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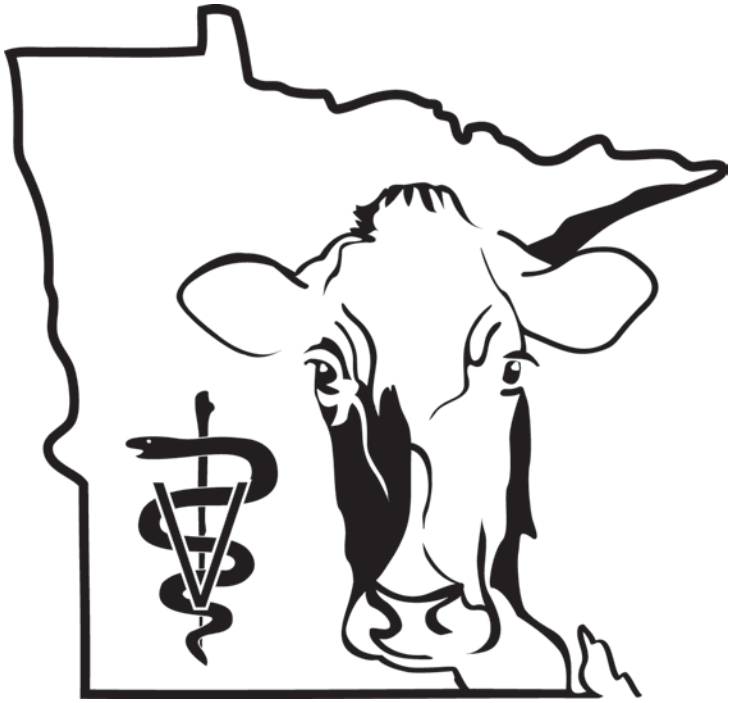

ST. PAUL, MINNESOTA
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Modifying Milk Fat Composition of Dairy Cows to Enhance Fatty Acids Beneficial to Human Health[1]

Adam L. Lock[1] and Dale E. Bauman[2]

[1]Department of Animal Science, University of Vermont, Burlington, VT

[2]Department of Animal Science, Cornell University, Ithaca, NY

ABSTRACT

There is increased consumer awareness that foods contain micro-components that may have beneficial effects on health maintenance and disease prevention. In milk fat these functional food components include EPA, DHA, and CLA. The opportunity to enhance the milk content of these fatty acids has improved as a result of recent advances that have better defined the interrelationships between rumen fermentation, lipid metabolism and milk fat synthesis. Dietary lipids undergo extensive hydrolysis and biohydrogenation in the rumen. Milk fat is predominantly triglycerides and de novo fatty acid synthesis and the uptake of circulating fatty acids contribute nearly equal amounts (molar basis) to the fatty acids in milk fat. Transfer of dietary EPA and DHA to milk fat is very low (<4%); this is, to a large extent, related to their extensive biohydrogenation in the rumen and partly because they are not transported in the plasma lipid fractions that serve as major mammary sources of fatty acid uptake (triglycerides and non-esterified fatty acids). Milk contains over 20 isomers of CLA but the predominant one is *cis*-9, *trans*-11 (75-90% of total CLA). Biomedical studies with animal models have shown this isomer has anti-carcinogenic and anti-atherogenic activities. *cis*-9, *trans*-11 CLA is produced as an intermediate in the rumen biohydrogenation of linoleic acid but not linolenic acid. However, it is only a transient intermediate and the major source of milk fat CLA is from endogenous synthesis. Vaccenic acid, produced as a rumen biohydrogenation intermediate from both linoleic acid and linolenic acid, is the substrate and Δ9-desaturase in the mammary gland and other tissues catalyzes the reaction. Diet can markedly affect milk fat CLA and there are also substantial differences among individual cows. Thus, strategies to enhance milk fat CLA involve increasing rumen outflow of vaccenic acid and Δ9-desaturase activity, and through these, several-fold increases in milk fat content of CLA can be routinely achieved. Overall, concentrations of CLA, and to a lesser extent EPA and DHA, can be significantly enhanced through the use of diet formulation and nutritional management of dairy cows.

[1] Adapted from Lock, A. L. and D. E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids. 39:1197-1206.

INTRODUCTION

Historically, the goal of agricultural research has been to increase yield and productive efficiency, with little focus given to improving the nutrient profile of food products. Mounting research evidence and consumer awareness of the potential health benefits of various micro-components in foods has given rise to the concept of functional foods and helped create a demand for foods with improved nutrient profiles (1). Thus, producers and scientists are interested in research and agricultural practices that may improve the nutrient profile of food products (2). One such example is the dairy industry and recent efforts to modify the composition of milk fat.

Milk and dairy products are recognized as important sources of nutrients in human diets, providing energy, high quality protein, and essential minerals and vitamins (3, 4). Milk fat is responsible for many of the sensory, physical and manufacturing properties of dairy products (5). However, milk fat is relatively more saturated than most plant oils, and this has led to a negative consumer perception and a public health concern related to excessive intake of saturated fats.

Milk fat content and fatty acid composition can be significantly altered through nutrition offering the opportunity to respond to market forces and human health recommendations (6). The impact of nutrition on fat content and fatty acid composition of milk has been extensively reviewed (6-12). Due to increased consumer awareness of the link between diet and health, research has focused on altering the fatty acid composition of cows' milk to achieve a fatty acid profile consistent with consumer perceptions and health recommendations. In the past, much of this work has involved studies in which whole-scale changes have been the goal, whereby large shifts in the saturated to PUFA ratio have been sought-after. Modest changes have been achievable, but this can lead to problems relating to product quality and stability.

Recent research has demonstrated that generalizations about fat and fatty acids are of little value and often lead to misleading and erroneous public understanding. Rather one must consider the biological effects and nutritional value on the basis of individual fatty acids. The focus of this review is on the nutritional manipulation of omega-3 fatty acids and CLA and possible constraints to their enhancement in milk and dairy products. These fatty acids are of particular interest because of their potential benefits to human health. However, the modification of the fatty acid content of milk fat in dairy cows is impacted significantly by the extensive metabolism of lipids that occurs in the rumen. Hence, we will also provide a brief background on lipid metabolism in the rumen and milk fat synthesis.

RUMINANT LIPID METABOLISM

Lipid metabolism in the rumen

Our understanding of lipid metabolism in the rumen and the effect of specific fatty acids on ruminant metabolism has increased significantly in recent years. Analytical developments that improved our ability to quantify the intermediates of fatty acid metabolism have played a major role in these advances. Diets consumed by lactating dairy cows are low in fat content, generally containing only about 4-5% lipid. The predominant PUFA in ruminant diets are

linolenic acid and linoleic acid, the former derived principally from forage crops and the latter being a major component of the oil seeds and concentrates that are fed to dairy cows.

The rumen of a dairy cow has a 40 to 50 liter fluid volume with 10^{10} to 10^{11} bacteria and 10^5 to 10^6 protozoa per ml of rumen contents. When dietary lipids enter the rumen, the initial step in lipid metabolism is the hydrolysis of the ester linkages found in triglycerides, phospholipids, and glycolipids, and this is primarily carried out by hydrolases produced by rumen bacteria. The extent of hydrolysis is generally high (>85%), and a number of factors that affect the rate and extent of hydrolysis have been identified (see reviews by Doreau and Ferlay (13), Doreau and Chilliard (14), and Harfoot and Hazelwood (15)).

Biohydrogenation of unsaturated fatty acids is the second major transformation that dietary lipids undergo in the rumen and it requires a free fatty acid to proceed. As a consequence, rates are always less than those for hydrolysis, and factors that affect hydrolysis also impact biohydrogenation. In addition, the rate of rumen biohydrogenation of fatty acids is typically faster as the extent of unsaturation in the fatty acids increases (16). Biohydrogenation involves only a few of the species of rumen bacteria, and they carry out these reactions as a protection mechanism against the toxic effects of PUFA. Biohydrogenation is extensive and for most diets linoleic and linolenic acid are hydrogenated to the extent of 70-95% and 85-100%, respectively (13-15, 17). Classical pathways of biohydrogenation were established using pure cultures of rumen organisms (Fig. 1). Based on their metabolic pathways, the bacteria involved in biohydrogenation have been classified into two groups with Group A bacteria hydrogenating PUFA to *trans* 18:1 fatty acids, and Group B bacteria hydrogenating the *trans* 18:1 fatty acids to stearic acid (15). Thus, in general no single species catalyzes the full sequence of reactions to convert linoleic and linolenic acid to stearic acid (18). For a more in-depth review of rumen bacteria and their role in rumen biohydrogenation and lipid metabolism, the reader is directed to the recent review by Palmquist et al. (19).

As a consequence of the extensive hydrolysis and biohydrogenation occurring in the rumen, the fatty acids that reach the small intestine are mainly saturated free fatty acids. Some biohydrogenation intermediates can also escape from the rumen and two of the major ones are *trans*-11 18:1 (vaccenic acid) formed from both linoleic and linolenic acid and *cis*-9, *trans*-11 CLA formed during the biohydrogenation of linoleic acid (Fig. 1); these are discussed in more detail in a following section. However, as analytical techniques have improved, it was clear that biohydrogenation processes were considerably more complex than first thought because a remarkable range of *trans* 18:1 and CLA isomers have been identified in rumen outflow (see review by Bauman et al. (16)). Thus, in addition to the major pathway involving the formation of *cis*-9, *trans*-11 CLA and vaccenic acid as intermediates, there must be many more pathways of biohydrogenation. Furthermore, modifications in diet and rumen environment impact biohydrogenation pathways thereby causing substantial changes in fatty acid intermediates produced in the rumen.

Milk fat synthesis

Milk fat consists of droplets of triacylglycerol that are coated with cell membrane. Thus, 96-98% of milk fat is triglyceride with the remainder mainly representing small amounts of

phopholipids, cholesterol and cholesterol esters found in the milk fat globule membrane (20). The triglycerides are composed of over 400 fatty acids, with a large portion of these produced as intermediates during lipid metabolism in the rumen (9). However, most of these fatty acids are present in trace amounts and it is generally recognized that the major fatty acids in milk fat include saturated fatty acids from 4:0 to 18:0 plus palmitoleic, oleic, linoleic and *trans*-18:1 fatty acids.

The fatty acids that comprise milk triglycerides are derived from two sources; de novo synthesis and the uptake of preformed fatty acids (20). Substrates for de novo synthesis are acetate and β-hydroxybutyrate derived from rumen fiber digestion. They are used by the mammary epithelial cell to synthesize short and medium chain fatty acids (4:0 to 14:0) plus a portion of the 16 carbon fatty acids. Mammary uptake of circulating long-chain fatty acids is the other source of the fatty acids in milk. This source provides a portion of the 16 carbon and all of the longer chain fatty acids, and represents fatty acids absorbed from digestion and mobilized from body fat reserves. Those from the diet are transported as triacylglycerol in VLDL and mammary uptake depends on the action of lipoprotein lipase residing in the capillary wall. The long-chain fatty acids from body fat reserves are transported as non-esterified fatty acids (NEFA) and mammary uptake is proportional to plasma concentration. Under typical conditions, on a molar basis, about one-half of the fatty acids in milk are synthesized de novo within the mammary gland. Although plasma triglycerides and NEFA represent less than 3% of total plasma lipid, they contribute the remaining one-half (molar basis) of milk fatty acids. However, this can vary according to physiological state. In particular, the contribution of fatty acids from body reserves can vary from about 5% in a well-fed animal to over 20% of milk fatty acids in early lactation when cows are in negative energy balance (21).

OMEGA-3 FATTY ACIDS

Background

Milk fat content of EPA (20:5 n-3) and DHA (22:6 n-3) are of interest because of their potential benefits to human health. The effects of these omega-3 fatty acids on reducing risk of cardiovascular disease, type II diabetes, hypertension, cancer, and certain disruptive neurological functions and their potential mechanisms of action have been extensively reviewed (22-25). In human nutrition, there is an effort to increase consumption of these functional food components due to the low intake of omega-3 and the relationship of the intake of omega-3:omega-6 fatty acids; Western diets typically have a omega-6 to omega-3 ratio of 20-30:1 whereas the ideal ratio is thought to be 4:1 or less, (26). As a consequence opportunities to enhance omega-3 fatty acids in many foods, including dairy products, are being explored.

EPA and DHA are absent or a minimal level in traditional dairy cow diets, and consequently they are typically present in very low amounts in ruminant products (<0.1% of total fatty acids). However, fish oils, fish by-products and marine algae are often available as dairy feedstuffs and these are rich sources of EPA and DHA. Hence, there is an increasing use of fish oils and fish meal in dairy cattle diets. Despite this, only modest increases in the enrichment of EPA and DHA content in milk fat have been achieved. For example, EPA and

DHA in milk fat before fish oil supplementation averaged less than 0.1% of total fatty acids and after supplementation were only marginally increased to 0.2 to 0.3% of total fatty acids (see review by Chilliard et al. (27)). The extent to which dietary supplements of fatty acids enhance their secretion in milk fat is referred to as "transfer efficiency". In general, the literature indicates that the transfer efficiency of EPA and DHA to milk fat is low. A review of several studies showed that transfer efficiencies averaged 2 and 4% for EPA and DHA, respectively (27), and similar values were observed in more recent studies (Table 1). Interestingly, the transfer efficiency for docosapentaenoic acid (22:5 n-3) appears to be higher than for EPA and DHA (27); transfer efficiencies of 20 to 30% were observed following the supplementation of 250 g/d fish oil (28, 29).

Transfer efficiencies to milk fat are greater when fish oil is directly administered post-ruminally or fed in a 'rumen-protected' form. McConnell et al. (33) reported average transfer efficiencies of 30 and 25% for EPA and DHA, respectively, when 150 g/d of a fractionated fish oil (~40 g/d each of EPA and DHA) was infused into the abomasum. Similar values were reported during duodenal infusions of fish oil (Table 1). Rumen-protected formulations of fish oil have produced similar results; transfer efficiencies were 32% for EPA and 18% for DHA during supplementation of a formaldehyde-protected tuna orbital oil supplement in lactating dairy cows (34). Likewise, rumen-protected marine algae resulted in a transfer efficiency of 17% for EPA (35). Transfer efficiencies also tend to be greater when the fatty acids are supplied by feeding fish meal (36), compared to dietary treatments in which fish oil has been used; thus, the matrix within which the oil is encased provides a degree of protection from rumen biohydrogenation, similar to that seen when oil seeds are fed.

Limitations in the transfer to milk fat

The basis for the low transfer efficiencies for EPA and DHA into milk fat is an active area of research and two possibilities have been proposed. One hypothesis suggests the low transfer efficiency is because EPA and DHA are biohydrogenated by rumen bacteria. Support for this comes from comparisons showing that the transfer efficiency is about 10-fold greater when the EPA and DHA are provided in a manner to by-pass metabolism in the rumen (Table 1). Studies utilizing duodenal or omasal sampling in sheep, steers and lactating dairy cows during fish oil supplementation have quantified the extent of biohydrogenation as 78 to 100% for EPA and 74 to 98% for DHA (28, 37-39).

Results from *in vitro* studies have been less consistent. Gulati et al. (40) and Ashes et al. (41) have suggested that there is little biohydrogenation of EPA and DHA; however, more recent *in vitro* studies have reported substantial biohydrogenation of these fatty acids (42, 43). Such discrepancies are likely due to the concentration of fish oil used in different studies. It is known that when the proportion of fish oil increases, the percent biohydrogenation declines (40, 42, 43), which is most likely due to the toxic effects of PUFA to certain rumen bacteria. For example, Gulati et al. (40) reported very little biohydrogenation when greater than 5 mg/ml (fish oil/ml rumen fluid) was incubated *in vitro*, but biohydrogenation was extensive when less than 1 mg/ml was used. Overall it would appear that, within the normal range of omega-3 fatty acids supplied in dairy cow diets, EPA and DHA will be extensively

biohydrogenated unless suitable technologies can be developed to provide protection from rumen bacteria.

The second possibility to explain the low transfer efficiency of EPA and DHA to milk fat may be a result of their partitioning into plasma lipid fractions that are less available to the mammary gland (29, 44, 45). As discussed earlier, the triglyceride fraction of chylomicrons and VLDL is the form of esterified lipid used most extensively by the mammary gland. Brumby et al. (45) first suggested that EPA and DHA were packaged into lipid classes not readily used by the mammary gland. Recent studies have shown that supplements of EPA and DHA give only marginal enhancements in the triglyceride and free fatty acid lipid classes of plasma compared with the cholesterol ester and phospholipid fractions (29, 44).

Transfer efficiencies of abomasally infused EPA and DHA (27, 33, 46) are similar to transfer efficiencies for abomasally infused isomers of CLA, which have been reported to have transfer efficiencies ranging from 20 to 32% (47-51). Such values are still low compared to transfer efficiencies reported for linoleic and linolenic acid. Although linoleic acid transfer efficiencies have been shown to be variable (10-90%), the majority of low values reported in the literature are when large amounts of linoleic acid were infused in cows in early to peak lactation; the majority of studies in established lactation observed transfer efficiencies in the 40-70% range; similar values have been reported for linolenic acid (see review by Chilliard et al. (46)). Overall, these results suggest that preferential packaging of EPA and DHA into plasma cholesterol ester and phospholipid fractions may limit mammary uptake and their use for milk fat synthesis.

Finally, potential limitations to the use of fish oils in ruminant diets to increase the EPA and DHA content of milk must be considered. In some instances, fish oil can cause a shift in rumen biohydrogenation which leads to a reduction in milk fat synthesis in the mammary gland and the production of milk with a low fat content. Although this may be beneficial in some circumstances (see Griinari and Bauman (52)), milk production with a low fat content is often detrimental, both financially and for many manufacturing processes (21). The second possible drawback to enhancing the omega-3 content of milk fat relates to organoleptic properties of milk. Off-flavors due to fatty acid oxidation are of prime concern because of the shift towards greater unsaturation of the milk fat when fish oils are fed. While some studies have reported flavor problems in milk when fish oil supplements were fed to dairy cows (53), other investigations have observed no adverse effects on flavor score or peroxide indexe (54-56).

CONJUGATED LINOLEIC ACIDS

Background

The intake of CLA in humans is of interest because of the potential health benefits these fatty acids may confer. The anticarcinogenic activity of CLA has been clearly established with *in vitro* cell culture systems and *in vivo* animal models for a wide range of cancer types (see reviews by Banni et al. (57), Belury (58) and Ip et al. (59)) . As biomedical studies with animal models expanded in scope, an impressive range of additional health effects were discovered for CLA, including anti-diabetogenic, anti-atherogenic, immunonmodiulation, anti-obesity and modulation of bone growth (58). The predominant source of CLA in human diets is ruminant-derived food products, with dairy products contributing about 75% of the total (60-62). CLA is a component of the fat in milk and hence research has concentrated on increasing the CLA content per unit of fat. Processing has little effect on CLA, so the content in food products is related to the CLA concentration in the starting fat (61).

The presence of CLA in milk fat was first noted in the 1930's by scientists at the University of Reading, UK (63, 64), with Parodi (65) later identifying *cis*-9, *trans*-11 CLA as a milk fatty acid that contained a conjugated double bond pair. Subsequent research established that *cis*-9, *trans*-11 CLA was the major CLA isomer in ruminant fat representing about 75 to 90% of the total CLA (61, 66), and the common name of "rumenic acid" has been proposed for this isomer because of its unique relationship to ruminants (67). As analytical techniques improved, numerous other isomers of CLA were identified in ruminant fat; these are present at much lower concentrations and they differ by position (e.g. 7-9, 8-10, 9-11, 10-12, 11-13) or geometric orientation (*cis-trans*, *trans-cis*, *cis-cis,* and *trans-trans*) of the double bond pair (Table 2). Thus far, the biological effects have been extensively examined for only two of the CLA isomers, *cis*-9, *trans*-11 and *trans*-10, *cis*-12, and there are clear differences. In the context of dairy products, biomedical studies with animal models have documented the anti-carcinogenic and anti-atherogenic effects of *cis*-9, *trans*-11 CLA (58, 59, 68). Since *cis*-9, *trans*-11 CLA is, by a considerable margin, the most predominant CLA isomer in milk fat, enhancing the CLA content of milk is realistically only related to increases in this isomer. The anti-obesity effects of CLA are due to the *trans*-10, *cis*-12 isomer; while this isomer can vary in milk fat it never represents more than 1 or 2% of total CLA and thus, food products derived from ruminants are unlikely to provide sufficient amounts of this isomer to have biological effects on body fat.

Origin of CLA

The major pathways for rumen biohydrogenation of linoleic and linolenic acid are shown in Fig. 2. Note that *cis*-9, *trans*-11 CLA is the first intermediate in the pathway and it is only formed during the biohydrogenation of linoleic acid. In contrast, vaccenic acid is an intermediate formed from both linoleic and linolenic acid. Vaccenic acid and CLA are both present in ruminant fat and it was generally assumed they were of rumen origin and represented intermediates that had escaped complete biohydrogenation. However, *cis*-9, *trans*-11 CLA is only a transitory intermediate in rumen biohydrogenation, whereas vaccenic acid tends to accumulate. Based on this and other considerations, Griinari and Bauman (69)

proposed that endogenous synthesis could be an important source of *cis*-9, *trans*-11 CLA in milk fat, with synthesis involving the enzyme Δ9-desaturase and vaccenic acid as the substrate.

The importance of endogenous synthesis of *cis*-9, *trans*-11 CLA has been examined in a series of in vivo investigations encompassing a range of diets characteristic for lactating dairy cows (see reviews by Palmquist et al. (19) and Bauman et al. (66). One of the strategies to investigate the importance of endogenous synthesis was a direct inhibition of Δ9-desaturase. For these *in vivo* studies, sterculic oil isolated from the nuts of *Sterculia foetida* was used. Sterculic oil contains about 60% sterculic acid (8-(2-octyl-1-cyclopropenyl) octanoic acid), a cyclopropenoic fatty acid that is a potent inhibitor of Δ9-desaturase. Another approach was to compare values of estimated rumen outflow of CLA with the quantity of CLA secreted in milk fat. This indirect method provided an estimate of the maximum proportion of milk fat CLA that could originate from rumen production. Overall, results from these different approaches and investigations were consistent; endogenous synthesis accounted for the majority of the *cis*-9, *trans*-11 CLA present in milk fat with over 70 to 90% originating from the conversion of vaccenic acid catalyzed by Δ9-desaturase (19, 66).

In 1998, Yurawecz et al. (70) discovered that ruminant fat contained *trans*-7, *cis*-9 CLA. This CLA isomer had not been reported previously because it co-elutes with *cis*-9, *trans*-11 CLA under typical conditions with GC analysis. The milk fat content of *trans*-7, *cis*-9 CLA is generally about 10% of *cis*-9, *trans*-11 CLA making it the second most prevalent CLA isomer. Endogenous synthesis is also the source of this CLA isomer, with it being derived by the action of Δ9-desaturase on *trans*-7 18:1 that is produced in the rumen (71, 72). In contrast to *cis*-9, *trans*-11 and *trans*-7, *cis*-9 CLA, other CLA isomers in milk fat appear to be exclusively of rumen origin. There are no specific mammalian desaturases analogous to Δ9-desaturase that could account for their presence. Rather, they are detected in rumen and duodenal contents, and estimates of rumen outflow are sufficient to account for the trace amounts of these CLA isomers secreted in milk fat (28, 72).

Two of the minor CLA isomers merit further mention, *trans*-11, *cis*-13 CLA and *trans*-10, *cis*-12 CLA. Kraft et al. (74) recently reported that *trans*-11, *cis*-13 CLA was found at concentrations of approximately 8% of the total CLA present in milk fat produced by dairy cattle grazing Alpine regions of Switzerland. The rumen biohydrogenation pathways that produce this CLA isomer and the physiological basis for the relatively greater concentrations in milk fat from cattle grazing Alpine pastures are unknown. The *trans*-10, *cis*-12 CLA isomer is of interest because it is often produced in relatively greater quantities in the rumen of cows fed diets associated with milk fat depression. Indeed, this isomer has been shown to be a potent inhibitor of milk fat synthesis (see reviews by Bauman and Griinari (21) and Bauman et al. (66)).

Altering Milk Fat Content

Diet is the major determinant of milk CLA content and over the last decade, numerous experiments have been carried out with the objective of enhancing the milk fat content of CLA (see reviews by Chilliard et al. (27, 46), Bauman et al. (79) and Stanton et al. (80)). The key to increasing milk CLA is to increase rumen vaccenic acid output, allowing for increased endogenous synthesis in the mammary gland. Maximizing rumen output of vaccenic acid can be achieved in two ways - increasing the supply of 18-carbon PUFA precursors and by inhibiting vaccenic acid reduction to stearic acid. Increasing the dietary supply of 18-carbon PUFA substrates is most easily achieved by the addition of plant oils high in linoleic and/or linolenic acids. The effects of different types and amounts of plant oils have been investigated, and a range of plant oils have been shown to be effective in increasing milk CLA content. The coat of oil seeds offers some protection from rumen biohydrogenation and thus the use of different oil seeds and processing techniques have also been investigated (81-83). Oil seeds high in linoleic and/or linolenic acid, processed so that oil was accessible to biohydrogenating bacteria, resulted in greater increases in milk CLA compared with whole oil seeds, but were not as efficient as using the pure oil. Plant oils are often added to dairy cattle diets as calcium-salts of free fatty acids and Chouinard et al. (82) fed calcium salts of canola (rape), soybean and linseed oil; all three increased the CLA content of milk fat, however those containing the greatest amounts of linoleic and linolenic (soybean and linseed, respectively) caused the greatest increases.

The lipid content of ruminant diets is generally restricted to less than 7% because higher amounts adversely affect the metabolism of rumen bacteria, thereby impairing rumen fermentation and animal performance (84, 85). Thus, there is a limit to the extent that one can provide dietary lipid supplements to increase rumen output of vaccenic acid and CLA. Even at a modest level of plant oil supplementation, the rumen environment can be modified so that a portion of the biohydrogenation pathways are shifted to produce *trans*-10 18:1 and *trans*-10, *cis*-12 CLA as intermediates. These dietary situations are associated with milk fat depression and increasing the CLA content of fat while reducing total milk fat secretion is often unacceptable to producers (see reviews by Bauman and Griinari (21, 86)).

Dietary factors that affect rumen bacteria involved in biohydrogenation, either directly or indirectly via changes in rumen environment, can also affect the CLA content of milk fat. These changes typically involve an inhibition of the final biohydrogenation step which converts vaccenic acid to stearic acid. Several examples have been well characterized including alterations in the forage:concentrate ratio, dietary supplements of fish oil and restricted feeding (79). The most effective of these is the use of fish oil and the general trend is that equivalent amounts of dietary fish oil compared with plant oils result in a greater increase in milk CLA content (28-30, 87-89). Fish oils supply only minimal amounts of 18-carbon PUFA, therefore the increases are most likely a result of a reduction in the final biohydrogenation step where vaccenic acid is converted to stearic acid (69). In support, DHA has been shown to promote vaccenic acid accumulation in mixed ruminal cultures when incubated with linoleic acid (90).

Lastly, a combination of dietary supply of PUFA and modification of the rumen environment can be especially effective in increasing the CLA content of milk fat. Seasonal effects on milk CLA have been known for some time (91-94) and these appear related to this. Fresh pasture results in a 2- to 3-fold increase in the CLA content of milk fat, but the effect diminishes as the pasture matures (81, 92, 93, 95, 96). These results cannot be totally explained in terms of the fatty acid composition and supply of PUFA that grass provides; therefore, there must be additional factors or components of grass that promote the production of vaccenic acid in the rumen, and these lessen in effect as the pasture matures.

Physiological factors that effect milk fat content of CLA have also been examined and differences among individuals are particularly striking. Even when diet and other physiological variables are similar, there is still a 2- to 3-fold range among individuals in the milk fat concentration of CLA (93, 97-99). A similar level of variation also occurs in the milk fat desaturase index (a proxy for Δ9-desaturase activity), with a several-fold range among cows (93, 97-99). These animal differences appear to have a genetic basis, although this has not been examined rigorously. Cows maintain a consistent hierarchy in milk CLA content and desaturase index over time when fed the same diet and when switched between diets (99). The effect of breed (Holstein vs Brown Swiss) was examined in a large study (>200 cows) by Kelsey et al. (97) and no differences were observed. Although some have proposed breed differences in milk CLA content of milk fat (89, 100-102), these studies have often involved very few animals, were confounded by diet, or both. Thus, if breed differences do exist in CLA content of milk fat and desaturase index, they must be minor compared with the effect of diet and individual animal variation (19, 66). Differences in milk CLA among cows are presumably related to individual variation in expression of the Δ9-desaturase gene and rumen outflow of vaccenic acid and CLA. Examination of other physiological factors has established that milk fat content of CLA and desaturase index have little relation to milk or milk fat yield, parity or stage of lactation (97, 103).

We have used such feeding regimes and taken advantage of individual animal variation to produce CLA-enriched butter for use in biomedical studies with animal models. Ip et al. (104) first showed that *cis*-9, *trans*-11 CLA was a potent anticarcinogen when supplied as a natural food component; dietary consumption of CLA-enriched butter was effective in reducing the incidence of tumors in a rat model of mammary carcinogenesis. We have subsequently shown that vaccenic acid derived from CLA-enriched butter increased tissue content of CLA and reduced mammary tumorogenesis (105) and that the anticarcinogenic effect of vaccenic acid is mediated predominately, perhaps exclusively, by its conversion to *cis*-9, *trans*-11 CLA (106). These findings highlight the fact that vaccenic acid should also be considered as a fatty acid with particular benefits to human health. Given that humans convert approximately 20% of dietary vaccenic acid to *cis*-9, *trans*-11 CLA via Δ9-desaturase (107), Parodi (61) has suggested that CLA intake x 1.4 would provide an estimate of the effective physiological dose of CLA derived from ruminant products.

CLA-enriched dairy products have been evaluated for taste, organoleptic properties and storage characteristics in comparisons with standard dairy products. Off-flavors due to fatty acid oxidation are of prime concern because of the shift towards greater unsaturation of the milk fat. We recently produced 2% fat milk with high CLA and vaccenic acid (47 and 121

mg/g fatty acids, respectively) and compared this with a standard 2% milk (5 and 14 mg/g fatty acids, respectively). Evaluations of the milk for up to 14 d post-pasteurization indicated no differences in sensory and triangle taste tests, or in susceptibility to develop oxidized flavors under both light and dark storage conditions (56). Similar results have been observed by others, with milk that had a less dramatic enrichment of CLA (54, 55, 108-110).

In summary, there are four areas to consider when designing diets to increase the CLA content of milk fat; (i) increase 18-carbon PUFA precursors in the diet (linoleic and linolenic acids); (ii) maintain normal biohydrogenation pathways (vaccenic acid pathway); (iii) inhibit the final biohydrogenation step (vaccenic acid to 18:0); (iv) increase desaturation of vaccenic acid to *cis*-9, *trans*-11 CLA in the mammary gland. Future strategies will thus involve establishing dietary and nutritional conditions that maximize rumen outflow of vaccenic acid and *cis*-9, *trans*-11 CLA, optimizing the amount and activity of Δ9-desaturase in mammary tissue, and identifying the genetic basis for the large differences among individuals in CLA-related variables. Obviously, before CLA-enriched foods are widely marketed, studies would need to examine commercial applications and be extended to a wide range of food products.

CONCLUSIONS

Consumers are increasingly aware of "functional food" components that can have positive effects on health maintenance and disease prevention. A number of specific fatty acids are now recognized as having beneficial effects on human health, and these include omega-3 fatty acids and *cis*-9, *trans*-11 CLA that are present in milk fat. Enhancing their content in milk fat requires an understanding of the interrelationship between dietary supply of lipid, rumen fermentation and mammary synthesis of milk fat. Milk and dairy products normally contain very low amounts of EPA and DHA, and increasing their content is limited primarily because their biohydrogenation in the rumen is extensive and secondarily because they circulate in specific plasma lipid fractions that contribute minimally to the mammary supply of fatty acids. These challenges must be addressed to achieve substantial increases in EPA and DHA levels in milk fat, and the formulation of supplements of EPA and DHA that are protected from metabolism by rumen bacteria has potential to address the biohydrogenation problem.

The predominant source of *cis*-9, *trans*-11 CLA in milk fat is endogenous synthesis from vaccenic acid, and thus strategies center on enhancing rumen output of vaccenic acid and increasing tissue activity of Δ9-desaturase. Diet has a major effect on milk fat content of CLA, and there is also a wide variation among individuals. Through modification of dairy cow diets and selection of cows with the highest milk CLA content it is possible to produce milk that is significantly enriched with CLA. Vaccenic acid is also present in milk fat and its contribution to CLA must be considered because it supplies precursor for the endogenous synthesis of CLA in humans. On this basis, vaccenic acid would also be considered as a functional food component in dairy products with potential benefits to human health. Finally, the education of the public that not all fatty acids are equal is required. This is of special importance with the introduction of *trans* fatty acid labeling of foods as undesirable and the fact that both vaccenic acid and *cis*-9, *trans*-11 CLA are *trans* fatty acids.

ACKNOWLEGEMENTS

The authors gratefully acknowledge the helpful discussions with C. McConnell and D. Palmquist during the preparation of the manuscript.

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Table 1. EPA and DHA Transfer Efficiencies in Ruminal and Post-Ruminal Fish Oil Supplementation Studies

	Source	EPA (% transfer)	DHA (% transfer)
Rumen			
Offer et al (29)	Fish oil	2.0	2.0
Chilliard et al. (27)	Fish oil	2.0	4.0
Shingfield et al. (28)	Fish oil	1.4	1.9
McConnell et al. (30)	Menhaden fish oil	2.0	4.0
Post-Rumen			
Chilliard et al. (31)	Menhaden fish oil; duodenal infusion	20.0	18.0
Hagemeister et al. (32)	Menhaden fish oil; duodenal infusion	35.0-40.0	35.0-40.0
McConnell et al. (33)	Menhaden fish oil; abomasal infusion	26.0-35.0	22.0-30.0

Table 2. Range of Positional and Geometric Isomers (% of Total Isomers) Of *trans* 18:1 and Conjugated 18:2 Fatty Acids in Milk and Dairy Products[a]

Trans 18:1		Conjugated 18:2	
Isomer	% of total *trans* 18:1 isomers	Isomer	% of total CLA isomers
trans-4	0.30-2.30	*trans*-7, *cis*-9	1.20-8.89
trans-5	<0.01-1.40	*trans*-7, *trans*-9	0.02-2.39
trans-6-8	0.50-11.30	*trans*-8, *cis*-10	0.06-1.47
trans-9	3.00-18.20	*trans*-8, *trans*-10	0.19-0.37
trans-10	3.40-29.80	*cis*-9, *trans*-11	72.56-91.16
trans-11	24.50-74.90	*trans*-9, *trans*-11	0.77-2.87
trans-12	1.90-17.60	*trans*-10, *cis*-12	0.03-1.51
trans-13 + 14	<0.01-23.10	*trans*-10, *trans*-12	0.28-1.31
trans-15	3.30-11.10	*cis*-11, *trans*-13	0.18-4.70
trans-16	1.70-12.50	*trans*-11, *cis*-13	0.07-8.00
		trans-11, *trans*-13	0.28-4.24
		cis-12, *trans*-14	0.04-0.80
		trans-12, *trans*-14	0.33-2.76
		cis-cis isomers	0.06-4.80

[a]Data derived from eight studies where fatty acid analysis was carried out on milk samples (28, 72-76), butter (77) and cheese (78).

FIG. 1. Classical biochemical pathways for the biohydrogenation of linoleic and linolenic acid in the rumen. Adapted from Harfoot and Hazelwood (15)

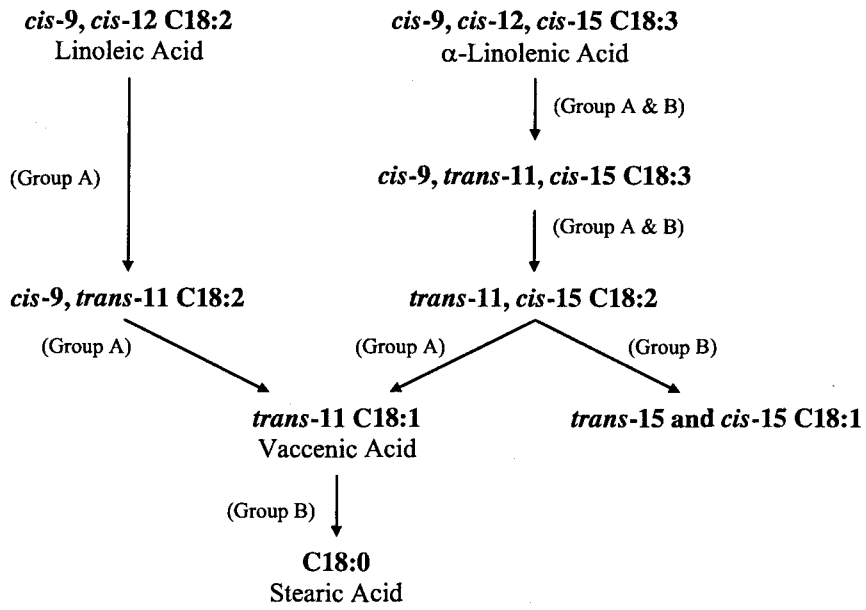
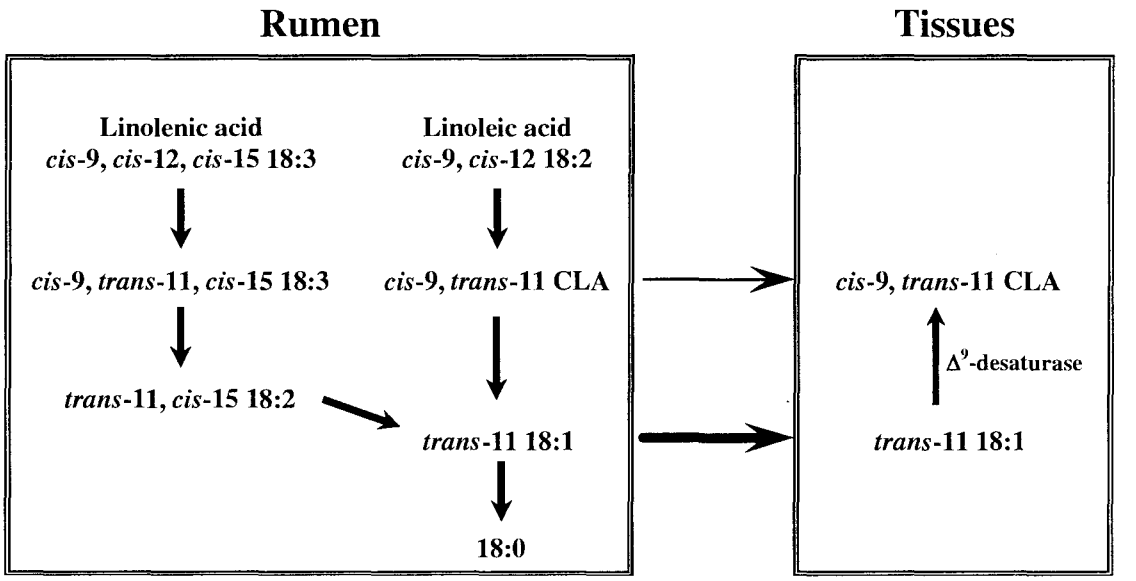
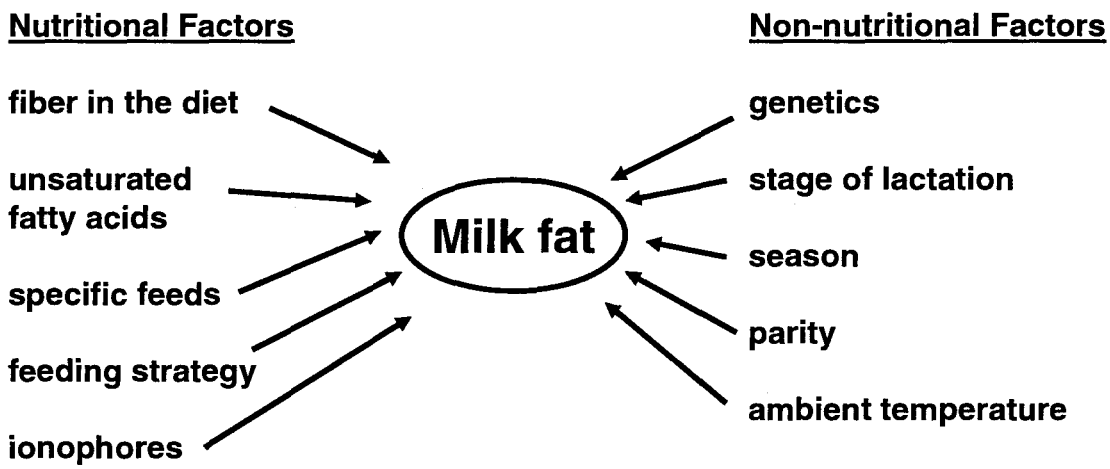


FIG. 2. Pathways for ruminal and endogenous synthesis of *cis*-9, *trans*-11 CLA in the dairy cow. Adapted from Bauman et al. (66)



Supplemental Fig. A. Nutritional and non-nutritional factors affecting milk fat content in ruminants.



Supplemental Fig. B. Milk fat synthesis in mammary cells of ruminants (McGuire and Bauman, 2002). Acetyl CoA carboxylase, ACC; β-hydroxybutyrate, βHBA; endoplasmic reticulum, ER; fatty acid binding protein, FABP; fatty acid synthase, FAS; glycerol phosphate, glycerol-P; lipoprotein lipase, LPL; milk fat globule membrane, MFGM; nonesterified fatty acid, NEFA; saturated fatty acids, SFA; triglycerides, TAG; unsaturated fatty acids, UFA.