Nutrition as a Tool to Alter Milk Composition

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Take Home Messages

- The composition of bovine milk is markedly influenced by the diet of the cow.
- Milk fat is more readily influenced through the nutrition of the cow than other milk components.
- Nutrition can alter both the amount and composition of milk fat thus allowing changes in the protein to fat ratio as well as opening up opportunities to produce milk with higher levels of fats such as CLA and omega-3 fatty acids.

Introduction

Milk from ruminant animals is an excellent source of nutrients and has been an important component of the human diet for thousands of years. There are many situations in which it might be advantageous to change the composition of milk. For instance, dairy farmers might be interested in changing the concentration of a particular milk component for economic reasons. Consumer demand for milk fat and milk protein changes over time and producers may wish to maximize their income by using nutritional strategies to alter the protein to fat ratio in milk. Dairy processors may be interested in strategies that increase casein for maximization of cheese yield, or in methods that increase unsaturated fatty acids, resulting in softer butter. Another example might be the desire of nutritionists to change milk composition in ways that promote better health. Possibilities include reducing the saturated fatty acid concentration or enhancing components with particular functional properties such as omega-3 fatty acids, conjugated linoleic acid (CLA), or bioactive peptides. Finally, some of the most profound possibilities for altering milk composition arise from genetic technologies. These include the use of transgenic cows designed to produce milk containing high-value pharmaceuticals of benefit to human medicine.

The great diversity of possible approaches to alter milk composition is a consequence of the complexity of the physiological processes that underpin lactation. In theory, there seems to be no limit to the possibilities for altering milk composition. The potential of the milk-secreting cells (i.e., mammary epithelial cells) is enormous. In practice however, the functional and biological constraints of the mammary epithelium pose real limitations on the opportunities for altering its secretory composition. Limits to manipulating milk composition are imposed, for example, by functional characteristics of mammary cells, the finite life span of mammary cells, functional trade-offs between milk components, the requirement for osmotically active secretion, the need to produce carbohydrates in a form suitable for storage, maternal nutritional requirements, and the need to maintain anti-microbial properties that protect the mammary gland. But even in view of these limitations there remains great potential to alter the composition of cow’s milk.

A great deal of research has focused on the influence of nutrition on the percent and composition of bovine milk fat because the fat component of milk is particularly amenable to dietary manipulation. 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. More recently, particular emphasis has been given to finding feeding regimens that will increase the concentration of CLA, a powerful anticarcinogen found naturally in ruminant milk fat. Despite the large volume of knowledge that has accumulated on how to modify the composition of cow’s milk, very little of this information has been applied in a commercial setting. Yet, the ability to modify the composition of milk fat represents an opportunity for the development of novel dairy products such as CLA-enriched milk.

This paper will give a brief overview of the principal nutritional factors that affect milk composition, followed by a more detailed survey of the effect of nutrition on milk fat.

■ Dietary Approaches to Alter Milk Composition

Nutrition offers the most effective means of rapidly altering milk composition. Almost all components of milk are subject to manipulation; however, the potential for change varies according to the component. In general, fat percent and the fatty acid composition of milk fat are most amenable to change, whereas lactose is least amenable and protein is intermediate. Changes in milk composition are not always obvious. For example, total protein concentration could remain constant but significant changes could occur in the ratio of casein to non-protein nitrogen. Similarly, substantial changes could occur in the fatty acid composition of milk fat without alterations in milk fat percent.

Influence of Diet on Milk Protein

Milk protein concentration and composition are influenced by many factors, but the magnitude of changes is less than that observed for milk fat content and composition. The dairy cow is a relatively inefficient converter (25 to 30%) of dietary nitrogen into milk protein. Attempts to increase milk protein content through protein or amino acid (AA) supplementation often result in smaller than anticipated responses, likely due to our lack of understanding of AA metabolism within the cow (Bequette et al., 1998). This lack of knowledge about how dietary protein and amino acids influence the composition and yield of milk protein makes it difficult to formulate diets that are biologically efficient and thus economical. Identification and characterization of the regulatory processes underlying amino acid and protein metabolism during lactation should open the door for us to improve the ability of the cow to convert amino acids into milk protein.

Hristov et al. (2004) conducted an analysis of 256 feeding studies to determine the impact of dietary components on milk protein yield. They found that milk protein yield was dependent on dry matter intake, and the dietary concentrations of crude protein, metabolizable protein, neutral detergent fibre, and cow body weight. They also found that days in milk had only a modest influence on protein yield. Milk protein yield was negatively correlated to crude protein, which suggests that protein yield may actually decrease with an increase in dietary crude protein. If the dietary crude protein contains a large proportion of non-protein nitrogen, then metabolizable protein may actually be lower than that of a low crude protein, low non-protein nitrogen diet. Milk protein yield was positively correlated to metabolizable protein. As metabolizable protein increases, amino acid absorption increases, and thus availability to the mammary gland increases as well. The drawback to this analysis was that amino acids were not included in the model. Because amino acids are the building blocks of milk protein, it is imperative that they be considered in any prediction of milk protein synthesis.

Doepel et al. (2004) conducted a similar study to that of Hristov but they examined studies in which AA or casein were infused postruminally, and concentrated on the effects of individual amino acids on the milk protein response. They found that milk protein yield was primarily dependent on the supply of absorbable histidine, lysine, and methionine, the supply of net energy, and on days in milk. While models such as these give an indication of the factors influencing protein yield, they are certainly not 100% accurate in their predictions. This is evident when we examine studies involving protein or amino acid supplementation in dairy cattle (for review see Bequette et al., 1998). In studies where increases in milk protein yield have been observed, the increases have generally been less than predicted. For example, in a series of experiments in which casein was infused, the recovery of the casein in milk protein was only 21% (Hanigan et al., 1998). The question that needs to be answered is: why is the response so small; is it tissue partitioning (competition between tissues for available AA), or the pattern, amount, or mammary uptake of amino acids that limits milk protein synthesis? Before we can consistently alter milk protein content, we must understand the regulation behind its synthesis.

The response to amino acid supply changes with advancing lactation (a negative relationship), which probably reflects alterations in tissue sensitivity to hormones and other metabolic regulators. It has been shown that mature cows have lower milk protein percentages but higher protein yields compared to heifers. Decreasing the rate and extent of digestion of dietary protein in the rumen has been shown to increase milk protein percent, although a greater positive effect is seen on milk yield and milk protein yield (Robinson et al., 1992). Alterations in rate of degradation of dietary protein do not always increase milk protein percent, but sometimes increase non-protein nitrogen content at the expense of casein and (or) whey protein (Khorasani et al., 1994; Robinson et al., 1991).

Increasing the supply of energy-generating compounds also influences milk protein synthesis. Ruminal infusion of butyrate results in an increase in milk protein percent (Huhtanen et al., 1993), whereas propionate infusion is without effect (Hurtaud et al., 1993). Increasing the level of concentrate (greater than 50% of dietary dry matter) in the diet generally results in higher milk protein yield. Similarly the source of starch (barley vs. corn) can influence characteristics of the protein fractions in milk (Khorasani et al., 1994).

Dietary supplementation with lipid usually has a negative effect on milk protein percent, but not all additions cause changes in milk protein yield (Robinson and Burgess 1990; Khorasani et al., 1991). Total daily production of milk protein may remain constant, or increase, in situations where milk yield is increased by fat supplementation. The effect of source of forage or grain is small, yet occasionally differences due to forage source (Khorasani et al., 1993), and grain source (Khorasani et al., 1994) do occur. Although dietary-induced changes in milk fat content may have a relatively small effect on milk protein concentration, they can have a substantial impact on the protein to fat ratio and thus the relative yields of protein and fat.

Influence of Diet on Milk Fat

Milk fat is a complex lipid containing more than 400 distinct 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). Milk fat is synthesized in the mammary gland through the esterification of free fatty acids to glycerol, resulting in triacylglycerols, which make up 97 - 98% of the milk lipid. The fatty acids are either synthesized in the mammary gland from precursors, or they enter the gland as preformed fatty acids, which come either from the diet or from mobilization of body fat stores. Of all the components in bovine milk, fat is the most variable in terms of amount and composition. The factors associated with this variation include genetics, stage of lactation, nutrition and other factors influencing rumen fermentation characteristics.

The main nutritional factors affecting milk fat 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 by ruminally producing specific fatty acids that either inhibit or stimulate de-novo synthesis.

Forages have an influence on milk fatty acid composition in two ways. Firstly, forage provides much of the substrate for cellulytic bacteria, the main producers of acetate and butyrate in the rumen. Secondly, forages can contribute a significant amount to the total fatty acid intake, primarily in the form of polyunsaturated fatty acids.

Dietary additives that modify rumen conditions can also have an impact on the milk fatty acid profile. Kennelly et al. (1999) evaluated the effect of the addition of buffer to diets containing 50:50 or 25:75 forage:concentrate. The addition of buffer prevented the elevation in milk *trans* fatty acids and the associated depression in milk fat percentage with the high concentrate diet. Monensin, an ionophore antibiotic, has also been shown to cause depressions in milk fat content, and to alter the composition of milk fat.

The addition of supplemental fats and oils to the diet has the greatest influence on the amount and composition of milk fat. Some of the common supplemental fats and oils that have been tested include whole and processed oilseeds or the extracted oil, animal fats, marine oils, and rumen-protected fats. A great deal of work has been done on the effect of feeding oilseeds, or the oil from oilseeds, to dairy cows. In general, feeding oil or oilseeds like canola, soybean, sunflower, safflower, or flaxseed results in a reduction in milk 4:0 - 16:0 and an increase in 18:1, 18:2, or 18:3; the extent of change in fat composition will depend on the amount and type of oilseed supplemented. However, the degree of enrichment for these mono- and polyunsaturated fatty acids is much lower than might be predicted from the fatty acid composition of the oilseed. The reason for this lies in the ability of the rumen microbes to hydrogenate unsaturated fatty acids. Researchers have attempted to get around this problem by protecting the oil from microbial biohydrogenation, most often by encapsulating the lipid in formaldehyde-treated casein or by feeding the fatty acids as calcium salts. Animal fats such as beef tallow have been used as an effective means of increasing the energy density of the diet. The major fatty acids in beef tallow are 16:0 (23-27%), 18:0 (14-29%), and 18:1 (36-50%). Supplementing beef tallow tends to decrease 6:0-14:0 and increase 18:1 in milk fat (Chilliard et al., 2001). Because of its low content of polyunsaturated fatty acids, beef tallow likely has limited value for the specific purpose of increasing the level of unsaturated fat in milk. Furthermore, it also suffers from the negative image surrounding the feeding of animal products to animals. A great deal of interest has been shown in the potential to modify milk fat composition through supplementation of marine oils. Marine oils tend to be rich in the very long chain polyunsaturated fatty acids, 20:5 and 22:6. Fish oil feeding generally tends to decrease the proportion of 4:0–14:0, 16:0, 18:0, and 18:1 and increase the *trans* 18:1, 20:5 and 22:6 in milk (Chilliard et al., 2001).

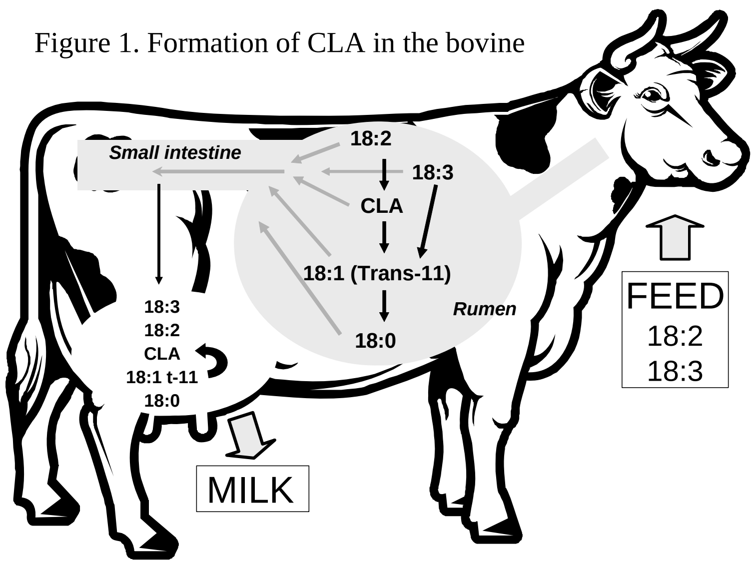
▪ Conjugated Linoleic Acid (CLA)

The topic of CLA as it relates to ruminant production has been reviewed previously (Griinari and Bauman, 1999; Dhiman, 2000; Chilliard et al., 2001). Conjugated linoleic acid 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.

Biosynthesis of CLA in the Cow

The term conjugated linoleic acid refers to a group of 18 carbon conjugated fatty acids. The *cis*-9, *trans*-11 is the predominant CLA isomer in bovine milk accounting for approximately 80% of the CLA, however, 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. Conjugated linoleic acid is formed in the rumen as an intermediate product in the digestion of dietary fat. Kepler and Tove (1967) showed that *cis*-9, *trans*-11 18:2, 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 et al., 1998). The forages and grains fed to dairy cows are characterized by a relatively high content of linoleic (18:2) and linolenic (18:3) acid. 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).

Figure 1. Formation of CLA in the bovine



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, 1999). This is possible through the action of stearoyl-CoA desaturase (Δ^9-desaturase), an enzyme capable of adding a *cis*-9 double bond to 18:1 *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 18:1 *trans*-11. Milk and meat from ruminants therefore contain 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, 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., 2001). Variation in Δ^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 little altered by processing, storage or cooking and hence, the concentration in food depends primarily on the concentration in the raw material.

Table 1. CLA content of various foods

Food	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 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 in animal models to be one of the most potent of all naturally occurring anti-carcinogens. This fatty acid was discovered in 1979 by work in the laboratory of Michael Pariza, who identified an anticarcinogenic compound in ground beef and further work identified this compound as CLA (Pariza et al., 1979, Pariza and Hargreaves, 1985). To prove that the anticarcinogenic effects were indeed due to CLA they tested a synthetically prepared mixture of the CLA isomers on a 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, 2002). 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. 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 (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 synthetic preparations of CLA that vary in the CLA isomers present. Presently research is expanding to study the individual isomer effect in a number of disease states such as obesity, bone disease, kidney disease, neuromuscular disorders, cardiac disease and atherosclerosis (Procter et al., 2004; Taylor et al., 2004; Anderson and Aminot-Gilchrst, 2004).

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 synthetic CLA isomers, protected in some way from microbial biohydrogenation in the rumen.

Manipulation of the Diet. The concentration of CLA in bovine milk fat can vary quite substantially depending on the feeding strategy adopted. 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 and the availability of the oils to the rumen microbes. 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. 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. 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 CLA 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.

Feeding Synthetic CLA. Mixtures of CLA isomers have been found to have an inhibitory effect on milk fat synthesis (Loor and Herbein, 1999; 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 (1999) have also shown that rumen concentrations of trans-10, cis-12 are negatively correlated with milk fat percentage in diets that 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, under some market conditions, be useful as a tool to increase the protein to fat ratio in milk (Griinari and Bauman, 2001), and potentially improve the energy balance of early lactation cows (Bennal-Santos et al., 2001). 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 be protected from rumen digestion by encapsulating the CLA in formaldehyde-treated casein (Gulati et al., 2000). This type of product is already available commercially for feeding to swine because of its ability to improve lean gain in the growing animal. In view of the ability of the trans-10, cis-12 isomer to reduce body fat in animals (Park et al., 1999), 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.

■ Study 1

Using 28 lactating Holstein cows 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) Control including monensin at 24 ppm (MON); (3) control including safflower oil at 6% DM (SAFF); (4) control including safflower oil at 6% DM plus monensin at 24 ppm (SAFF/M). 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 SAFF tended to have a lower lactose percentage compared to MON and SAFF/M. Both percentage and yield of fat were significantly affected by treatment. The MON, SAFF, and SAFF/M treatments produced milk with a lower milk fat percentage than CTL, the greater reduction being seen for the safflower diets.

The CTL diet, representative of diets fed in Alberta, resulted in milk fat with a *cis*-9, *trans*-11 CLA concentration of 0.45%, similar to that typically reported for whole milk. Cows fed the SAFF/M diet produced milk fat with 5.15% *cis*-9, *trans*-11 CLA, more than ten times greater than the CTL diet. Although the yield of fat was lower in the SAFF/M treatment, the yield of CLA was still approximately nine times greater than the yield of CLA for the CTL treatment. Cows fed the SAFF and SAFF/M diets also had significantly higher levels of trans fatty acids (especially 18:1 *trans*-11) in their milk. In the past decade there has been an accumulation of evidence that suggests that trans fatty acids (TFA) may contribute to the development of coronary heart disease (CHD). Investigations found that TFA increased blood cholesterol levels, which are believed to be an important risk factor for CHD.

Table 2. Yield and composition of milk from cows fed control diet (CTL), control including monensin at 24 ppm (MON); control including safflower oil at 6% DM (SAFF); control including safflower oil at 6% DM plus monensin at 24 ppm (SAFF/M)

	Diet				
	CTL	MON	SAFF	SAFF/M	sem
DMI, kg/d	20.45	20.25	18.39	19.52	0.74
Milk yield, kg/day[2]	26.87	27.58	26.78	27.83	1.48
Fat, %[3]	4.02[a]	3.57[a]	2.83[b]	2.95[b]	0.16
Fat yield, kg/day	1.05[a]	0.97[a]	0.74[b]	0.81[b]	0.05
Protein, %	3.32	3.38	3.11	3.23	0.10
Protein yield,	0.87	0.92	0.82	0.88	0.05
Lactose, %	4.30	4.54	4.26	4.50	0.09
Lactose yield,	1.13	1.24	1.14	1.24	0.08
c-9, t-11CLA [5]	0.45[a]	0.52[a]	3.36[b]	5.15[c]	0.23

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

[2]Average milk yield over the last eight days of the treatment period.

[3]Values for fat, protein, and lactose represent average values for milk taken from the last two days of the treatment period.

A study reported by Willett et al. (1993), which followed more than 80,000 women for 8 years, found an association between high intakes of TFA and coronary heart disease. This created the impetus to make labeling of TFA on food packaging mandatory. Approximately 75 to 80% of the TFA 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 (Allison et al., 1999). However, the study reported by Willett et al. (1993) showed that the association between TFA and CHD was specific for TFA from industrial hydrogenated fats, whereas TFA of animal origin were not correlated with CHD. A recent study, however, on the trans-11 18:1 fatty acid has shown that it had a strong anti-carcinogenic effect on mouse mammary tumors. Tumor formation was inhibited by up to 50% while normal mammary cells were unaffected by a diet high in trans-11 18:1 (Lock et al., 2004). Clearly, the issue of TFA in food is controversial and requires further investigation. The planned changes in food labeling to include TFA, along with the current nutritional advice suggesting a decrease in TFA intake is sure to present a confusing message to consumers with the promotion of "high-trans" CLA-enriched milk. This is an important issue that needs to be considered with the development of CLA-enriched dairy products.

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 SAFF and SAFF/M milk compared to CTL or MON had approximately 33 to 35% lower 14:0 and 41 to 44% lower 16:0. There was also an increase of approximately 50% 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 could be modified to give a more favorable fatty acid composition. Furthermore, we demonstrated the feasibility of producing milk with substantially elevated levels of CLA through dietary manipulation.

Follow-up studies at the University of Alberta have confirmed and extended the above results. It was questioned whether monensin would lose its effectiveness over time through adaptation of the rumen microbial populations. In a follow-up study we found that monensin maintained its effect on milk CLA over a two-month period. Other sources of oilseeds were also tested for their ability to increase milk CLA levels. The high 18:2 n-6 oilseeds linola, safflower, and sunflower were the most effective. Providing the 18:2 n-6 as oil was generally more effective than using the processed whole seed. An exception to this rule was seen in studies involving unprocessed whole sunflower seed. Feeding unprocessed whole sunflower seed resulted in similar milk CLA levels as that achieved using sunflower oil.

Butter made from the CLA-enriched milk reflected the CLA content of the milk (Table 3) and it was softer and more spreadable than the control butter. Work is in progress to further refine the feeding strategies aimed at enhancing CLA in milk as well as testing of the subsequent dairy products. In addition, studies are underway that will attempt to gain a greater understanding of the rumen biohydrogenation process and the biology of CLA incorporation into milk fat. These studies include analyzing changes in the population of rumen microbes in response to varying diets and also investigating the biochemical pathways involved in CLA formation in the mammary gland and in addition, establishing reasons for animal variability seen in each CLA-enhancing treatment.

Table 3. CLA content of milk fat, buttermilk, and butter from cows fed either control, or control including linola seed, linola oil, canola seed, flaxseed, safflower oil, soy oil, sunflower oil

Treatment	Milk	Buttermilk	Butter
Control	0.65	0.65	0.66
Linola seed	2.03	2.25	2.22
Linola oil	3.22	3.69	3.18
Canola seed	0.63	0.72	0.75
Flax seed	0.88	1.12	0.88
Safflower oil	2.18	2.24	2.18
Soy oil	1.29	1.38	1.32
Sunflower oil	3.33	3.47	3.27

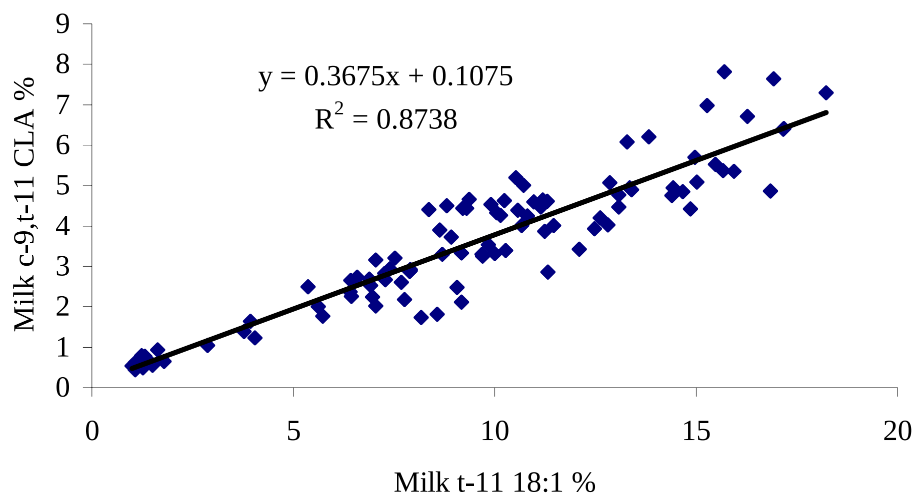


Figure 2. Relationship between cis-9, trans-11 CLA and trans-11 18:1 in milk fat

▪ Study 2

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 infusions 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 11day periods in a 4x4 Latin square design. Data from the last two days of each period were used for statistical analysis.

Infusion of the synthetic CLA product had dramatic effects on milk production and composition (Table 4). 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 CLA isomers increased significantly as a result of CLA infusion. 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 4. 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]	TALL	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

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.

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 Streptococcus/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) where 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 and this 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.

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 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 (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 SAFF/M diet (Table 2), one serving of whole milk (420mg CLA) and a sandwich with butter (334mg CLA) and cheddar cheese (660mg CLA) would provide 1414 mg 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 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 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. As indicated elsewhere in these proceedings, consumers are becoming more conscious of the health attributes of the food they consume. Conjugated linoleic acid 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.

■ Concluding Remarks

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 and milk enriched in omega-3 fatty acids. This recognizes the trend among consumers towards an increased desire to make diet choices that promote good health. Immense potential exists to modify the composition of milk fat using nutrition. Furthermore, the potent health promoting effects of CLA has been an unanticipated discovery. The ability to enrich the level of CLA and other potentially beneficial fatty acids in dairy products may provide exciting new market opportunities for milk and milk products such as butter and cheese.

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