Conjugated Linoleic Acid: Cancer Fighter in Milk

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Introduction

As an important source of nutrients, milk provides high-quality protein, energy, calcium and a variety of vitamins and minerals. Recent research has focused on altering the fat and protein content of milk and other dairy products in order to improve the nutrient content of these foods so that they more aptly reflect current dietary recommendations and trends. For example, diet is a contributing factor to the onset or progression of some cancers, with epidemiological studies indicating diet composition may be related to 35 percent of human cancer deaths.[11] A few substances in our diet have been identified as anti-carcinogens, but most are of plant origin and are only present in trace concentrations. However, conjugated linoleic acid (CLA), a component of milk fat, introduces an exciting twist to what we know about diet and cancer. Unlike most naturally occurring anti-carcinogens, CLA is potent at extremely low levels and present in foods from ruminant animals.

Discussion

The National Academy of Sciences publication,[38] Carcinogens and Anti-carcinogens in the Human Diet, concluded "...conjugated linoleic acid (CLA) is the only fatty acid shown unequivocally to inhibit carcinogenesis in experimental animals." Conjugated linoleic acid refers to a series of positional and geometric isomers of linoleic acid with conjugated double bonds.[16] These are fatty acids with 18 carbons and two double bonds separated by one single bond. CLAs as a group are important because they have been recognized as the only fatty acids shown unequivocally to inhibit carcinogenesis in experimental animals,[38] and it is heartening to note that CLA is a potent anti-carcinogen in all cancer models tested.[22,44]

While no intervention study in humans has examined the role of CLA in cancer prevention or treatment, epidemiologic evidence from Finland[29] demonstrated a protective effect of milk intake on the risk of breast cancer in women, and milk is a major source of CLA in the human diet.[6] Beef is also an important source of CLA in the human diet.[6] In fact, the first examination published[40] concerning the effect of CLA on cancer used partially purified extracts of CLA from grilled ground beef and demonstrated a reduction in the number of skin cancers, as well as the number of mice that had skin cancers. Ip *et al*[22] showed that dietary CLA is a potent inhibitor of mammary tumor development and growth in rats (Figure 1). Further, Ip *et al*[26] demonstrated that CLA works as an anti-carcinogen regardless of dietary fat content or type of fat fed (corn oil vs lard).

Recent work demonstrated that CLA could inhibit the growth of human breast[50] and prostate[5] cancer cells when implanted into immune-deficient mice.

Furthermore, consumption of CLA may play beneficial roles in aspects of atherosclerosis, growth and diabetes. First, Lee *et al*[31] demonstrated that rabbits fed 0.5 g CLA per day exhibited lower circulating LDL cholesterol (the bad cholesterol) and triglyceride concentrations; examination of the aortas showed less atherosclerosis. Interestingly, recent epidemiological evidence[14] shows no increase in risk of coronary heart disease with greater butter consumption, and intake of margarine (a food lacking CLA) actually has been associated with an increased risk of heart disease. Thus, although more studies are needed, it may be possible that CLA is beneficial in prevention of atherosclerosis in humans.

The second biological phenomenon, investigated more recently, concerns alterations in growth and body composition. During periods of catabolic stress caused by endotoxin injections, chickens[8] and mice[37] lose less

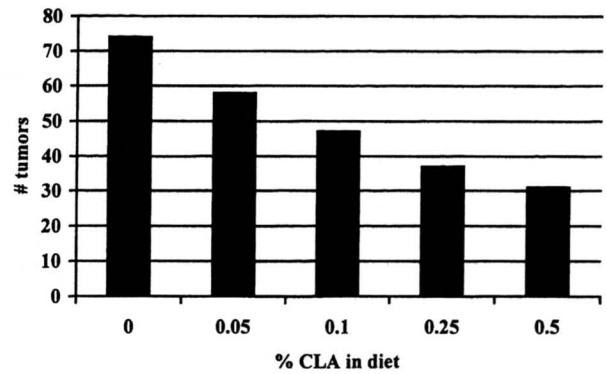

Figure 1. Response of mammary tumors in rats fed diets with varying levels of CLA (Ip *et al*[25]).

body weight when fed a diet containing CLA. These observations suggest that CLA could alter tissue loss. Chin *et al*[7] demonstrated that CLA may also affect tissue gain: rat pups nursing dams fed diets containing CLA were larger than pups nursing dams fed diets without CLA. While no consistent response in milk protein content was observed, the CLA content of rat milk was enriched in the dams fed CLA. No data were presented on the fat content of the milk. Further, Chin *et al* demonstrated that postnatal weight gains increased 7% in rats fed CLA. On the other hand, Belury and Kempa-Steczko[1] noted slower growth rates in young adult mice fed CLA. Although body composition was not measured, lipid content of liver increased with greater CLA intake. Very recent evidence suggests that CLA may, in fact, alter body composition. Mice fed diets containing 0.5% CLA exhibited nearly 60% less fat with an almost 10% increase in protein.[41] Similar studies have demonstrated improved lean growth in growing pigs fed CLA-enriched diets.[39] In the very near future, pork could become a source of CLA in the human diet.

The third area of biological effect is in control of diabetes. Feeding CLA to rats prone to developing diabetes normalized glucose tolerance and improved hyperinsulinemia as effectively as current medications.[20] However, the study was short-term and needs to be replicated before the results can be applied to human health. Nonetheless, if CLA can inhibit body fat accretion as demonstrated in mice, rats and pigs, then improvements in lean-to-fat ratios in the human body may be beneficial to diabetes-prone humans prone. This remains an exciting area of research.

CLAs: Multiple Isomers, Multiple Biological Actions

Many isomers of CLA are made during industrial production, such as those utilized to produce CLA supplements available in health food stores. These mixes of CLAs have been the testing material for cancer, atherosclerosis, growth and diabetes experiments. Is it possible or probable that one isomer could cause such diverse metabolic events as reduction in tumor development or growth, protection of arterial walls from plaque formation, alterations in circulating lipoproteins and cholesterol, promotion of lean growth while diminishing fat deposition, and regulation of milk fat synthesis? We believe it unlikely that one isomer is responsible for all biological activities and, thus, encourage further research and discussion concerning CLAs and their biological importance to focus on effects of each specific isomer.

What is Rumenic Acid?

Rumenic acid is the term given to the CLA that possesses a double bond arrangement of *cis*-9 and *trans*-11.[30] The name "rumenic acid" was chosen because this CLA is present in the human diet primarily in foods (beef and dairy) derived from ruminant animals.[6] While other CLAs can be found in foods for humans, rumenic acid is the predominate form found naturally. Rumenic acid represents greater than 90% of the CLAs present in milk fat and over 75% of the CLAs present in beef fat.[6] However, as mentioned previously, rumenic acid intake can now be increased by the ingestion of industrially-produced CLA supplements available from health food stores. Unfortunately, these supplements also provide other CLAs, as well as additional identified and unidentified fatty acids.

Should We Fortify Or Enrich Foods With Rumenic Acid?

We have examined the presence of CLA in human milk and infant formula.[36] Human milk contained more CLA than did all brands of infant formula tested and over 80% was rumenic acid. Because of the potential for altered growth and body composition, it may be important to fortify infant formula with CLA.

For other ages, beef and dairy products are the predominate dietary sources of rumenic acid,[46] with dairy products the most substantial contributors (Figure 2).

Greater consumption of foods rich in rumenic acid increases circulating concentrations.[3,21] Further, lactating women consuming large quantities of dairy products and beef increased the concentration of rumenic acid in their milk,[42] again confirming the importance of rumenic acid intake in determining human rumenic acid status.

However, the species of bacteria that make rumenic acid from linoleic acid can be found in the human colon.[4] To test if circulating status of rumenic acid can be altered by consumption of linoleic acid, Herbel *et al*[19] fed subjects an oil and vinegar dressing for 6 weeks that increased linoleic acid consumption 2.5-fold. At the end of the dietary intervention, no change in circulating concentration of rumenic acid was detected on average, but it is noteworthy that a small number of subjects did respond to this intervention. This suggests that rumenic acid needs to be present in the diet of most humans to impact human rumenic acid status.

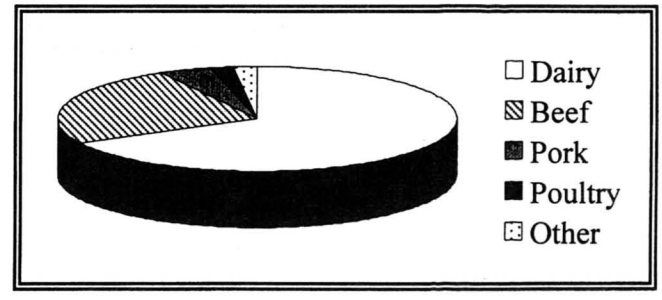

Figure 2. Sources of rumenic acid in the human diet.[46]

The question is whether we, as animal scientists, should strive to increase the natural CLA content of beef and dairy products or simply focus on greater promotion of consumption of those currently available products. From the Knekt *et al*[29] study, it is clear that minor increases in consumption of milk (e.g. 2 glasses/day) can decrease the risk of breast cancer. Therefore, it may be unnecessary to alter rumenic acid content of beef and dairy products to benefit human health. On the other hand, Finnish people are avid milk fat consumers. Although additional milk consumed that reduced the risk of breast cancer provided an estimated 55 mg/d of rumenic acid, basal intake of rumenic acid was approximately 300 mg/d.[29] This has significant implications for Americans, because rumenic acid intake has been estimated to be 50 to 180 mg/d in adult men and women[21,46] and 225 mg/d in lactating women.[42] Thus, it may be more reasonable that we focus simply on increasing the basal intakes of dairy products and beef foods in the United States. For example, if margarine were replaced with butter in the American diet, rumenic acid intake would increase approximately 54 mg/d: a number amazingly close to the amount of CLA present in the milk consumed by Finnish women with the lowest risk of breast cancer.[29]

Further evidence that dairy products can help prevent cancer was recently provided by investigations by Ip *et al*.[23] Using the standard MNU mammary tumor model, Ip *et al*[23] fed rats from weaning to 50 days of age a diet without additional CLA or with CLA from various sources. Reduction in tumor incidence and number occurred regardless of whether the CLA was provided by commercial sources or through butter. The *cis*-9, *trans*-11 CLA (i.e., rumenic acid) provided as the relatively pure isomer or in butter possessed the ability to inhibit cancer. This is the CLA isomer found naturally in dairy products and beef. Anticarcinogenic properties for other isomers of CLA have yet to be proved.

Increasing the Rumenic Acid Content of Dairy and Beef Products

Although it is unclear whether increasing the rumenic acid content in bovine products will be desirable in the longrun, we will review variations in rumenic acid concentrations of foods and ways in which its concentration can be manipulated. Since content of rumenic acid in beef and dairy products is altered little by processing,[47,48] concentrations in the foods consumed by humans are primarily dependent on the rumenic acid content of the raw material. Therefore, rumenic acid enrichment of dairy products and beef would need to be implemented in cattle production systems before processing. To date, attempts to alter rumenic acid content of unprocessed milk have focused on the nutrition of the cow,[26,36] and are highlighted.

Working out the pathways of biohydrogenation in the rumen using cultures of mixed and pure rumen bacteria, rumen microbiologists have shown that rumenic acid is the product of isomerization of linoleic acid (*cis*-9, *cis*-12 C18:2).[18] Many species of bacteria can perform this rearrangement of the double bond in the 12 carbon position. Further, many of these same species perform the first hydrogenation step by removing the *cis* double bond in the 9 position. However, other species of bacteria complete the hydrogenation of linoleic acid to stearic acid by removing the *trans* double bond in the 11 position. Thus, changes in the concentration of rumenic acid in milk are basically due to changes in the rate of this biohydrogenation and passage of these intermediates from the rumen.

Effect of season / pasture

Seasonal variation in content of conjugated, unsaturated fatty acids was noted by Booth *et al*.[2] They reported that conjugated fatty acids in milk fat increased in cows turned out to pasture after winter. Riel[45] detected seasonal variation in the content of conjugated dienoic acids in milk, with nearly double the content during summer months compared to winter months. Parodi[43] suggested seasonal variation was actually a nutritional effect of lush pastures.

Dhiman *et al*[10] extended the examination by feeding cows solely pasture or giving nutrients in addition to pasture. Concentration of rumenic acid in milk fat decreased as concentrates were added. Further, when the pasture was dried, made into hay and then fed to cattle, concentration of rumenic acid in milk fat was reduced. Possibly some component of the lush pasture responsible for the high rumenic acid concentration was lost when the pasture was dried into hay.

Effect of dietary fatty acids

Kelly *et al*[27] utilized different oil sources to confirm that rumenic acid is formed from linoleic acid. In this work, dietary lipid was 8% for all diets whose major source of lipid in the diet was either peanut oil (51.5% oleic acid), sunflower oil (69.4% linoleic acid), or linseed oil (51.4% linolenic acid). Concentration of CLA in milk fat was 13.7, 24.3 and 17.4 mg/g fat for the peanut, sunflower and linseed oil treatments, respectively. They concluded that linoleic acid is the predominate substrate for rumenic acid synthesis. However, linseed oil yielded rumenic acid concentrations greater than peanut oil, even though peanut oil has nearly twice as much linoleic acid.

During the hydrogenation of linolenic acid, *trans*-11 C18:1 (vaccenic acid), a similar intermediate in the hydrogenation of linoleic acid, is formed. While it is doubtful the rumen could form rumenic acid from vaccenic acid, it is well known that the mammary gland has a desaturase enzyme that typically forms oleic acid

from stearic acid by adding a *cis* double bond in the 9 position.[28] Mahfouz *et al*[33] demonstrated that the delta-9 desaturase enzyme can produce rumenic acid from vaccenic acid in microsomal preparations from rat liver. We suggest that the desaturase enzyme in the mammary gland can use the vaccenic acid as a substrate to form rumenic acid. Feeding vaccenic acid protected from further hydrogenation in the rumen, or feeding diets that produce large quantities of vaccenic acid in the rumen, could increase rumenic acid concentration in milk. In fact, when Corl *et al*[9] infused *trans* vaccenic acid, rumenic acid concentration in milk fat increased.

Effect of level of linoleic acid

To determine if a dose response relationship between lipid intake and rumenic acid concentration of milk would occur, we[34] fed cows 4 different levels of corn oil (50% linoleic acid). Rumenic acid content increased from 2.3 to 6.9 mg/g milk fat as the dose of dietary lipid increased from 3% to 7.2%. However, a surprisingly large variation among cows was detected in concentration of rumenic acid, even though feed intakes and milk yields were similar among the eight cows.

In another examination[10] of dietary lipid level, cows were fed a diet containing corn silage and high-moisture ear corn from either typical or a high-oil corn variety. Estimated increase in lipid content of the diet using high oil corn was marginal, as the lipid content of high oil corn silage typically increases from 3% to 5%, while grain content increases from 4% to 7.5%. Thus, overall lipid in the diets increased from 4.2% to 5.1%. Compared to our results[34] with addition of corn oil we would expect the content of CLA in milk to increase only slightly, which it did.[10] In conclusion, these subtle increases in dietary lipid do not dramatically alter concentrations of rumenic acid.

Effect of rumen fermentation

Factors that alter rumen fermentation and the microbial population are keys to controlling regulation of rumenic acid synthesis. Griinari *et al*[15] determined that a source of unsaturated fatty acids (linoleic acid) was required to produce high concentrations of RA in milk fat. Presence of fiber enhanced the concentration of rumenic acid in milk fat.[15] Ionophores such as monensin doubled the concentration of rumenic acid in a rumen culture system.[12] However, rumenic acid concentration in milk fat from lactating cows fed monensin was unaltered.[10] One would suspect that *in vitro* results would support the biology found *in vivo*, but further research is needed to confirm this.

Effect of rumenic acid infusions

Technologies exist to protect nutrients from rumen degradation. Sources of CLAs containing rumenic acid may soon be commercially available in quantities sufficient for livestock-feed ingredients. If rumenic acid were delivered post-ruminally in greater quantities, one would expect increased rumenic acid content in milk. Evidence from Loor and Herbein[32] and Chouinard *et al*[8] (Table 1) demonstrates that if CLAs are infused post-ruminally, dramatic increases in the concentration of several CLAs can occur. Chouinard *et al*[8] also detected a dramatic reduction in milk fat concentration during the 3-d infusion. It is interesting that their 50 g/d infusion administered approximately 30.5 g of CLAs per day. By individual CLA isomer, infusion of rumenic acid was 7.2 g/d and the *trans*-10, *cis*-12 CLA isomer was 10.5 g/d. In contrast, rumenic acid exposure at the mammary gland in our corn oil study was approximately 8.5 g/d without any effect on milk fat concentration. Also note that in our human lactation study,[42] rumenic acid intake was positively – not negatively – related to milk fat concentration. This suggests that something other than rumenic acid in the infusion mixture used by Chouinard *et al*[8] caused the dramatic and rapid reduction in milk fat synthesis.

Effect of rumen-protected CLA

We[17] have utilized available technology to produce calcium salts of a CLA mixture similar to what Chouinard *et al*[8] infused. Feeding this material increased CLA content of milk and decreased milk fat percent in a similar way to abomasal infusion, suggesting that cows can be fed to specifically "design" milk fat.

Milk fat can be "designed" for consumer benefit, but its impact on lactating dairy cows has not been fully examined. Fat secretion in milk constitutes greater than 50% of milk energy costs of lactating cows. Milk-fat depression is typically considered an undesirable result of inhibited rumen fermentation and acidosis. However, stimulating milk-fat depression while preserving rumen health may promote increased milk yield and/or energy balance during early lactation. We[13] conducted a study

Table 1. Effect of abomasal infusion of conjugated linoleic acids on concentrations (mg/g fat) of fatty acids and milk fat percent (Chouinard *et al*[8])[1]

Variable	Infusion rate (g/d)			
	0	50	100	150
cis-9, *trans*-11[2]	5.4	9.5	15.2	19.1
trans-10, *cis*-12	0.2	3.1	8.2	11.3
Milk fat, %	2.81	1.43	1.38	1.23

[1]Material (Natural Lipids, Norway) infused was approximately 61% CLAs of which *cis*-9, *trans*-11 represented 23.7% and *trans*-10, *cis*-12 represented 34.5% of the CLAs present.
[2]Rumenic acid

to evaluate response of lactation performance and parameters describing energy balance while stimulating milk fat depression. Twenty multiparous Holstein cows were paired by previous lactation 305-d ME milk yield and fat percentage. A cow of each pair was randomly assigned to receive a daily supplement containing either 128 g calcium salts of CLA-60 (provided 50 g CLA) or Megalac® ("CON"). The remainder of the diet was identical for both groups and was held to 3.9% fat to eliminate potential effects of other lipid sources on milk fatty acid profile.

Mean dry matter intake did not differ between CON and CLA cows before treatment (20.1 kg/day vs. 19.9 kg/day; 44.2 lb/day vs. 43.8 lb/day) or thereafter (26.0 kg/day vs. 26.2 kg/day; 57.2 lb/day vs. 57.6 lb/day). Mean milk yield (Figure 3, top panel) during the treatment period was higher for cows fed CLA (50.1 kg/day; 110.2 lb/day) than CON (47.7 kg/day; 104.9 lb/day) and was affected by a significant interaction between treatment and days in milk.

Time at which milk yield for cows fed CLA increased over that of cows fed CON coincided with decreased milk fat depression (Figure 3, bottom panel). This may indicate a transfer of energy output in milk from fat to yield when milk fat depression is initiated by feeding of CLA.

Fat percentage of milk was similar between groups before treatment began, and by one week after treatment was stopped (Figure 3, bottom panel). However, milk fat percentage was lower (2.68% vs 3.21%) during the treatment period for cows fed calcium salts of CLA, compared with cows fed Megalac®. Milk fat depression began after 28 DIM or approximately 2.5 wk after supplementation began.

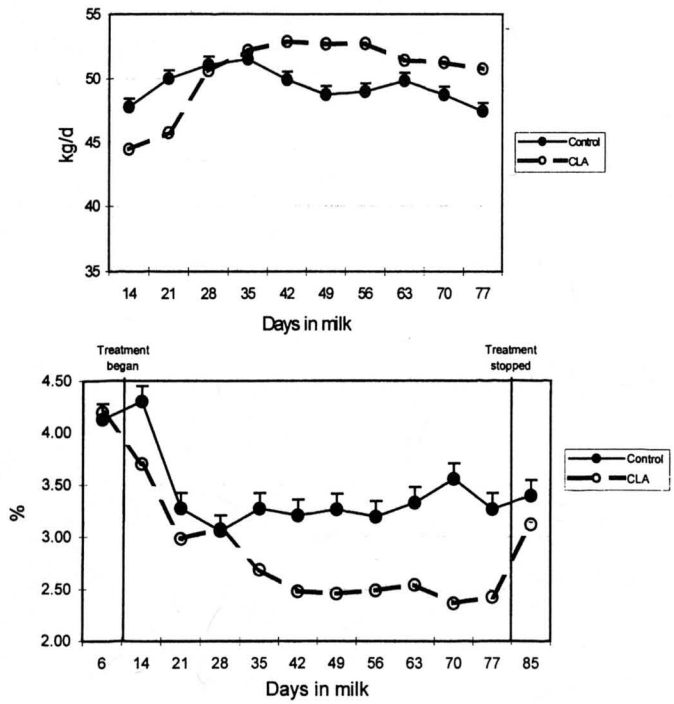

Figure 3. Response of milk yield (top panel) and milk fat percentage (bottom panel) in early lactation cows to supplementation of calcium salts of CLA (CLA) or Megalac® (Control).

Previous feeding of CLA to mid- and late- lactation cows[17] resulted in milk fat depression within 24 hours of first supplementation. Depression of milk fat synthesis by CLA is through suppression of *de novo* fat synthesis in the mammary gland.[8,15] Delayed milk fat depression of cows in early lactation may be a result of utilization of pre-formed fatty acids as the predominant source of fatty acids for milk fat synthesis. Milk fat depression did not occur until after cows fed CLA maintained estimated energy balance greater than zero. Maintaining positive energy balance would reduce lipolysis, thereby limiting the amount of pre-formed fatty acids available to the mammary gland. In fact, plasma non-esterified fatty acids reached their low point at 28 to 35 DIM. Energy balance did not differ between groups before treatment, but was greater throughout the collection period for CLA (5.06 Mcal/d) than CON (0.60 Mcal/d). Supplementing early- lactation cows with calcium salts of CLA caused milk fat depression and promoted higher milk yield and improved energy balance.

Conclusion

Knowledge of the presence of rumenic acid in milk and beef will likely enhance public perception of these foods. It may not be necessary to enrich animal products with rumenic acid, as substitution of milk fat for margarine and beef for other meats may provide the additional rumenic acid necessary to confer anti-carcinogenic benefits to humans. Of course, further studies are required to determine whether rumenic acid is responsible for this beneficial effect of milk intake on breast cancer risk. After all, rumenic acid is just one of many nutrients present in dairy products that benefit human health and well-being.

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