.

Ip, C., Singh, M., Thompson, H.J., Scimeca, J.M. (1994) Conjugated linoleioc acid suppresses carcinogenesis and proliferative activity of the mammary gland in the rat. Cancer research, 54:1212.

Kelly, M.L., Bauman, D.E. (1996) Conjugated linoleic acid: A potent anticarcinogen found in milk fat. Proceedings Cornell Nutr. Conf. Feed Manuf., Pages 68-74. 58th Cornell Nutrition Conference, Ithaca, New York, 1996.

Kelly, M.L., Berry, J.R., Dwyer, D.A., Griinari, J.M., Chouinard, P. Y., Van Amburgh, M.E., Bauman, D.E. (1998) Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr. 128:881-885.

Lee, K.N., Kritchevsky, D., Pariza, M.W. (1994) Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis 108:19-25.

Nicolosi, R.J., Rogers, E.J., Kritchevsky, D., Scimeca, J.A., Huth, P.J. (1997) Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. Artery 22:266-277.

Pariza, M.W., Ashoor, S.H., Chu, F.S., Lund, D.B. (1979) Effects of temperature and time on mutagen formation in pan-fried hamburger. Cancer Letters, 7:63-69.

Pariza, M.W., Hargreaves, W.A. (1985) A beef-derived mutagenesis modulator inhibits initiation of mouse epidermal tumors by 7,12-dimethylbenz[a]anthrazene. Carcinogensis Vol. 6 no.4:591-593.

Park, Y., Albright, K.J., Liu, W., Storkson, J.M., Cook, M.E., Pariza, M.W. (1997) Effects of conjugated linoliec acid on body composition in mice. Lipids, 32:853-858.

Ritzenthaler, K., McGuire, M.K., Falen, R., Shultz, T.D., McGuire, M.A. (1998) Estimation of conjugated linoleic acid (CLA) intake, FASEB J. 12 A527.

Salminen, I.M., Mutanen, M., Jauhiainen, M., Aro, A. (1998) Dietary trans fatty acids increase conjugated linoleic acid levels in human serum. J. Nutr. Biochem. 9:93-98.

Scimeca, J.A. (1999) Cancer inhibition in animals. In: *Advances in Conjugated Linoleic Acid Research, Volume 1*, Eds. Yurawecz, M.P., Mossoba, M.M., Kramer, J.K.G., Pariza, MW., Nelson, G.J. AOCS press, Illinois, Chapter 34, pages 420-443.

Shultz, T.D., Chew, B.P., Seaman, W.R. (1992) Differential stimulatory and inhibitory responses of human MCF-7 breast cancer cells to linoleic acid and conjugated linoleic acid in culture. Anticancer research, 12:2143-2146.

Watkins, B.A., Li, Y., Seifert, M.F. (1999) Bone Metabolism and Dietary Conjugated Linoleic Acid. In: *Advances in Conjugated Linoleic Acid Research, Volume 1* Eds. Yurawecz, M.P., Mossoba, M.M., Kramer, J.K.G., Pariza, MW., Nelson, G.J. AOCS press, Illinois, Chapter 25, pages 327-339.

Wattenberg, L.W. 1992. Inhibition of carcinogenesis by minor dietary constituents. Cancer Research (suppl.) 52:2085-2091.

Willet, W.C., Stamper, M.J., Manson, J.E., Colditz, G.A., Speizer, F.E., Rosner, B.A., Sampson, L.A., Hennekens, C.H. (1993) Intake of trans fatty acids and risk of coronary heart disease among women. The Lancet, Vol.341, March, 6 pages 581-585.