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# Conjugated Linoleic Acid: Incorporation into Bovine Milk Fat and Effects on Human Health

## Introduction

Milk from ruminant animals has been an important component of the human diet for thousands of years. Ruminants have the unique ability to take organic material that is indigestible to humans and convert it into milk, a food with high nutritional value. Ancient civilizations learnt to exploit this potential through the domestication of various species of ruminant including the cow, sheep, goat, buffalo, and camel. Processing of milk into products like butter and cheese also has a long history. Cheese making is thought have been practiced as early as 4000 BC in regions of the Middle East and was a well-developed skill in the Roman era. Strategies that alter the basic composition of milk are a much more recent endeavor, encouraged by the rapid advancement of scientific knowledge over the past century.

## Biosynthesis of milk fat

Milk fat is a complex lipid containing more than 400 distinct fatty acids. Most of these fatty acids are esterified to glycerol as triacylglycerols, which make up 97 - 98% of the milk lipid. The remainder is mainly comprised of much smaller amounts of phospholipids, cholesterol, cholesterol esters, diacylglycerols, monoacylglycerols, and free fatty acids. The prominent features of bovine milk fat include the presence of short chain fatty acids, the presence of odd and branch-chain fatty acids, a relatively high proportion of saturated fatty acids, a low proportion of polyunsaturated fatty acids, and a relatively high proportion of *trans* fatty acids, including conjugated linoleic acid (CLA).

Bovine milk fat is synthesized in the mammary gland through the esterification of free fatty acids to glycerol. The fatty acids originate either through de-novo synthesis from acetate and β-hydroxybutyrate, or from preformed fatty acids, which come either from the diet or mobilization of body fat stores. De-novo synthesis of fatty acids produces most of the short and medium chain saturated fatty acids from 4:0 to 14:0 and approximately half the 16:0. The main nutritional factors affecting milk fit composition include the effect of forages, rumen modifiers, and supplemental fats and oils. These factors can influence the milk fatty acid composition by providing dietary preformed fatty acids, by influencing the rumen production of precursors for de-novo synthesis, by affecting rumen microbial fatty acid synthesis, and through the rumen production of specific fatty acids that either inhibit or stimulate de-novo synthesis.

Researchers have been interested in modifying the composition of milk fat for decades. Much of this work has focused on feeding various sources of dietary lipid for the purpose of increasing the level of particular fatty acids: most often n-3 and n-6 polyunsaturated fatty acids (see reviews by Ashes, et al, 1997; Chilliard, et al., 2001; Jensen, 2002; Kennelly, 1996; Kennelly and Glimm, 1998; Mansbridge and Blake, 1997). More recently, particular emphasis has been given to feeding regimens that will increase the concentration of CLA, a powerful anticarcinogen found naturally in ruminant milk fat.

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## Biosynthesis of Conjugated Linotele Acid (CLA) in the Cow

The topic of CLA as it relates to ruminant productiom has been reviewed previously (Griinari and Bauman, 1999a; Dhiman, 2000; Chilliard, et al., 2001). Conjugated linoleic acid (CLA) is a component of milk fat 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. Conjugated linoleic acid is formed in the rumen as an intermediate product in the digestion of dietary' fat. The forages and grains fed to dairy cows are characterized by a relatively high content of linoleic (18:2) and linolenic (18:3) acid. Kepler and Tove (1967) showed that *cis*-9. *trans*-11 18:2, the major isomer of CLA, is the first intermediate formed in the biohydrogenation of linoleic acid by the rumen bacteria, *butyrivibrio fibrisolvins* (Figure 1.). This initial reaction involves the isomerization of the *cis*-12 double bond to *trans*-11 by *cis*-9, *trans*11 isomerase. The next step is the conversion of this diene to the *trans-11* monoene *(trans-11* 18:1). These initial steps occur rapidly. The conversion of *trans* -11 18:1 to 18:0 appears to involve a different group of organisms and occurs at a slower rate (Griinari, et al. 1997). For this reason, *trans*-11 18:1 typically accumulates in the rumen. *Trans*-11 18:1 and *cis-9, trans*-11 18:2 account for approximately 50% of the *trans* fatty acids found in milk fat (Griinari, 1998). Although the *cis*-9, *trans*-11 is the predominant CIA isomer in bovine milk, other isomers can be formed with double bonds in positions 8/10, 9/11, 10/12. or 11/13. Each of these double bonds can be in a *cis* or *trans* configuration, giving a range of possible CLA isomers. The term conjugated linoleic acid refers to this whole group of 18 carbon conjugated fatty acids. Alphalinolenic acid goes through a similar biohydrogenation process producing 18:1 *trans*-11 and 18:0, but does not appear to produce CLA as an intermediate.

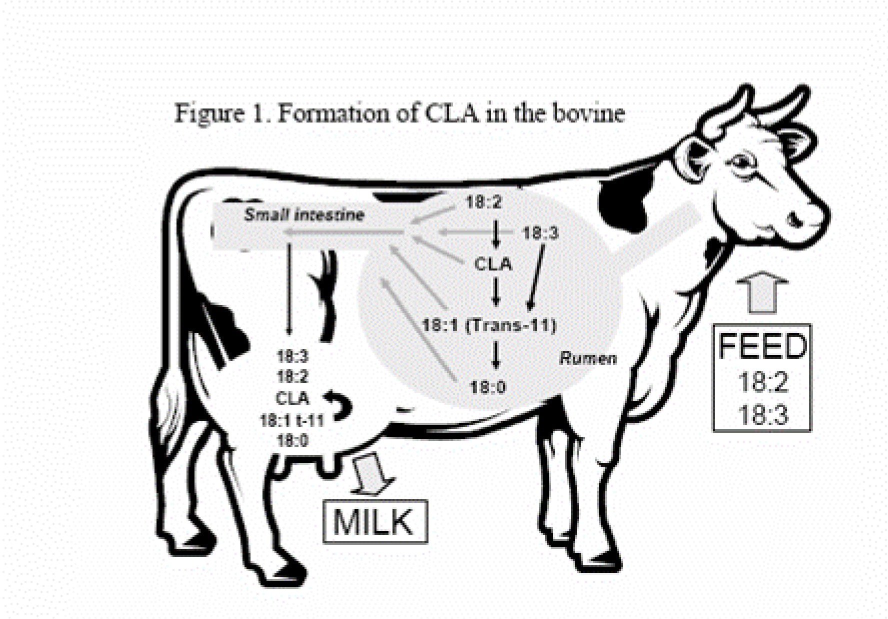
Figure 1. Formation of CLA in the bovine

Oleic acid (18:1 *cis*-9*)* is also a typical fatty acid found in ruminant feeds. This fatty acid is mostly hydrogenated to stearic acid and is not a precursor for CLA in the rumen. However, recent evidence has shown that oleic acid can also be converted to *trans* 18:1 isomers by rumen bacteria (Mosely, et al 2001). Because of the extensive biohydrogenation of linoleic and linolenic acid to 18:1 *trans*-11. several studies have suggested that there may be little accumulation of CIA 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 18:1 *trans*-11 (Griinari and Bauman, I999a). This is possible through the action of stearoyl-CoA desaturase ($\Delta^9$-desaturase), an enzyme capable of adding a *cis*-9 double bond to 18:1 *trans*-11 to give *cis*-9, *trans*-11 CLA. Cork et al.

(2000) showed that the level of *cis*-9, *trans*-11 in milk was reduced by over 60% after abomasal infusion of sterculic oil, a potent inhibitor of $\Delta^9$-desaturase. 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 18:1 *trans*-11.

Milk and meat from ruminants therefore contains more CLA than that of non-ruminants. The amount of CLA found in whole milk is generally about 4.5 to 5.5 mg/g fat, although variation of as much as 2.5 to 18 mg/g fat has been reported. Some researchers have also reported variation associated with breed. White, et al (2001) found that Holstein cows tended to have a higher concentration of CLA in their milk than Jersey cows. In another study, milk from Brown Swiss cows was reported to contain more CLA than Holstein milk, although Brown Swiss milk appeared to be less responsive to dietary manipulation (Whitlock, et al., 2002). Variation in $A^9$-desaturase may explain much of this difference between breeds. Age of the dairy cow and stage of lactation may also 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.

## Potential Health Benefits of CLA

Most substances in nature that demonstrate anti-carcinogenic 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 (Belbury, 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 its cancer-fighting properties. Some of these effects include a role in reducing atherosclerosis (Lee et al. 1994a, Nicolosi et al. 1997) and in the treatment of diabetes (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 enhance 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. These mixtures are composed mainly of the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers but a range of other isomers are also present. As research continues, the specific physiological effects of each of the isomers will 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 microbial biohydrogenation in the rumen. Both approaches will be discussed below.

### *Manipulation of the Diet*

The concentration of CLA in bovine milk fat can vary quite substantially depending on the feeding strategy adopted. For instance, pasture feeding has been found to result in a much higher milk fat CLA concentration than that achieved with typical total mixed rations (TMR) based on conserved forage and grain (Dhiman, et al., 1999; White, et al., 2001). Dhiman, et al. (1999) reported the CLA concentration of milk to be 22.1mg/g fat with pasture feeding compared to 3.8mg/g fat with TMR feeding. Kelly et al. (1998) supplemented the basal diet with 53g/kg 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. It appears that the availability of the oils to the rumen microbes is an important determinant of subsequent CLA production. Chouinard, et al. (2001) showed that processing soybeans, especially by extrusion, increased milk CLA above that obtained by feeding ground soybeans. The extrusion process ruptures the seed, likely making the oil more available for rumen biohydrogenation. The amount of CLA, and type of CLA isomers, produced as a result of feeding supplemental fat varies to a large extent depending on the ruminal conditions. A study at 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 18:1 and trans-10, *cis*-12 CLA isomers.

Dietary fish oil supplementation has also been found to increase the concentration of CLA in bovine milk from 0.2-0.6% in control diets to 1.5-2.7% in supplemented diets (reviewed in Chilliard, et al., 2001). This was somewhat surprising as fish oils are generally high in fatty acids of 20 or more carbons (especially 20:5 and 22:6) but low in 18 carbon polyunsaturated fatty acids. It is thought that the supplemental fish oils interfere with the biohydrogenation of 18:2 and 18:3 from the basal diet, specifically inhibiting the conversion of *trans*-11 18:1 to 18:0. As discussed already, trans-11 18:1 can be desaturated to trans-11 CLA in the mammary gland. Fish oil supplementation has been shown to increase ruminal production of trans-octadecanoic acids (Pennington and Davis, 1975; Wonsil, et al., 1994). Moreover, studies using fish supplementation that reported milk CLA values showed that the increase in CIA was almost exclusively in the *cis*-9, *trans*-11 isomer (Chilliard, et al., 1999; Offer et al., 1999). Feeding fish oil in combination with a source of 18:2 or 18:3 would therefore be expected to increase the level of milk CLA much more than would be achieved with 18:2 or 18:3 alone, This hypothesis was tested by Abu-Ghazaleh, et al (2002) who fed diets containing 0.5% fish oil, 2.5% soybean oil, or a combination of 0.5% fish oil and 2% soybean oil. They reported levels of *cis*-9, *trans*-11 CLA (g/100g fatty acids) of 0.33, 0.47, 0.79, and 1.39 for control, fish oil, soybean oil, and the combination, respectively. Butter from milk containing these higher than average levels of CLA and other polyunsaturated fatty acids is softer and the flavor characteristics of both the milk and butter appear to be unchanged by altering the milk fatty acid profile (Baer, et al., 2001; Ramaswamy, et al., 2001).

We have carried out a series of feeding studies to determine if we could manipulate the animal's diet in a way that would increase the CLA content more than had previously been achieved (Bell and Kennelly, 2000). Table I shows the fatty acid composition from one particular study where cows were fed a diet that is typical for Alberta, Canada (Control, CTL), as well as the fatty acid composition of cows fed a diet designed to increase the concentration of CLA (Treatment diet, TRT). Details of the diets used in these studies can not be given at this time for reasons of patent confidentiality. Generally, feeding the CTL diet resulted in milk fat with a *cis-9, trans*-11 CIA concentration of approximately 0.4 to 0.6%, similar to that typically reported for whole milk. Cows fed CLA-producing diets produced milk fat with as much as 5% *cis*-9, *trans*-11 CIA, approximately 10 times greater than the CTL diet. Although the yield of fat was lower in TRT, the yield of CLA was still approximately nine times greater than the yield of CLA for the CTL treatment (Table 1).

Cows fed the TRT diet also had significantly higher levels of *trans* 18:1 fatty acids in their milk. In the past decade there has been an accumulation of evidence that suggests that *trans* fatty acids may contribute to the development of coronary heart disease (CHD). Investigations found that *trans* fatty acids increased blood cholesterol levels, which are believed to be an important risk factor for CHD. This was supported by strong epidemiological evidence. A study reported by Willett et al. (1993), which followed more than 80.000 people for 8 years, found an association between high intakes of *trans* fatty acids and coronary heart disease. This created the impetus for plans to make labeling of *trans* fatty acids on food packaging mandatory. However, the study reported by Willett et al (1993) showed that the association between *trans* fatty acids and CND was specific for *trans* fatty acids from industrial hydrogenated fats, whereas *trans* fatty acids of animal origin were not correlated with CHD.

Approximately 80 to 90% of the *trans* fatty 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 a rationale for the differences seen in the epidemiological associations. The primary *trans* fatty acids in bovine milk are 18:1 *trans-11* and CLA, whereas partially hydrogenated vegetable oils are characterized by a range of trans fatty acids such as 18:1 *trans*-8, *trans*-9, *trans*-10, *tram*-11, *trans*-12 and *trans*-13. As noted earlier, CLA has been found to inhibit cholesterol-induced atherosclerosis in rabbits and hamsters. Furthermore, there is evidence that 18:1 *trans*-11 can be desaturated to *cis*-9, *trans*-11 CLA in human tissues (Salminen et al., 1998).

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

**Table 1. Fatty acid composition of milk fat from cows fed either a control diet (typical of diets fed in Alberta, Canada), or a high CLA-producing diet.**

| Fatty acid[1] | Control diet (CTL) | CLA-producing treatment diet (TRT) | sem |
|---|---|---|---|
| 4:0 | 5.17$^{a}$ | 4.06$^{b}$ | 0.200 |
| 6:0 | 3.38$^{a}$ | 2.14$^{b}$ | 0.120 |
| 8:0 | 1.98$^{a}$ | 1.13$^{b}$ | 0.064 |
| 10:0 | 4.23$^{a}$ | 2.06$^{b}$ | 0.154 |
| 11:0 | 0.65$^{a}$ | 0.23$^{b}$ | 0.027 |
| 12:0 | 4.80$^{a}$ | 2.40$^{b}$ | 0.130 |
| 13:0 | 0.25$^{a}$ | 0.15$^{b}$ | 0.017 |
| 14:0 | 13.78$^{a}$ | 9.16$^{b}$ | 0.232 |
| 14:1 | 1.36$^{a}$ | 1.01$^{a}$ | 0.121 |
| 15:0 | 1.68$^{a}$ | 0.98$^{b}$ | 0.055 |
| 16:0 | 33.$^{36a}$ | 18.66$^{b}$ | 0.900 |
| 16:1 n-7 | 1.87$^{a}$ | 1.20$^{b}$ | 0.166 |
| 18:0 | 5.73$^{a}$ | 8.02$^{b}$ | 0.360 |
| 18:1 trans | 1.40$^{a}$ | 13.53$^{b}$ | 0.760 |
| 18:1 n-12 | 0.71$^{a}$ | 1.50$^{b}$ | 0.091 |
| 18:1 n-9 | 11.59$^{a}$ | 16.72$^{b}$ | 0.474 |
| 18:1 n-7 | 0.61$^{a}$ | 0.83$^{b}$ | 0.024 |
| 18:2 n-6 | 1.38$^{a}$ | 2.58$^{b}$ | 0.082 |
| 18:3 n-3 | 0.39$^{a}$ | 0.34$^{b}$ | 0.015 |
| 20:0 | 0.12$^{a}$ | 0.14$^{a}$ | 0.008 |
| 20:1 n-12 | 0.10$^{a}$ | 0.11$^{a}$ | 0.006 |
| 20:1 n-9 | 0.03$^{a}$ | 0.07$^{b}$ | 0.003 |
| CLA c-9, t-11 | 0.45$^{a}$ | 5.15$^{b}$ | 0.232 |
| CLA t-10, c-12 | nd$^{a}$ | 0.08$^{b}$ | 0.006 |
| CLA | 0.03$^{a}$ | 0.15$^{b}$ | 0.008 |
| Other FA | 4.94$^{a}$ | 7.57$^{b}$ | 0.162 |

[a,b,c] Within a row, values with different superscripts are significantly different ($P < 0.05$).

[1] All values presented as percentage of fatty acid ethyl esters.

nd = Not detected

*Feeding 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 CIA concentration in bovine milk if protected in sonic way from the rumen environment. Methods available to reduce biohydrogenation in the rumen include encapsulation of the fat in formaldehyde-treated casein or feeding the fat as a calcium salt.

Mixtures of CIA isomers have been found to have an inhibitory effect on milk fat synthesis (Loor and Herbein, 1998; Chouinard. et al., 1999). The *trans*-10, cis-12 CLA appears to be the isomer responsible for this effect (Baumgard, et al., 2000). Abomasal infusion of *trans*- 10, *cis*-12 at levels up to 14 g/day for five days produced a dose response reduction in milk fat yield and concentration in dairy cows (Baumgard, et al., 2001). Griinari et al (1999b) has also shown that rumens concentrations of *trans*-10, *cis*-12 are negatively correlated with milk fat percentage in diets which cause milk fat depression. Decreases in acetyl Co-A carboxylase (ACC) and fatty acid synthase (FAS) activity and ACC mRNA abundance are associated with this depression in milk fat (Piperova, et al., 2000). The use of rumen-protected CLA isomers as a method of depressing milk fat may he useful as a tool to increase the protein to fat ratio in milk (Bauman and Griinari, 2001), and potentially improve the energy balance of early lactation cows (Perfield II et al., 2002). Feeding trials using calcium salts of CLA have demonstrated that they are an effective method of reducing milk fat percentage (Giesy et al., 1999; Sippel, et al., 2001). A study using goats showed that CLA could also he protected from rumen digestion by encapsulating the CLA in formaldehyde-treated casein (Gulati, et al., 2000). In view of the ability of the *trans*-10, *cis*-12 isomer to reduce body fat in animals (Park, et al., 1997), interest has been shown in whether this isomer could have a benefit for weight reduction in humans. Feeding rumen-protected CLA could be a means of elevating the concentration of these fatty acids in bovine milk fat, thereby increasing the supply of these specific fatty acids in the human diet.

In view of the potential of synthetic CLA to increase the concentration of CLA in bovine milk, we carried out a study to evaluate the effect of this product on milk yield and composition (Bell and Kennelly, 2003). Four Holstein cows received abomasal infusion of: (1) control, no fat infusion (CTL), (2) 150g/day of synthetic CLA, 31.7% c-9, t-11; 30.4% t-10, c-12 (CLA), (3) 150g/day of safflower oil (SAFF), and (4) 150g/day of tallow (TALL). Infusion was carried out for 20-22 hours/day for 11 day periods in a 4x4 Latin square design. Data from the last two days of each period was used for statistical analysis.

Infusion of CLA had dramatic effects on milk production and composition (Table 2). Milk yield was 35 to 40% lower over the last two days of the period 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. The concentration of CIA isomers increased significantly as a result of CLA infusion (Table 3). The concentration of linoleic acid (18:2) was significantly increased with infusion of safflower oil (76% linoleic acid). 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 (data not shown).

**Table 2. Yield and composition of milk from cows receiving abomasal infusion of control (CTL), beef tallow (TALL), safflower oil (SAFF), or conjugated linoleic acid (CLA).**

| | CTL[2] | FALL | SAFF | CLA | Sem |
|---|---|---|---|---|---|
| Milk yield (Kg/day)1 | $24.2^{a}$ | $23.0^{a}$ | $26.6^{a}$ | $15.0^{b}$ | 1.928 |
| Lactose % | $3.86^{a}$ | $3.86^{a}$ | $4.04^{a}$ | $3.36^{b}$ | 0.075 |
| Lactose yield (kg/day) | $0.94^{a}$ | $0.90^{a}$ | $1.07^{a}$ | $0.45^{b}$ | 0.074 |
| Fat % | $2.36^{a}$ | $2.46^{a}$ | $2.39^{a}$ | $1.66^{b}$ | 0.117 |
| Fat yield (kg/day) | $0.53^{a}$ | $0.54^{a}$ | $0.62^{a}$ | $0.21^{b}$ | 0.035 |
| Protein % | $3.04^{a}$ | $2.98^{a}$ | $3.14^{a}$ | $4.35^{b}$ | 0.191 |
| Protein yield (kg/day) | $0.70^{ab}$ | $0.65^{a}$ | $0.82^{b}$ | $0.55^{a}$ | 0.047 |

[a,b,c] 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 I50g/day of synthetic CLA.

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 mammary gland. However, we believe that infection was not the cause of the high SCC observed with CLA infusion. 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. The cows showed no physical signs that may have indicated an infection. For instance, there was no effect on dry matter intake. Furthermore, there were no visible signs of mastitis during milking at any time in the course of the experiment. Bacterial analysis of the milk showed counts of streptocuccus/enterococcus well within the normal range for raw milk and no signs of staphylococcus aureus. We did not observe these types of effects in the feeding trial described above (Bell and Kennelly, 2000) were we had a large enrichment of *cis*-9, *trans*-11 CLA in the milk. This may suggest that-the effects observed with the synthetic product were due to the *trans-10, cis-12* isomer. As discussed already, *trans*-10 isomers of CLA have a potent inhibitory effect on milk fat synthesis. 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 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. The changes observed are similar to what occurs during the early stages of involution of the mammary gland. Although purely speculative, it is possible that infusion of these synthetic CLA isomers was initiating the dry-off mechanisms in the mammary gland. More studies will be necessary to further explore these interesting effects of CLA on lactation.

**Table 3. Fatty acid composition of milk fat from cows receiving abomasal infusion of control (CTL), beef tallow (TALL), safflower oil (SAFF), or conjugated linoleic acid (CLA).**

| Fatty acid | CTL[2] | TALL | SAFF | CLA | Sem |
|---|---|---|---|---|---|
| 4-15 | 23.6$^{a}$ | 22.3$^{a}$ | 21.9$^{a}$ | 18.2$^{b}$ | 0.910 |
| 16:0 | 32.3$^{a}$ | 31.9$^{a}$ | 29.9$^{a}$ | 39.5$^{b}$ | 1.120 |
| 16:1 | 1.41$^{ab}$ | 1.58$^{a}$ | 1.12$^{b}$ | 1.39$^{ab}$ | 0.120 |
| 17:0 | 0.62 | 0.58 | 0.57 | 0.62 | 0.024 |
| 18:0 | 11.0$^{a}$ | 11.0$^{a}$ | 11.2$^{a}$ | 13.5$^{a}$ | 0.801 |
| 18:1 trails | 2.48 | 2.70 | 2.58 | 2.21 | 0.157 |
| 18:1 cis-9 | 24.4$^{ab}$ | 25.6$^{a}$ | 22.8$^{b}$ | 18.2$^{c}$ | 0.696 |
| 18:2 | 1.8$^{a}$ | 2.1$^{a}$ | 7.6$^{b}$ | 2.3$^{a}$ | 0.203 |
| 18:3 | 0.15 | 0.15 | 0.17 | 0.19 | 0.022 |
| Cis-9, trans-11 CIA | 0.59$^{a}$ | 0.61$^{a}$ | 0.58$^{a}$ | 1.77$^{b}$ | 0.108 |
| Trans-10, cis-12 CLA | ND$^{a}$ | ND$^{a}$ | ND$^{a}$ | 0.85$^{b}$ | 0.060 |

[a,b,c] 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 CIA.

ND = Not detected

This study demonstrated that post-ruminal delivery of CLA isomers could significantly increase the concentration of these fatty acids in milk. However, it also showed that the extent of enrichment possible for *trans*-10 isomers of CLA is limited because of other unacceptable effects on milk yield and composition. This may place a constraint on the degree to which bovine milk could be used as a vehicle to increase the supply of *trans*-10*, cis*-12 CLA in the human diet.

## CLA-Enriched Dairy Products - New Product Opportunities?

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 ill 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 (Ip, et al., 1994), although others suggest that direct extrapolation may be an overestimation (Ma, et al., 2000). Using the CLA percentage achieved with the TRT diet (Table 1), 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 benefit health, without the need for unrealistic changes to eating habits.

Of course, consumers could increase their CLA intake by taking synthetic CIA in pill form, which is already available in health food stores. The main difference between the CIA 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 will be to convince the processing sector to invest in new product development. The long-term viability of the dairy industry depends on producing products to meet changing consumer demand. 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 he the payment of a premium price for the milk.

## Conclusions

The potential to use nutrition as a tool to alter the composition of milk, especially regarding the milk fatty acid profile and fat percent, has not yet been fully exploited. 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. The potent health promoting effects of CLA has been an unanticipated discovery. Enriching the level of CLA and other potentially beneficial fatty acids in dairy products may provide new market opportunities for milk and milk products such as butter and cheese.

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