EFFECT OF PROCESSING AND STORAGE ON THE CONJUGATED LINOLEIC ACID (CLA) LEVELS OF DAIRY PRODUCTS MANUFACTURED FROM CLA ENHANCED MILK

By

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A thesis
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ABBREVIATIONS

AOAC	-Association of Analytical Chemist
BHA	-butylated hydroxyanisole
CHD	-coronary heart disease
CLA	-conjugated linoleic acid
DHA	-docosahexaenoic acid
DM	-dry matter
DMBA	-7,12 dimethybenz[a]anthracene
DNA	-deoxyribonucleic acid
EPA	-eicosapentaenoic acid
FF	-fresh forage
FFR	-full fat rapeseed
FFS	-full fat soybean
FM	-fishmeal
GLM	-general linear model
GC	-gas chromatography
HDL	-high density lipoprotein
HMW	-high molecular weight
HPLC	-high performance liquid chromatography
HTST	-high temperature short time
IDF	-International Dairy Federation
IQ	-heterocyclic amine 2-amino-3methulimidazo
LDL	-low density lipoprotein
LMW	-low molecular weight
LPL	-lipoprotein lipase
MFGM	-milk fat globular membrane
MRS	-de Man-Rogosa-Sharpe broth
NMU	-methylnitrosourea
MUFA	-monounsaturated fatty acids
PUFA	-polyunsatured fatty acid
REF	-reference
SFA	-saturated fatty acids
SNF	-solids non-fat
TMR	-total mixed ratio
TVA	-trans vaccenic acid
UHT	-ultra high temperature
VLDL	-very low density lipoprotein
WPC	-whey protein concentrate

ABSTRACT

Dairy products, high in conjugated linoleic acids (CLA), show promise as functional foods due to their reported numerous health benefits. Conjugated linoleic acids, specifically the cis-9, trans-11 isomer have been shown to have activity as anti-oxidants, anti-carcinogens and anti-atherosclerotic agents. They also have been reported to have beneficial functions in reducing body fat and in modulating the immune system.

For this study three experiments were conducted to determine the effect of dairy lactation diets, processing technologies and storage on CLA levels in milk and processed dairy products. The processing variables examined were heat treatment, the addition of whey protein concentrate and probiotic cultures. Sensory evaluations of products were performed to compare the taste of dairy products manufactured from CLA-enhanced milk to those made from control milk.

Conjugated linoleic acid-enhanced milk was obtained from dairy cows fed forage and barley grain based diets supplemented with either fresh forage and solin (FF-solin) or fishmeal and solin (FM-solin). Control and CLA-enhanced milk were processed into pasteurized milk, butter and fermented dairy products using standard procedures. Fatty acids of milk and dairy products were analyzed by gas chromatography.

Results indicated that supplementing dairy cow diets with solin and fishmeal significantly increased CLA levels in milk (2.78%) compared to control diets (0.40%). Processing milk into pasteurized milk and fermented dairy products, and their subsequent storage had no significant effect on CLA levels. Similarly, various heat

treatments of milk had no significant effect on the levels of CLA. Processing milk into butter, however, significantly decreased the level of CLA by 4.5%. The addition of different probiotic cultures in yogurt and kefir production had no significant effect on CLA levels when compared to products without probiotics. Conjugated linoleic acids decreased by 9% when whey protein concentrate was added to yogurt at either 6 or 10%. Sensory panelists found the flavour of CLA-enhanced dairy products to be acceptable and comparable to control products.

Results from feeding trials and sensory studies indicate the feasibility of producing CLA-enhanced dairy products. This will position the dairy industry as an important contributor to the functional food industry.

1.0 INTRODUCTION

Historically milk has been considered an important source of nutrients including protein, calcium, vitamins, minerals and energy. It was only recently that the high ratio of saturated to unsaturated fatty acids in dairy fat was associated with increasing serum cholesterol and coronary heart disease (Ney, 1991). This brought about the development of several "reduced fat", "low fat" and "fat free dairy products and the launch of a new line of dairy products in the market. As we begin a new millennium, the nutritional interests of consumers are changing towards nutraceutical foods (foods with disease prevention and therapeutic properties). Thus, as we find out more about conjugated linoleic acids (CLA) and their desirable health attributes, the positive nutritional potential of milk fat is being reconsidered.

Conjugated linoleic acids are naturally occurring fatty acids in milk fat that have been recognized for their anti-carcinogenic and anti-atherosclerotic properties (Parodi, 1994). Recent studies have also shown that CLA can enhance immunologic functions, affect body fat partitioning (reduce body fat gain while increase lean body mass) and prevent diabetes (Parodi, 1999). Conjugated linoleic acids are found predominantly in products of ruminant animals with milk and milk products being the richest source (Chin et al., 1992). This makes them a unique group of compounds, since most anti-carcinogens or disease preventative micronutrients are normally from plant origin (Parodi, 1994).

Conjugated linoleic acids are a mixture of positional and geometric isomers of linoleic acid (cis-9, trans-12 octadecadienoic acid) that contain a conjugated double

bond system (Pariza et al., 2000). The double bonds in CLA can be found in positions 9 and 11, 10 and 12, 11 and 13, 8 and 10, and 7 and 9 in all possible cis or trans configurations (Parodi, 1994). Although, numerous CLA isomers are found in milk fat the cis-9, trans-11 isomer was first thought to be the most predominant one, accounting for up to 90% of the total CLA in milk fat (Parodi, 1994). It was also thought that this isomer was the only biologically active isomer because it was found incorporated in the phospholipid fraction of rat tissues (Ha et al., 1989; Ip et al., 1991). However, recent studies (Corl et al., 2002; Yurawecz et al., 2002) indicate that early reported values for cis-9, trans-11 levels in dairy foods could represent a mixture of the cis-9 trans-11 and trans-7, cis-9 isomers since new analytical techniques are permitting better separation of positional and geometric CLA isomers. Furthermore, current literature also indicates that all CLA isomers have the potential of being biologically active however, the exact roles and mechanisms that each one is involved in still remains unknown. (Pariza et al., 2000).

Conjugated linoleic acid levels in milk are dependent on the biohydrogenation of unsaturated fatty acids in the rumen (Palmquist, 2001). The cis-9, trans-11 isomer is formed as a first intermediate in the ruminal biohydrogenation pathway of linoleic acid to stearic acid by the bacteria *Butyrivibrio fibrisolvens* (Kepler and Tove, 1967; Palmquist, 2001). It is also endogenously synthesized in tissues from trans vaccenic acid (TVA), another ruminal intermediate. The latter is believed to contribute to the majority of CLA in milk fat (Palmquist, 2001).

Numerous factors have been shown to influence CLA levels in milk fat including; dietary oils, dietary restriction, forage to concentrate ratio, seasonal variation, animal breed and lactation number. These factors affect CLA levels in milk fat by altering the rumen ecosystem and hence ruminal biohydrogenation (Palmquist, 2001). Results from compiled studies provide strong evidence that supplementing dairy cow diets with feed sources high in unsaturated fatty acids can increase CLA levels in milk fat (Lawless et al., 1998; Abu-Ghazeleh et al., 2001, Dhiman et al., 2000). Other studies have indicated that processing technologies could affect CLA levels in milk and milk products (Ha et al., 1989; Shantha et al., 1992; Garcia-Lopez et al., 1994). However, few studies (Dhiman et al., 1999; Baer et al., 2001) have investigated the effects of processing CLA-enhanced milk into various dairy products. In addition, there is a lack of information regarding the sensory properties of CLA-enhanced dairy products.

The objectives of this study were: 1) to determine the effect of different bovine diets on CLA levels in milk; 2) to determine the effect of processing CLA-enhanced milk on the CLA levels in manufactured dairy products; 3) to determine the stability of CLA in manufactured products after storage; and 4) to evaluate and compare the sensory quality of milk and dairy products manufactured from CLA-enhanced and control milk. Results from this study should provide useful information for the dairy industry regarding the feasibility of producing CLA-enhanced milk and dairy products.

2.0 LITERATURE REVIEW

2.1 Milk Fat Overview

Milk is an oil in water emulsion, composed of water, lipids, carbohydrates, proteins, salts and a number of other constituents (Table 2.1). Of the constituents, milk fat has always been of primary interest to a number of researchers. Milk fat has gained a reputation for being the most complex and unique of all natural fats and oils. This is primarily due to its widely distributed fatty acid composition and their arrangement within the triacylglycerol molecules. The diverse array of fatty acids in milk and their specific positioning on the glycerol molecule give milk fat its distinctive physical, chemical and biological properties not found in other common fats.

The majority of the milk fat exists as an emulsified globule enveloped by a bi-molecular phospholipid cell membrane referred to as the milk fat globule membrane (MFGM) (Jensen et al., 1991). The MFGM acts as an active interface barrier between the lipid globule and milk serum. The membrane's functions consist of controlling physical and chemical interactions, as well as maintaining the stability of the emulsion. Destabilizing the membrane by churning, heating or freeze-thawing results in globule fusion or fat coagulation (Jensen et al., 1991).

Bovine milk contains between 2.5 to 5.5 percent total lipids. In general, milk fat globules are composed of 98% triacylglycerols, 1% phospholipids and 0.5% sterols. The latter two lipid classes are mainly found in the globular membrane (Table 2.2). Diacylglycerols, monoacylglycerols and free fatty acids are also present in minute

quantities as a result of lipolysis. Cholesterol is the major sterol present in milk fat with a concentration of approximately 15 mg/dl or 0.46% of total lipids (Jensen et al. 1991).

Over 400 fatty acids have been found in milk; however, most of these are present in amounts of less than one percent of total fat (Jensen et al. 1991). Among these acids, approximately 70% are saturated fatty acids (SFA), 25% monounsaturated fatty acids (MUFA) and 5% polyunsaturated fatty acids (PUFA) (Grummer, 1991). The ten major fatty acids are C4:0, C6:0, C10:0 C12:0, C14:0, C16:0, C16:1, C18:0, C18:1, and C18:2. The fatty acid composition of milk typically contains 10% short chain SFA (<C12:0), 10% myristic acid (C14:0), 26% palmitic acid (C16:0), 12% stearic acid (C18:0) and 25% oleic acid (C18: 1) (Ney, 1991). The fatty acid profile typically found in cow's milk is exhibited in Table 2.3.

Fatty acids in milk originate from two major sources, de novo fatty acid synthesis in mammary tissues and from circulating blood lipids obtained from feed. It is suggested that nearly all C4:0 to C14:0 and approximately fifty percent of C16:0 in milk are derived from de novo fatty acid synthesis occurring in the mammary cell via the fatty acid synthetase complex. The remaining C16:0 and virtually all the long chain fatty acids are derived from blood lipids (Grummer, 1991). In the mammary tissues, both acetate and β-hydroxybutyrate contribute equally as sources of carbon for fatty acid synthesis (Jensen and Clark, 1988). Blood lipids transported in the form of very low-density lipoproteins (VLDL) and chylomicrons are derived from the digestion and absorption of dietary fat or from fatty acids stored in adipose tissue. However, it was found that the former is a much greater contributor of plasma lipids. Palmquist and

Table 2.1. Approximate composition of bovine milk.

Component	Average content in milk (% w/w)	Range (% w/w)
Water	87.1	85.3 - 88.7
Solids-non-fat	8.90	7.9 - 10.0
Lactose	4.6	3.8 –5.3
Fat	4.00	2.5 - 5.5
Protein	3.30	2.3 - 4.4
Casein	2.60	1.7 - 3.5
Mineral substances	0.70	0.57 - 0.83
Organic acids	0.17	0.12 - 0.21

(Adapted from Walstra et al., 1999)

Table 2.2. Lipids of bovine milk fat globules and membranes.

Lipids	Fat globule	Fat globule membrane
Free fatty acids	0.3	6.7
Monoacylglycerols	...	Traces
Diacylylglycerols	...	8.9
Triacylglycerols	98.6	61.7
Phospholipids	0.26	22.1
Unsaponifiable lipids	0.28	0.89

(Adapted from Jensen et al., 1991)

Table 2.3. The fatty acid profile typically found in cow's milk.

Fatty Acid	Percentage of total by weight
C4:0	3.3
C6:0	1.6
C8:0	1.3
C10:0	3.0
C12:0	3.1
C14:0	9.5
C16:0	26.3
C16:1	2.3
C18:0	14.6
C18:1	29.8
C18:2	2.4
C18:3	0.8
C20:0 – C22:0	trace

(Adapted from Fox and McSweeney, 1998)

Mattos (1978) estimated that 88% of the fatty acids derived from blood was from dietary origin and 12% from endogenous contribution. Dietary triacylglycerides packed within VLDL and chylomicron carriers are hydrolyzed by lipoprotein lipase (LPL) to free fatty acids, which allows their uptake by the mammary gland (Grummer, 1991). In the mammary cell, the fatty acids are reesterified in a non-random fashion to form milk triacylglycerides.

The fatty acid composition of milk plays a major role in contributing to the flavour, physical characteristics and nutritional properties of processed dairy products (Baer, 1991; Hillbrick and Augustin, 2002). Short chain fatty acid C4:0 to C8:0 along with many other components such as lactones, methyl ketones and aldehydes give milk fat its very desirable and distinct flavour (Kaylegian et al., 1993). The physical characteristics of milk fat such as its melting point is also strongly influenced by the type and arrangement of fatty acids present in milk triacylglycerols (Fox and McSweeney, 1998). The melting point of fatty acids decrease with decreases in chain length or with increases in the number of unsaturated double bonds. Cis isomers have lower melting point than corresponding trans isomers. The melting point of cis and trans isomers increases as the double bond moves from the carboxyl group towards the omega carbon. Furthermore, symmetrical triacylglycerides have higher melting points than asymetrical molecules with the same fatty acids (Fox and McSweeney, 1998). Variations in the fatty acid content of milk such as increases in unsaturated fatty acids affect the rheological properties of milk fat. In general, butter made from milk containing higher levels of

unsaturated fatty acids is softer and more spreadable than that made from milk with lower levels (Baer, 1991).

In terms of human health, milk fat has been identified as a hypercholesterolemic fat that may contribute to atherosclerosis and coronary heart disease (CHD) (Ney, 1991). This is mainly due to its high content of saturated fat and more specifically its content of lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (16:0), which have been found to increase plasma cholesterol (Ney, 1991). This reason alone serves as a driving force to alter the fatty acid profile of milk.

2.2 Introduction to CLA

Conjugated linoleic acids are a group of naturally occurring fatty acids found in milk and animal fat that have recently gained attention due to their anticarcinogenic properties. Conjugated linoleic acids are a mixture of positional and geometric isomers derived from linoleic acid (Fig. 2.1). They contain a conjugated double bond system that can be found in positions 7 and 9, 8 and 10, 9 and 11, 10 and 12, and 11 and 13, in all possible cis or trans configurations (Parodi, 1994).

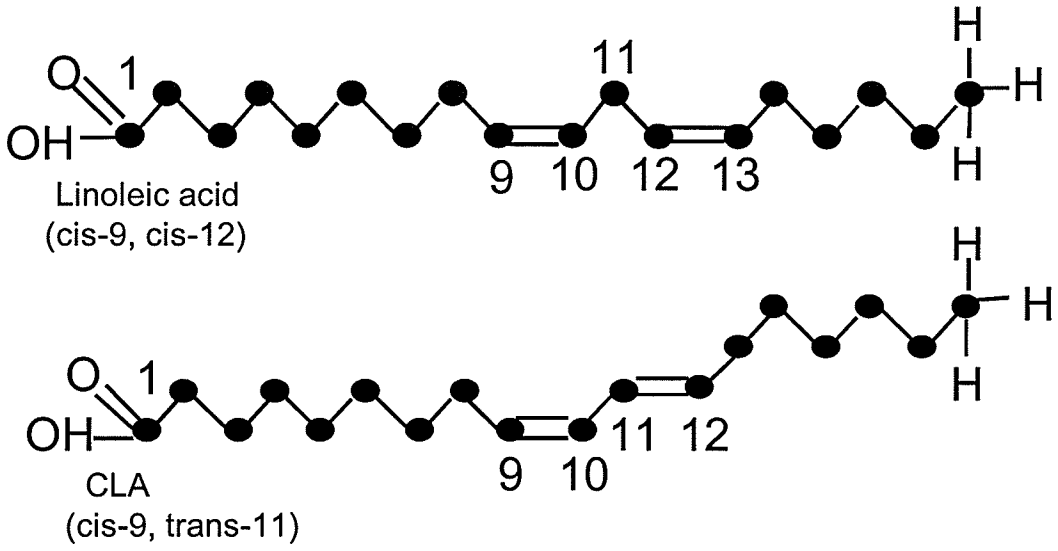

Figure 2.1 Chemical structure of linoleic acid and CLA.

2.3 Food Sources of CLA

Conjugated linoleic acids have been found in various foods and animal tissues (Garcia-Lopez, 1994). However, they are found in higher amounts in foods from animal origin compared to plant origin and in higher amounts in products from ruminant animals than those from non-ruminants. Among all foods, milk and dairy products provide the highest natural food source of CLA (Jiang et al., 1996) with the majority (>90% of total CLA) in the form of cis-9, trans-11 and trans-7, cis-9 octadecadienoic acid (Corl et al., 2002). In general, the evel of total CLA isomers in foods have been reported to range from as low as 0.2 mg CLA/g fat in corn and peanut oil to as high as 30 mg/g fat in milk fat. Negligible levels have also been detected in seafoods and vegetable oils (Riel, 1963; O'Shea, 1998). A study conducted by Chin et al. (1992) determined the CLA content of 14 types of dairy products. They found that the content ranged between 0.6 mg/g fat in a non-fat frozen dairy dessert to 7.0 mg/g fat in condensed milk. In addition, they also examined the CLA content of 13 varieties of natural cheeses and found the CLA content to range between 2.9 mg/g fat in Romano to 7.1 mg/g fat in brick cheese. Ha et al. (1989) determined the CLA content of various natural and processed cheeses. Among the natural cheese the CLA content ranged from 0.6 mg/g fat in blue cheese to 1.9 mg/g fat in Parmesan. However, processed cheese contained almost four times more CLA (8.8 mg/g fat) than the natural cheese. Similar results were reported by Shantha et al. (1992), who found the CLA content of processed cheese to range from 3.2 to 8.9 mg/g fat (Table 2.4). The CLA levels in non-dairy foods

have also been investigated by a variety of researchers (Fogerty et al., 1988; Ackman et al., 1981; and Chin et al., 1992). Examples of CLA values are illustrated in Table 2.5.

Table 2.4. Levels of total CLA in dairy products.

Food	Total CLA (mg/g fat)
Homogenized milk	5.5
Condensed milk	7.0
Butter	4.7
Sour cream	4.6
Plain yogurt	4.8
Romano	2.9
Medium cheddar	4.1
Mozzarella	4.9
Ricotta	5.6
Colby	6.1
Brick	7.1
Processed cheese	3.2 - 8.9

(Adapted from Chin et al., 1992)

Table 2.5. Levels of total CLA in other food products.

Food	Total CLA (mg/g fat)
Ground beef	4.3
Beef tallow	2.6
Veal	2.7
Pork	0.6
Chicken	0.9
Seafood	0.5
Canola oil	0.5
Corn oil	0.2
Olive oil	0.2
Peanut oil	0.2

(Adapted from Chin et al., 1992)

2.4 CLA and Its Health Benefits

Conjugated linoleic acids have been shown to have a variety of potential health benefits. Pariza and his colleagues first discovered CLA in 1978 while investigating the formation of possible mutagens in cooked ground beef. Instead they found a mutagenic inhibitor, which later was shown to possess anticarcinogenic properties. Since then, numerous studies have been devoted to investigating CLA and its biological activities. The CLA isomers used for the majority of the studies were synthesized by a base-catalyzed isomeration of linoleic acid (Pariza et al., 1979).

2.4.1 Animal model studies

Ha et al. (1987) reported that a synthetically prepared topical application of CLA inhibited the initiation of mouse skin cancer induced by 7,12-dimethybenz[a]anthracence (DMBA). Similar results were reported when a 1.0 or 1.5% dietary supplement of CLA was provided in the same model (Belury et al. 1996). In a later study, Ha and his coworkers (1990) observed that a mixture of synthetic CLA isomers administered by gavage could inhibit mouse forestomach tumors induced by benzo[a]pyrene. Zu and Schut (1992) examined the inhibitory ability of CLA on the heterocyclic amine 2-amino-3-methylimidazo [4,5-f] quinoline (IQ) (a potent mutagen and carcinogen) administered to mice. They found that mice fed CLA prior to IQ administration reduced IQ DNA adducts in numerous organs. Similarly, rats fed CLA were found to be protected against IQ adduct formation in the colon (Liew et al., 1995).

Ip and his colleagues (1991) found that 1% dietary CLA was effective in suppressing mammary tumor development in rats induced by DMBA. Conjugated linoleic acids provided a dose-dependent protection at levels of 1% and lower, with no further beneficial effect at levels higher than 1%. In a subsequent experiment Ip et al. (1995) found that the timing and duration of CLA feeding was important in modulating mammary cancer risk. Rats exposed to CLA during early post weaning and adolescence (21 to 42 days of age) conferred a lasting protection against mammary carcinogenesis induced by methylnitrosourea (MNU). In contrast, feeding rats CLA for a short-term (one or two months) post MNU was ineffective in cancer protection. It was found that for CLA to be effective post MNU, rats needed to be fed an uninterrupted supply of CLA in their diet. More recently, a one percent dietary dose of CLA inhibited local tumor growth and prevented metastatic spread in immunodeficient mice injected with human breast adenocarcinoma cells (Visonneau et al., 1997).

2.4.2 Cell culture studies

Shultz et al. (1992) reported that CLA inhibited the growth of human malignant melanoma (M21-HPB), colorectal (HT-29) and breast (MCF-7) cancer cell lines in a dose and time-dependent manner. Schonberg and Krokan (1995) found that CLA exerted a dose-dependent reduction in the proliferation of three human lung adenocarcinoma cell lines. More recently, CLA was shown to inhibit the growth of various cancer cell lines including melanoma, leukemia, mesothelioma and glioblastoma

together with breast, prostate, colon and ovarian cancer cell lines (Visonneau et al., 1997).

In addition to possessing anticarcinogenic properties CLA has been shown to have many other nutraceutical functions. Lee et al. (1994) discovered that CLA possessed antiatherogenic properties. They found that supplementing 0.5g CLA per day for 22 weeks to rabbits fed an atherogenic diet significantly reduced their plasma triacylglycerides, LDL-cholesterol (LDL-C) and LDL-C/HDL-C ratio compared to control animals. Furthermore, fewer aortic fatty lesions were found in the CLA supplemented group. Nicolosi and Laitinen (1996) observed similar results in hamsters.

Cook et al. (1993) found that dietary CLA prevented immune-induced cathexia (weight loss) in chicks without compromising other immune functions. Later findings indicated that CLA enhanced immune functions in several animal models while providing protection from immune cytokin-induced wasting (De Voney et al., 1997; Cook and Pariza, 1998). The role of CLA in immune related functions is still not completely understood. However, it is believed that CLA has a key role in modulating eicosanoid synthesis due to its relatedness to linoleic acid (Cook and Pariza, 1998). Briefly, eicosanoids such as prostanglandin E_2 (PGE_2) are metabolites of arachidonate that are involved in signaling processes for immune responses and arachidonic acid (C20:4) is a desaturated and elongated product of linoleic acid (Cook and Pariza, 1998).

Animal studies using rats, mice and chicks have demonstrated that dietary CLA improves feed efficiency, reduces body fat and increases body protein composition (Chin et al., 1994; MacDonald, 2000). The effect CLA on body composition has been

attributed to its involvement in decreasing lipoprotein lipase activity (fat deposition) and increasing lipolysis in adipocytes coupled with enhancing fatty acid oxidation in adipocytes and muscle cells (Park et al., 1997; MacDonald, 2000).

Although numerous animal and cell culture studies provide strong evidence that support the health benefits of CLA, further research is still necessary to determine its effects on humans. Furthermore, since most researchers have used a mixed synthesized form of CLA for their studies, more work is still required to examine the effect of a natural source of CLA. Dairy products, being the highest natural food source of CLA that are widely consumed in all parts of the world, would be the most practical choice for future studies. Research is also needed to precisely determine which isomers are biologically active and their specific roles and mechanisms involved in disease prevention and treatment. Finally, scientists still have to determine the most effective doses and timing required for expression of beneficial biological activities by CLA.

2.5 Biosynthesis of CLA

Conjugated linoleic acid in milk fat was first thought to originate solely from the ruminal biohydrogenation of linoleic to stearic acid by *Butyrivibrio fibrisolvens*, because it is formed as a first intermediate in the pathway. However, it was found that the availability of CLA from the rumen was inadequate to account for levels present in milk fat. Thus, it is now generally believed that CLA must also be synthesized in tissues from another precursor of rumen origin (Griinari and Bauman, 1999).

Several decades of compiled studies using radio-labeled or pure fatty acids *in vivo* or *in vitro* have allowed the identification of major pathways of ruminal biohydrogenation and hence a better understanding of CLA synthesis (Griinari and Bauman, 1999). The predominant pathways of ruminal biohydrogenation of unsaturated fatty acids are illustrated in Fig. 2.2.

Hydrogenation of linoleic acid to stearic acid involves two distinct steps and populations of ruminal bacteria (Griinari and Bauman, 1999). First, linoleic acid (cis-9, trans –12 octadecadienoic acid) is isomerized to CLA (cis-9, trans-11 octadecadienoic). It is then rapidly hydrogenated to form trans-11 vaccenic acid (TVA) prior to being further hydrogenated to form stearic acid. Unlike CLA, vaccenic acid reduction has been found to be the rate limiting step in biohydrogenation of unsaturated C18:0 fatty acids and for this reason tends to accumulate in the rumen.

Linoleate isomerase (C 5.2.1.5) is the enzyme responsible for isomerization of linoleic acid to CLA. This enzyme is bound to the bacterial cell membrane of certain ruminal bacteria such as *Butyrivibrio fibrisolvens* and has an absolute subtrate specificity for cis -9, cis -12 diene arrangements plus a free carboxyl group (Griinari and Bauman, 1999).

Although, the cis-9, trans-11 CLA isomer is the most predominant isomer found in milk fat numerous, other positional octadecadienoic acids have been detected. The formation of these isomers is believed to result from either: 1. double bond migration during the hydrogenation of CLA to TVA; or 2. the activity of specific cis, trans isomerases in ruminal bacteria (Griinari and Bauman, 1999).

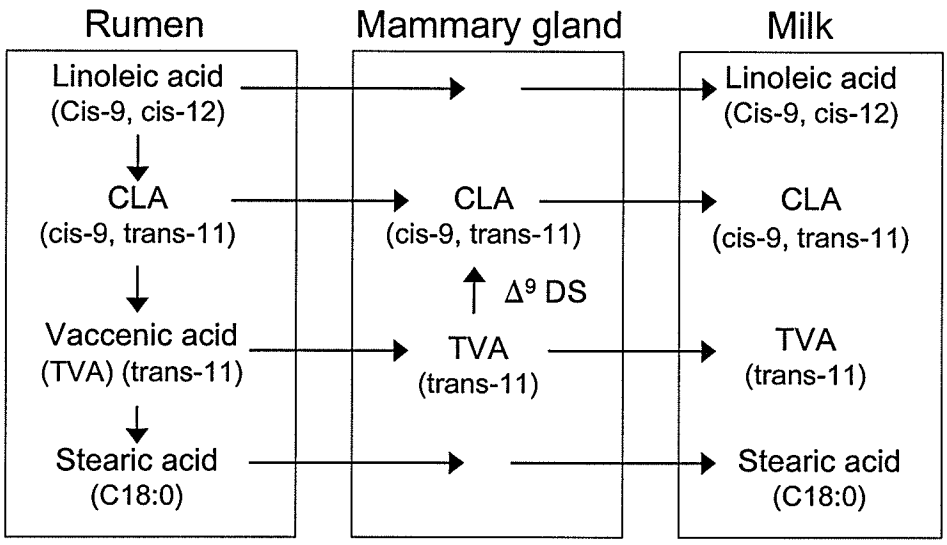

Figure 2.2 Pathways of CLA biosynthesis.

Griinari and Bauman (1999) speculated that TVA derived from the rumen is the precursor of milk fat CLA. Moreover, CLA is produced endogenously in the mammary gland from TVA by Δ^9 desaturase. The basis of this theory came from observing the constant ratio of TVA and CLA present in milk fat from cows fed a wide range of diets.

Later, these researchers tested their hypothesis in lactating dairy cows using postruminal infusion. Results from their study showed an increase in both trans-11 and its desaturase product cis-9, trans-11 in milk fat of cows infused with trans-11, which clearly supported CLA endogenous synthesis via Δ^9 desaturase.

2.6 Factors that Contribute to Elevated CLA Levels in Milk

Many factors have been reported to contribute to the elevation of CLA levels in milk and dairy products, including animal diet, breed, lactation number (age), seasonal variations and processing technologies. Among these factors, the effect of animal diet and dietary regimes a major impact and, therefore, have been studied extensively.

2.6.1 Animal studies

Research studies have repeatedly shown that feeding cows lipid sources rich in linoleic acid (C18:2) or linolenic acid (C18:3) in the form of either seed or oil could enhance the CLA content in milk. Murphy et al. (1995) observed that feeding full fat soybean (FFS) or full fat rapeseed (FFR) to lactating cows on pasture decreased the short to medium chain fatty acids and increased long chain fatty acids (C18:0, C18:1, C18:3) in milk fat. Later, Stanton et al. (1997) reported that CLA levels increased in

milk fat from cows on a high FFR supplemented diet (7.9 mg CLA/g fat) compared to the control (pasture) (4.8 mg/g) and low FFR diets (5.2 mg/g fat). Similarly, Lawless et al. (1998) found that cows on pasture supplemented with both FFR and FFS produced milk fat with higher CLA levels (24.0 mg CLA /g fat) and (19.6 mg/g) respectively compared to those fed a control diet (16.6 mg/g). In addition they concluded that FFR was more effective than FFS at increasing CLA. More recently, Dhiman et al. (2000) examined the effect of supplementing bovine diets with raw cracked soybean, roasted cracked soybean, soybean oil or linseed oil. They found that roasted cracked soybean, soybean oil and linseed oil treatments increased the CLA content of milk compared to an unsupplemented control diet. Raw cracked soybean had no effect on the CLA content of milk. This was attributed to the slow release of oil from the raw soybean in the rumen as compared to the heat-treated form. It was suggested that heat treatment may facilitate the release of soybean oil and hence increase its availability for rumen biohydrogenation. Furthermore, they concluded that the soybean oil was more efficient in elevating CLA content in milk than the linseed oil. Milk concentrations of CLA increased progressively as the dietary supply of soybean oil increased. Similar results were also reported by Kelly and Bauman (1996) who fed cows increasing levels of corn oil.

Other researchers examined the effect of diets, which differed in fat source (saturated vs. unsaturated) and in forage to concentrate ratio. They found that both a dietary supply of unsaturated lipids and forage to concentrate ratio had an impact on CLA levels in milk. It was observed that feeding cows a 50:50 forage to concentrate

ratio with a supply of unsaturated lipids was optimal for increasing CLA. (Kelly and Bauman, 1996). In contrast, Jiang et al. (1996) found that feeding cows a restricted diet with a lower forage to concentrate ratio (35:65) produced milk with higher concentration of CLA (11.28 mg CLA/g fat) than those fed a higher ratio (50:50) (5.0 mg/g fat) or unrestricted diets (6.6 mg/g fat).

Furthermore, it was also reported that milk content of CLA was higher in cows grazing pasture versus cows fed conserved diets (Kelly and Bauman, 1996). In general, milk fat produced from cows at pasture is softer and has higher concentrations of C18:1 and C18:2, and lower levels of C16:0 which is a result of the high levels of polyunsaturated fatty acid in fresh grass (Murphy et al., 1995). A study found that cows grazing pasture and receiving no supplemental feed had 500% more CLA in their milk (22.1 mg/g) than cows fed a total mixed ration (TMR) containing a forage to concentrate ratio of 50:50 (3.8 mg/g). Moreover, it was observed that increasing the proportion of grazed grass from pasture in the diet of dairy cows directly increased CLA content in milk. However, feeding dry pasture grass (hay) did not have the same effect in elevating CLA levels in milk (Dhiman et al., 1999). Similar results were later found by Stockdale et al. (2000) and Walker et al. (2001), who reported that fresh pasture feeding and increased pasture intake elevated CLA levels in milk. In addition, it has been noted that there are seasonal variations in the concentration of CLA in milk. The highest CLA levels in milk occur in the early summer when cows consume vegetative forage. (Kelly and Bauman, 1996).

Supplementing dairy cow diets with fishmeal instead of oilseed has also been investigated as a means of increasing CLA in milk fat. Dhiman et al., (1999) found that TMR diets supplemented with 3% menhaden fishmeal (DM basis) alone or in combination with 250g monensin/cow/per day marginally increased CLA levels in milk. These increases were small compared with those found from cows grazing pasture. Similarly, increases in CLA were also reported by Offer et al. (1999), Donovan et al. (2000), Jones et al. (2000) and Baer et al. (2001) who fed cows menhaden oil. Another study conducted by Abu-Ghazaleh et al. (2001) observed that the gradual replacement of soybean meal with fishmeal as a source of protein increased the beneficial fatty acids CLA, TVA and omega-3 in milk fat. The concentration of CLA, TVA and omega-3 in milk fat from cows fed a TMR diet supplemented with 100% fishmeal on an isonitrogenous basis were 0.72%, 1.54%, and 0.72% respectively compared to 0.39%, 1.09% and 0.54% from cows fed 100% soybean meal.

Dr. Kennelly and his research team at the University of Alberta have designed a feed formulation for dairy cows that resulted in milk cis-9, trans-11 CLA levels approximately 12 times greater than levels typically found in milk fat (0.49 %) (Bell and Kennelly, 2001). Details about the diet are unavailable since they are waiting for the patent on their feed formulation.

Another group of researchers Chouinard et al., (1999), investigated an alternative non-conventional method to feeding supplemented lipid sources as a means of increasing CLA in milk fat. They examined the effect of directly infusing a commercial source of CLA abomasally to bypass rumen fermentation. Their results indicated that all

the major CLA isomers in the supplement were transferred to the milk fat in a dose-dependent manner. However, it caused a dramatic reduction in milk fat content.

The amount of copper in the diet of dairy cows has been shown to influence ruminal biohydrogenation and hence CLA concentration (Palmquist, 2001). Morales et al. (2000) reported that a low copper diet increased milk CLA, whereas a high copper diet had the opposite effect (Engle et al., 2000).

Variations in CLA have also been related to both animal age and breed. A study found that cows with seven or more lactations produced significantly more CLA in their milk than cows with one to six lactations (Werner et al., 1992). Conjugated linoleic acid was higher in milk from French Montbeliardes and lowest in Jerseys (Lawless et al. 1998; Morales et al. 2000). These differences have been suggested to be a result of a lower Δ-9 desaturase activity. The coefficients of variation of CLA in milk fat were found to be 25-35% among cows in a herd and 15% among herds. (Palmquist, 2001).

2.6.2 Processing studies

Ha and his colleagues (1989) originally suggested that processing could contribute to elevating CLA levels in dairy products. They found that processed cheese formulated with whey proteins had greater concentrations of CLA than natural cheese (without whey protein) or milk. Furthermore, they postulated a mechanism for the formation of CLA in processed milk products. According to their proposal, linoleic acid is first converted to a linoleic acid radical then hydrogenated to form a conjugated double bond system. Processing factors that could influence CLA formation by this

mechanism are heating, mixing, churning and air incorporation and other oxidative reactions, which could increase the formation of free radicals and consequent shifts in double bonds. The addition of whey protein concentrate (WPC) may also aid in the formation of conjugated double bonds by acting as hydrogen donors (Ha et al., 1989).

Shantha et al. (1992) reported that high processing temperatures used under atmospheric conditions and the addition of WPC during the preparation of processed cheese increased the formation of CLA. During their study they found that Cheddar cheese further processed at 80°C and 90°C under atmospheric conditions contained significantly higher levels of CLA (4.99 and 5.18 mg CLA/g fat) compared with those found in unprocessed cheese (3.99 mg CLA/g). However, processing cheese under nitrogen at either 70°C or 85°C did not significantly increase CLA levels. These higher CLA levels were attributed to increases in the formation of linoleic and/or oxygen radicals that occur at higher temperatures in the presence of air. The presence of air during processing could cause the formation of oxygen radicals such as hydroxyl radicals that are known to initiate lipid oxidation and cause the formation of lipid free radicals. Increasing the temperature in the presence of air may further increase the formation of linoleic and/or oxygen radicals and hence formation of CLA.

Shantha et al. (1992) also found that the addition of WPC at a concentration normally present in processed cheese increased CLA levels. Increasing the concentration of total WPC or its low molecular weight (LMW) fraction from 0 to 6% increased the CLA content in processed cheese by 35% and 19 % respectively compared to processed cheese with no additives. Conversely, the high molecular weight (HMW)

fraction did not increase the CLA content of processed cheese. It was suggested that the components in WPC and its LMW fraction responsible for promoting CLA formation are lipid oxidation catalysts and hydrogen donors (Colbert and Decker, 1991).

Garcia-Lopez et al. (1994) evaluated the effect of processing by comparing the amount of CLA attributed to the raw ingredients, to that found in cheese at different points during processing. They concluded that CLA levels increased 14.4% on average during processing and that the increase was primarily due to the heating step. They also found that about 86% of the CLA content in cheese was contributed by the raw ingredients. These findings were consistent with those reported earlier by Shantha et al. (1992). Conversely, Werner and his colleagues (1992) reported that different starter cultures, processing conditions, and aging periods had negligible effects on the CLA concentration of Cheddar-type cheeses, which ranged from 5.05 to 5.39 mg CLA/g fat.

Shantha et al. (1995) suggested that results obtained from some earlier processing studies (Ha et al., 1989; Werner et al., 1992) may be subjective since the concentrations of CLA in unprocessed milk was not determined and is known to be highly variable. Results from their study on processing of dairy products indicated that CLA content increased in non-fat yogurt and in butter relative to unprocessed milk. However, no changes in CLA content were found in low fat and regular ice cream, low fat and regular yogurt, regular sour cream, Mozzarella, Gouda, or Cheddar cheeses. Furthermore, storage did not affect CLA levels in any of the products. Similarly, Lin et al. (1999a) reported that processing had a minor influence on CLA formation in Cheddar type cheese.

The same group of researchers, Lin et al. (1998) examined the effect of packaging type, milling pH, additives and aging on the CLA concentration in Cheddar cheese. They found that canned cheese aged six months had significantly higher CLA levels (3.03 mg/g) compared to vacuum pouch packed cheese (2.70 mg/g) of the same age. The higher CLA content in canned cheese was attributed to the greater free space and hence greater residual air in the interior of the can compared to the vacuum pouch. Air would increase lipid oxidation, which could increase the formation of free radicals and CLA. Changing the standard milling pH from 5.7 to either 5.5 or 5.9 resulted in a decreased CLA content in cheese aged 6 months. This suggests that pH 5.7 may be optimal for enzyme activity involved in CLA formation.

Furthermore, these researchers were interested in determining the effects of adding different antioxidants such as butylated hydroxyanisole (BHA), tyrosine and lysine on the CLA content in cheese. These compounds contain functional groups that are able to donate hydrogens to intermediates of free radical oxidation and biohydrogenation. Results from their experiment showed that the addition of BHA, tyrosine or lysine significantly lowered CLA content in cheese. Thus it was concluded that hydrogen donors might have enhanced CLA formation initially. However, further donation of hydrogen during aging of cheese may have caused the reduction of CLA to monoenoic or stearic acid. On the basis of their results, Lin et al. (1998) proposed that free-radical oxidation may be the predominant mechanism for CLA formation prior to aging, while biohydrogenation is the major mechanism during aging.

Other researchers have investigated the capability of commonly used dairy cultures to produce CLA. Jiang et al. (1998) screened 19 different strains of lactobacilli, lactococci, streptococci and propionibacteria for their ability to produce CLA from free linoleic acid in de Man-Rogosa-Sharpe (MRS) broth. Their results showed that only three strains of propionibacteria were capable of converting free linoleic acid to CLA. Of the CLA produced, the majority was found to be extracellular and more than 70% represented the cis-9, trans-11 isomer. More importantly it was also found that these propionibacteria strains were capable of producing CLA from free linoleic acid in skim milk media. Thus, this is indicative of their potential activity in a fermented dairy system.

These results prompted Lin et al (1999b) to investigate the ability of lactic cultures to produce CLA from linoleic acid using skim milk medium. They found that inoculating *Lactobacillus acidophilus* into skim milk supplemented with 1000 μg/ml linoleic acid with a 24-hour incubation period was most effective in promoting CLA formation. It was also observed that further increasing linoleic acid in culture medium and/or longer incubation times did not further enhance CLA levels. Increases in the addition of linoleic acid from 1000 to 5000 μg/ml to growth medium caused significant decreases in CLA formation. The inhibitory effect was attributed to the antimicrobial effect that free linoleic acid exerts on certain microorganisms including lactic acid bacteria. These results were consistent with those reported by Jiang et al. (1998) who observed a similar antimicrobial effect on propionibacteria. No significant differences in CLA were found when cultures were inoculated in skim milk medium without added

linoleic acid, suggesting that adequate linoleic acid substrate must be present for CLA formation by lactic cultures. These results were in agreement with those previously reported by Werner et al. (1992), who found that starter cultures did not influence the CLA concentration of Cheddar-type cheeses. Thus it was concluded that the yield of CLA produced from lactic bacteria was primarily dependent on the linoleic acid concentration and strain of starter culture used.

In a subsequent study, Lin (2000) examined the effect of various sweeteners and sodium chloride on cis-9, trans-11 CLA production in the skim milk medium with added linoleic acid. For this study, six different lactic cultures were used and the sweeteners tested were sucrose, lactose and fructose. The researcher's rational was that most fermented milk products are either sweetened in the West or salted in the Middle East and therefore it would be important to determine how these additives might affect CLA production. Their results indicated that among the six cultures tested, *Lactobacillus acidophilus* in 60 g/L fructose or lactose and 10 g/L sodium chloride-treated skim milk incubated for 24 h under aerobic conditions was the most effective in maintaining CLA levels. They also found that the different reducing powers exerted by lactose and fructose had a negligible effect on the oxidation of linoleic acid to CLA.

Ogawa et al. (2001) described an efficient method for producing CLA from linoleic acid and provided a possible explanation for the metabolic pathway involved. They found that CLA isomers 9c, 11t and 9t, 11c were efficiently produced from linoleic acid by washed cells of *Lactobacillus acidophilus* AKU 1137 under microaerobic conditions. The transformation of linoleic acid to CLA required more than a one step

isomeration. It first involved the production of two hydroxy fatty acids (10-hydroxy-cis 12 octadecaenoic acid and 10-hydroxy-trans-12 octadecaenoic acid). It was noted that the hydroxy octadecaenoic acid accumulated prior to CLA formation and then subsequently decreased with increased formation of CLA.

Other researchers have investigated the effect of both dietary regimen and processing on the CLA levels in milk and milk products. Dhiman et al. (1999) fed dairy cows full fat extruded oilseeds to determine their effect on CLA content in milk and Mozzarella cheese. They found that the CLA content in milk and cheese increased on average by 109% when full fat extruded soybean was fed and 77% when cottonseed was fed compared to cows fed a control diet containing soybean meal. Processing did not affect the CLA content or fatty acid composition of Mozzarella cheese.

Boylston et al. (2000) found that CLA levels in milk and yogurt were increased 2.8 fold, when cows were fed diets supplemented with soy oil and 2.0 fold with CLA supplementation in a free form. They suggested that supplementing soybean oil was more effective in increasing CLA levels in milk compared to free CLA, because it increased the content of substrate (linoleic and linolenic acids) for biohydrogenation reaction, whereas CLA supplementation was more likely to be further hydrogenated in the rumen. They also found that processing milk from cows on soybean oil diets into yogurt and subsequent storage of yogurt for 7 days did not alter the CLA concentration or fatty acid distribution of this product. These results were consistent with those of Dhiman et al. (1999). Baer et al. (2001) found that incorporating fish oil into the diet of dairy cows increased the concentration of CLA and TVA in milk and butter by 3.7 and

4.4 fold respectively, compared to control products. They also reported that the flavour of milk and butter manufactured from milk from cows fed fish oil supplemented diets was comparable to that of control products. However, butter made from milk obtained from cows fed fish oil was softer than the control at both 4 and 20°C.

Numerous studies have indicated the possibility of increasing CLA levels in milk by manipulating dairy cow diets. However, there is still a lack of knowledge regarding effects of processing CLA-enhanced milk into various dairy products. There is little information on the sensory quality and stability of CLA levels in dairy products made from CLA-enhanced milk. To our knowledge no study has investigated the incorporation of probiotic organisms in cultured products such as yogurt or Kefir to determine their effect on promoting CLA formation.

3.0 MATERIALS AND METHODS

Feeding studies were conducted by the Department of Animal Science at the University of Manitoba research farm to produce CLA-enhanced milk for processing studies.

3.1 Experiment I

3.1.1 Cows and dietary treatments

Twelve Holstein cows were divided into three groups. They were randomly assigned to three dietary treatments in a 3 x 3 Latin square design, each period lasting three weeks for a total of nine weeks. The first two weeks of each feeding period were used to allow cows to adjust to the treatment diets and milk collection occurred in the last week of each period. Cows were fed TMR diets containing high levels of fresh forage (forage to concentrate ratio of 60:40) to stimulate CLA production without the confounding effects of pasture management. Dietary treatments were i) a control diet (containing 57.1% grass hay, 30.6% concentrate, 8.9% canola meal and 4% tallow, ii) a diet containing 59.2% fresh grass (cut daily), 30.9% concentrate, 7.8% canola meal and 3.2% tallow (FF) iii) a diet containing 59.5% fresh grass (cut daily) 30.5 % concentrate, plus 10.2% crushed solin seed as a source of supplemental linoleic acid and protein (FF-solin) (refer to appendix 3, Table 1 for dietary treatments).

3.1.2 Milk collection

The milk was collectected in each period for five consecutive milkings. The milk was collected in 35 L stainless steel or plastic cans and kept on ice until

transported to the University of Manitoba dairy pilot plant where it was stored in a walk-in cooler at 5°C until processed. Milk collected during the five milkings and from different cows within a treatment was mixed in a 500 L tank to form a pooled composite of milk.

3.1.3 Pasteurized milk

Pooled milk was heated at 73°C for 27 s using a high temperature short time (HTST) processing system (APV, model HX, serial no. 696-885, Toronto, Ontario, Canada). After pasteurization, 125 kg of each type of milk was collected in clean sanitized cans and set aside for cheese manufacture. The rest of the milk was homogenized at 13,790 kPa, using a dual stage three piston stand-alone homogenizer (Cherry Burnell, series 200, serial No. 254416) cooled to 5°C, placed in 5 L plastic bags and stored at 5°C until tested for sensory, component and fatty acid analysis as described in sections 3.1.8 - 3.2.1.

3.1.4 Yogurt

Milk from different treatments was processed into plain yogurt according to a modified procedure adapted by the University of Manitoba from Kosikowski (1977). Whole milk was heated in a 30 L stainless kettle (Groen, model D-10, Elk Grove, Illinois, USA) at 90°C for five min. and homogenized at 13,790 kPa. Upon cooling to 40°C the milk was inoculated with a yogurt culture (DPL 651, Rhodia, Mississauga, Ontario, Canada). Yogurt culture was prepared by dissolving 2 g of bacterial culture

powder into 99 ml pasteurized milk tempered at 40°C. The culture was added at a rate of 2 ml/L of milk. Following inoculation, yogurt was packaged in 500 ml containers and incubated at 35°C for 7 h (or until yogurt reached a pH of 4.5) then placed at 5°C.

3.1.5 Cheddar cheese

Milk from the three treatments was used to manufacture Cheddar cheese, following a modified procedure adapted by the University of Manitoba from Kosikowski (1977). One hundred and twenty-five liters of milk was added to the cheese vat and heated to 34°C. A freeze-dried culture of *Lactococcus lactis ssp. lactis* and *Lactococcus lactis ssp. cremoris* (MA 011 or MA 014, Rhodia, Mississauga, Ontario Canada) was added at a rate of 4 units / 100 L of milk. The cheese milk was allowed to ripen for 55 min. (or until acidity increased by 0.01 %). A colorant annatto orange, and a coagulant CHY-MAX (CHR Hansen Ltd., Mississauga, Ontario, Canada) were added at a rate of 0.07 ml/L of milk and 0.082 ml/L of milk respectively and allowed to set. After 30 min. the coagulated milk was cut then cooked at 38°C for 30 to 40 min. The whey was drained when the acidity reached 0.15 %. Subsequently, the curd was salted, hooped, and allowed to press overnight at 379.2 kPa in a cheese press (Deval Ltd., model 18 FT, serial 3735, Peterborough, Canada). The next day the cheese was vacuum packaged and stored in at 5°C for aging (refer to appendix 2 for Cheddar cheese manufacturing log 1, 2 and 3).

3.1.6 Queso Blanco cheese

Milk was processed into direct acidified cheese following a modified procedure adapted by the University of Manitoba from Kosikowski (1977). Milk was pasteurized in a 30 L stainless steel kettle at 90°C for five min. before adding citric acid (acidifying agent) at a rate of 2 g/L of milk. The curd was left to settle and agglomerate for 5 min. after coagulation, then hooped and allowed to drain overnight at 5°C. The next day the cheese was vacuum packaged and stored in at 5°C.

3.1.7 Cultured cream

Milk was separated into cream and skim milk using a small centrifugal separator (International Harvest). Cream was pasteurized in a kettle at 85°C for 30 min. Upon cooling to 34°C the cream was inoculated with a freeze-dried culture of *Lactococcus lactis ssp. lactis* and *Lactococcus lactis ssp. cremoris* (MA 014, Rhodia, Mississauga, Ontario, Canada). The culture was added at a rate of 0.38 g / L of milk. Following inoculation, cultured cream was package in 500 ml containers and incubated at 34°C for 16 hours then refrigerated at 5°C.

3.1.8 Product storage and sampling

All products were stored in a walk-in cooler at 5°C. Milk, yogurt, Queso Blanco cheese and cultured cream were analyzed for their CLA content after 1, 7 and 14 days of storage. Cheddar cheese was analyzed at day 1 and after 6 and 9 months of aging. All samples with the exception of Cheddar cheese were obtained by randomly selecting

containers of product. Cheddar cheese samples were obtained by randomly selecting small blocks of cheese that were cut from an 18 kg block of cheese.

3.1.9 Component analysis

Milk samples were sent to the Manitoba Milk Producers Lab (Winnipeg, Manitoba) for component analysis. Fat, protein and solids non-fat (SNF) were analyzed using an infra-red spectroscopy analyzer (Milk-O-Scan, model 303) and somatic cell counts were determined using the Fossomatic 300 (Foss North America Technology, Cornwall, Ont.). The methods used to determine fat and protein in milk were according to International Dairy Federation (IDF) procedures IDF 1c, (1987) determination of fat content and IDF 20B, (1993) determination of nitrogen content. Solids non-fat was determined according to the AOAC 990.19 milk solids (total) in milk procedure. Fat and moisture content of cheese was analyzed using the Babcock method and oven drying method, respectively (AOAC, 1980).

3.1.10 Fatty acid analysis

Lipids of milk and milk products were extracted using a modified method of Bligh and Dyer (1959). Conjugated linoleic acids and fatty acid profiles were determined according to a modified procedure from Kramer et al. (1997). Briefly, lipids from dairy products were extracted using a chloroform and methanol (2:1, vol/vol) mixture. The chloroform layer containing lipids was separated from the aqueous phase through centrifugation and aspiration. The solvent was evaporated under nitrogen at

50°C and the lipid pellet was immediately redissolved in iso-octane. Fatty acids were methylated with a 0.5 N solution of sodium methoxide in an oven at 50°C for 15 min., then neutralized with 10% acetic acid in distilled water before iso-octane extraction. Fatty acid methyl esters were analyzed by gas chromatography equipped with a flame ionization detector (Hewlett-Packard, model 5890) using a SP-2560 fused silica capillary column (100 m x 0.25 mm) and H_2 as the carrier gas. The GC was programmed to operate at 70 °C for 2 min., then temperature was increased at 15°C/min to 155 °C, for 25 min., then increased to 215 °C at 3 °C/min and remained there for 8 min. Peaks were identified and quantified using reference standards (GLC-461, UC-59, NU- Chek-Prep, Inc., Elysian, MN) and an internal standard (methyl heptadecenoate in iso-octane solution) (refer to appendix 4, for examples of the gas chromatograms. Fig. 1. shows a gas chromatogram of CLA standards and Fig. 2. represents a gas chromatogram of control bovine milk fatty acids).

3.1.11 Sensory analysis

Milk, yogurt and Cheddar cheese manufactured from each treatment were evaluated for flavour. Whole milk and plain yogurt manufactured from the three treatments were evaluated using a constant duo-trio difference test (Larmond, 1977). Two sets of three samples per set were presented randomly to each panelist. Each set contained a control labeled as REF (reference) plus two other samples coded with a random three-digit number. The two coded samples included a treated sample (either FF or FF-solin) and the control. The panelists were instructed to taste the reference sample

and then the coded samples and select the coded sample that had a different flavour from the reference. Panelists were encouraged to comment on any differences among samples. They also completed a questionnaire indicating the frequency and type of milk products they normally consumed (refer to appendix 1, for sensory evaluation forms). Milk was evaluated after 4 and 10 days of storage. Yogurt was evaluated after 2 or 3 days of storage and after 27 days. Samples were measured or weighed (40 ml for milk and 30 g for yogurt), placed in plastic cups with lids and tempered to 7°C. The sensory panel consisted of 18-30 untrained panelists who volunteered to evaluate milk and/or yogurt samples.

Cheddar cheese was evaluated by a six member-judging panel with experience in tasting dairy products. The panelists were asked to evaluate and comment on the flavour of cheese made from treated milk. The evaluation of cheese was conducted as a round table type of discussion. Cheese was evaluated after 6 months of aging and was tempered at 21°C for 2 h prior to tasting.

3.1.12 Statistical analysis

All dairy products with the exception of cultured cream were manufactured (replicated) three times with each sample analyzed by GC in duplicate. Cultured cream was replicated twice with each sample analyzed in duplicate. The data was arranged as a factorial experiment with main factors being milk type and processing treatment. The fatty acid data was analyzed as complete randomized designs using the general linear models (GLM) procedure of SAS (1996). Mean differences among milk type and

processing treatments were tested using the Student-Neuman-Keul's multiple range test (Steele and Torrie, 1980) when treatment differences were identified $P< 0.05$. Data from sensory evaluation of milk and yogurt was analyzed using a one-tailed, $p =1/2$ t-test (Roessler et al., 1978).

3.2 Experiment II

For this experiment control and CLA-enhanced milk (produced from cows fed a solin and fishmeal diet) were manufactured into fluid milk, Queso Blanco cheese and cultured cream as previously described in experiment I. In addition, different variations of yogurt (with and without probiotics or WPC), Feta cheese and butter were also manufactured. Component and fatty acid analysis of milk and milk products were performed as described in experiment I.

3.2.1 Cows and dietary treatments

Milk was obtained from six cows fed a CLA promoting diet (supplemented with solin and fishmeal) and from cows from a commercial dairy farm (control). The CLA promoting diet (FM-solin) consisted of alfalfa silage, corn silage and barley grain based concentrate (forage to concentrate ratio of 44:56, DM basis) supplemented with 8.3% crushed solin oilseed (DM basis) and 6.2% fishmeal (DM basis). Since the research herd consistently produced milk containing relatively higher CLA levels than what is reported in the scientific literature as commercial milk levels, the control milk was obtained from a commercial milk producer. The commercial diet was a rolled barley,

hay based lactation ration supplemented with canola distiller pellets (Feed-Rite, Winnipeg, Manitoba) with no added oilseed.

3.2.2 Milk collection

CLA-enhanced milk was collected on the third and fourth week of the feeding trial. Control milk was collected from a commercial milk producer located near the University of Manitoba farm. The collection procedure was as previously described in experiment I.

3.2.3 Yogurt

Six variations of yogurt were processed from both control and CLA-enhanced milk in 5 L batches. The procedure used was as described previously with modifications being the addition of a probiotic culture or whey protein concentrate (WPC). The cultures used were *Lactobacillus acidophilus* (75622), *Lactobacillus casei R-215* (75492) and *Bifidobacterium bifidum* (75617) from Institute Rosell, Montreal, Canada. Whey protein concentrate was obtained from Armstrong Cheese Company Ltd., Winkler, Manitoba and its composition was 35 % protein, < 5 % fat, < 4.5 % moisture and approximately 6 % ash. The types of yogurt manufactured and rates in which the probiotic cultures and WPC were added are shown in Table 3.1.

Table 3.1. Types of yogurts produced using a standard culture, various probiotics and WPC.

Yogurt	Rate for probiotic addition
DPL 651 (Standard yogurt culture)	-
DPL 651 + *Lactobacillus acidophilus* (75622)	0.26 g/ 5 L
DPL 651 + *Lactobacillus casei* (75492)	0.01 g/ 5 L
DPL 651 + *Bifidobacterium bifidum* (75617)	0.26 g/ 5 L
DPL 651 + 6% WPC	-
DPL 651 + 10% WPC	-

3.2.4 Feta cheese

Control and CLA-enhanced milk were processed into Feta cheese following a modified procedure adapted by the University of Manitoba from Kosikowski (1977). One hundred and twenty-five liters of milk was added to the cheese vat and heated to 34°C. A freeze-dried culture of *Lactococcus lactis ssp. lactis* and *Lactococcus lactis ssp. cremoris* (MA 011or MA 014 from Rhodia, Mississauga, Ontario, Canada) was added at a rate of 4 units /100 l. The cheese milk was allowed to ripen for 55 min. (or until acidity increased by 0.01%). A coagulant (liquid rennet 75715MS, Glengarry Cheesemaking & Dairy supplies, Alexandria, Ontario, Canada) was added at a rate of 5 ml / 15 L of milk and allowed to set. After 30 min. the coagulated milk was cut using knives. The whey was drained when the acidity increased 0.01%. Subsequently, curd was hooped, and left to drain overnight at 20°C. The next day cheese was cut, placed in

containers filled with 10% salt brine and stored in a walk-in cooler at 5°C for aging (refer to appendix 2 for Feta cheese manufacturing log 1 and 2).

3.2.5 Butter

Control and CLA-enhanced milk was separated into cream and skim milk as previously described for the manufacture of cultured cream. After pasteurization cream was cooled and stored at 5°C. overnight. The following day, both control and high CLA cream, were re-heated to 11°C and churned in a small laboratory scale electric butter churn (Glengarry Cheesemaking and Dairy Supplies, Model GD2). The butter was then washed with filtered water at approximately 3°C and worked by hand to remove buttermilk.

3.2.6 Product storage and sampling

All products with the exception of Feta cheese were stored and sampled as previously described in experiment I. Feta cheese was analyzed at day 1 and after 4 months of aging at 5°C. Feta cheese samples were obtained by randomly selecting small blocks of cheese that were cut from an 18 kg block of cheese.

3.2.7 Sensory analysis

All products were evaluated by a six member-judging panel with experience in tasting dairy products. The panelists were asked to evaluate and comment on the

flavour of dairy products made from treated milk. The evaluation of dairy products was conducted as a round table type of discussion.

3.2.8 Statistical analysis

All dairy products with the exception of Feta cheese were manufactured (replicated) three times with each sample analyzed by GC in duplicate. Feta cheese was manufactured twice. The fatty acid data was analyzed as previously described in experiment I.

3.3 Experiment III

For this experiment control and CLA-enhanced milk were subjected to different heat treatments and was manufactured into Kefir. Milk collection and component and fatty acid analysis of dairy products were performed as described in experiment I.

3.3.1 Cows and dietary treatments

Milk was obtained from five cows fed a CLA promoting diet (supplemented with solin and fishmeal) and from cows from a commercial dairy producer (control). The CLA promoting diet consisted of alfalfa silage and grass hay based diet supplemented with 8.3 % crushed solin and 6.2 % fishmeal (DM basis). The commercial diet was as decribed in experiment II (section 3.2.1).

3.3.2 Heat treatment of milk

Control and CLA-enhanced milk were each subjected to three different heat treatments; 72°C for 16 seconds, 90°C for five min. and 121°C for 2 seconds. The first two treatments were conducted in an oil bath (Blue M Electric Company, model mw-1145a-1, serial no. OBBS-2500, Illinois, USA) and the third treatment was conducted in an autoclave (Amsco Eagle 3000 series, Erie, Pennsylvania, USA). Sample preparation and heat treatment were as follows: 10 ml of each milk were pipetted into test tubes, placed in a rack in the oil bath or autoclave and once samples reached the desired temperature, they were held for the appropriate times. Immediately after heating the tubes were cooled in an ice bath for approximately 5 min.

3.3.3 Kefir

Two variations of Kefir were processed from both control and CLA-enhanced milk, in 3 L batches. Kefir was produced with and without the addition of *Lactobacillus acidphilus*. The process used was according to a modified procedure adapted by the University of Manitoba from Kosikowski (1977). Milk was pasteurized in a kettle at 90°C for five min. and upon cooling to 22°C the milk was inoculated with Kefir starter culture 485 (Glengarry cheesemaking and dairy supplies, RR#2 Alexandria, Ontario K0C 1A0). The Kefir starter culture was added at a rate of 5 g/L of milk. The probiotic culture, *Lactobacillus acidophilus* (75622) from Institute Rosell, Montreal, Canada, was added at a rate of 0.086g/L. Following inoculation, Kefir was packaged in 500 ml

containers and incubated at 22°C (room temperature) for 16 hours and then placed at 5°C.

3.3.4 Product storage and sampling

Milk was stored and sampled as previously described in experiment I. Kefir was analyzed for its CLA content after 1, 7 and 14 days of storage at 5°C.

3.3.5 Sensory analysis

Kefir was evaluated by a six member-judging panel with experience in tasting dairy products. The panelists were asked to evaluate and comment on the flavour of Kefir made from both control and CLA-enhanced milk. The evaluation of dairy products was conducted as a round table type of discussion.

3.3.6 Statistical analysis

Each milk heat treatment was performed in triplicate. Kefir was manufactured (replicated) three times with each sample analyzed by GC in duplicate. The fatty acid data was analyzed as previously described in experiment I.

4.0 RESULTS AND DISCUSSION

The CLA values reported in this study are expressed as a percentage of total fatty acids and represent a mixture of cis-9, trans-11 and trans-7, cis-9 CLA isomers. Based on current literature (Corl et al., 2002) it is believed that all CLA isomers have the potential of being biologically active and it is estimated that the cis-9, trans-11 and trans-7, cis-9 isomers account for greater than 90% of total CLA in milk fat. To obtain separation of these two CLA isomers additional analysis using silver ion HPLC (Ag+-HPLC) is required.

4.1 Experiment I

The objectives of this experiment were; 1) to determine the effect of feeding fresh forage and fresh forage and solin on the CLA levels in milk; 2) to determine the effect of processing and storage on the CLA levels of dairy products manufactured from both control and CLA enhanced milk; 3) to evaluate and compare the flavour of dairy products made from control and CLA enhanced milk.

Baseline data for the CLA content found in various commercially available dairy products is given in Table 4.1. These products were obtained from a number of local supermarkets in Manitoba. The CLA content based on percent total fatty acids was similar among all products analyzed, and ranged from 0.59 % in butter to 0.75 % in 2% milk with *L. acidophilus*. Butter with the higher fat content would contain the highest total amount of CLA.

In earlier studies, researchers reported that some dairy products including yogurt, Cheddar cheese and processed cheeses contained higher levels of CLA than milk (Ha et al., 1989; Shantha et al., 1992; Shantha et al., 1995). They believed that the elevated CLA levels in these products were a result of certain processing treatments such as heating. However, more recent studies indicate that conventional processing methods used for the manufacture of dairy products have no effect on CLA levels (Lin et al., 1999a; Dhiman et al., 1999; Boylston et al., 2001). Our data for commercial dairy product (Table 4.1) is in agreement with results from the latter studies that have found no differences in CLA levels among products.

The discrepancy between the earlier and more recent findings could be attributed to the methods used for the analysis of CLA and also to the fact that many reported studies did not report controls (starting CLA levels in raw milk). Since these early studies, the development of methods for the analysis of CLA has been subject to much research. Researchers are continuously optimizing methods used for the analyses of CLA, which is enabling better separation and quantification of CLA isomers.

The composition of milk from cows fed control, FF and FF-solin diets is shown in Table 4.2. The fat, protein and SNF content of FF-solin milk were comparable to the levels found in control milk and to levels typically found in bovine milk (refer to Table 2.1). The fat content in FF milk (3.26 %) was however lower than that of the control (3.61 %) and FF-solin milk (3.66 %). It has been well documented that cow diets can change the pathway of rumen biohydrogenation and ultimately the composition and content of milk fat (Ashes et al., 1997). Thus, feeding cows a diet supplemented with

fresh forage may have promoted the formation of fatty acid intermediates such as t 10 C18:1 and t 10, cis 12 CLA known to cause milk fat depression (Chouinard, et al., 2001; Baumgard et al., 2000).

The fatty acid composition of milk from cows fed control, FF, and FF-solin diets are shown in Table 4.3. In general, supplementing dairy cow diets with either FF or FF-solin decreased the proportion of short to medium chain fatty acids (C4:0 – C16:0) by 1.4 to 9.2 % and increased the proportion of long chain fatty acids (C18:0 – C18:2) by 1.3 to 9.9 % in milk compared to the control diet. Similar trends have been reported by other researchers who have previously investigated the effect of supplementing fat to dairy cow diets (Grummer, 1991; Dhiman et al., 2000). High levels of long chain fatty acids including stearic, linoleic, and linolenic acids (C18:0, C18:2, C18:3) have been shown to increase secretion in milk with inhibited de novo synthesis of short and medium chain fatty acids (Grummer, 1991; Dhiman et al., 2000).

Milk from cows fed FF-solin diet had significantly lower short chain fatty acids (C4:0 – C8:0) (4.89 %) and medium chain fatty acids (C14:0 – C16:0) (25.78 %) and significantly higher long chain fatty acids (C18:0 – C18:2) (61.14 %) compared to control milk (5.46 %, 34.33 % and 51.27 % respectively). A similar trend was observed when comparing the fatty acid composition of FF milk to control milk however, for most fatty acids the differences were not significant (Table 4.3). In addition to increases in long chain fatty acids there was also an increase in the level of total unsaturated fatty acids. The FF-solin milk contained the highest content of unsaturated fatty acids (44.68 % of total fatty acids) followed by FF milk (41.80 %) and control (40.02 %). The level

of unsaturated fat in the FF-solin milk was about 10 to 15 % greater than levels normally found in milk (Grummer, 1991; Baer, 1991). In terms of human health, dairy products with a higher unsaturated fat content would be more desirable as unsaturated fats are associated with lowering serum cholesterol and preventing coronary heart disease.

The omega-3 linolenic (C18:3) fatty acid content in milk from the three dietary treatments was comparable. The CLA content of milk from cows fed the FF-solin diet was significantly higher than FF and control diets (Table 4.3). The average CLA content in FF-solin milk was 1.25%, followed by 0.99% in FF milk and 0.84% in control milk. The higher levels of CLA in milk from cows fed the FF-solin and FF diets compared to control diets were as expected. It has been repeatedly shown that cows grazing pasture or fed diets supplemented with oilseed have increased CLA content in their milk (Kelly and Bauman, 1996; Lawless et al., 1998; Dhiman et al., 1999, 2000; Stockdale et al., 2000; Walker et al., 2001). Solin is a cultivar of flaxseed that contains high levels of linoleic acid (C18:2) (White et al., 1999), which can serve as a substrate for ruminal biohydrogenation and hence increase formation of CLA. Fresh forage is known to contain high levels of linolenic acid (C18:3), which can also serve as a substrate for the microbial biohydrogenation (Parodi, 1999). However, CLA is not an intermediate formed in its biohydrogenation pathway, thus it is believed that linolenic acid increases the endogenous synthesis of CLA by increasing levels of its ruminal precursor TVA (Palmquist, 2001).

The CLA content of the FF-solin milk (1.25 %) was 2 times greater than that found in the commercial milk (Table 4.1) and 2.3 times more than the average value

reported for milk in a survey conducted by Chin et al. (1992) (Table 2.4). Meanwhile, Dr. Kennelly and his team at the University of Alberta using a patented feed formulation claim CLA values 10 to 12 times greater than concentrations normally found in bovine milk (Bell and Kennelly, 2001). In addition, the FF-solin milk contained significantly higher levels of TVA (C18:1 11t) (2.73 %) as compared to the FF (2.11 %) and control milk (2.03 %)(Table 4.3). This could also be beneficial for human health, since some studies (Salminen et al., 1998; Adlof et al., 2000) have found that ingestion of TVA increases CLA levels in human serum.

These results show the potential of producing high CLA milk by supplementing fresh forage and solin to dairy cow diets. Additional manipulation of cow diets could possibly lead to further increases in CLA levels in milk.

Table 4.1. Conjugated linoleic acid levels in various commercial dairy products purchased from supermarkets in Manitoba.

Product	CLA (% of total fatty acids)*	CLA (mg/g fat)
Homogenized milk	0.60	6.0
2% milk	0.66	6.6
2% ultra high temperature milk	0.62	6.2
2% milk with *L. acidophilus*	0.75	7.5
Plain yogurt	0.70	7.0
Half & half light cream (10% MF)	0.70	7.0
Sour cream	0.71	7.1
Butter	0.59	5.9
Cheddar cheese (mild)	0.70	7.0

*Total fatty acids = C4:0 - C20:0

Table 4.2. Composition of milk from cows fed the control, FF and FF-solin diets.

	Dietary treatment*		
Item	Control	FF	FF-solin
Fat, %	3.61	3.26	3.66
Protein, %	3.31	3.26	3.29
Solids non fat, %	8.61	8.48	8.53

*Means are of pooled milk from 15 milking times

Table 4.3. Effect of supplementing FF or FF-solin to dairy cow diets on the fatty acid profile of milk.

Fatty acids	Dietary treatment			SEM
	Control	FF	FF-solin	
	% of total fatty acids*			
C4:0	2.17[a]	2.16[a]	1.93[b]	0.05
C6:0	1.99[a]	1.95[a]	1.78[b]	0.04
C8:0	1.30[a]	1.27[ab]	1.18[b]	0.03
C10:0	1.64	1.62	1.54	0.05
C12:0	1.72	1.59	1.55	0.05
C14:0	7.48[a]	6.87[b]	6.10[c]	0.20
C14:1	0.81[a]	0.79[a]	0.52[b]	0.03
C16:0	26.04[a]	25.38[a]	19.16[b]	0.28
C16:1	1.83[a]	1.93[a]	1.28[b]	0.07
C18:0	15.14[b]	14.85[b]	19.86[a]	0.25
C18:1 11t	2.03[b]	2.11[b]	2.73[a]	0.16
C18:1 9c	31.65[c]	32.94[b]	34.79[a]	0.40
C18:1 11c	0.93[c]	0.96[ab]	0.99[a]	0.02
C18:2	1.52[b]	1.71[b]	2.77[a]	0.07
C18:3	0.41	0.37	0.35	0.02
C20:0	0.33[a]	0.32[a]	0.26[b]	0.01
CLA	0.84[c]	0.99[b]	1.25[c]	0.04
Unsaturated FA	40.02	41.80	44.68	

*Total fatty acids = C4:0 - C20:0, % total fatty acids x 10 = mg/g fat
[a,b,c]Means within a row without common superscripts differ ($P < 0.05$)
SEM = standard error of the mean, n = 12

Processing effects such as temperature, storage and recipe of manufacture for products such as yogurt, cultured cream, Cheddar cheese and Queso Blanco cheese were evaluated for effect on CLA levels in final products. The interaction of milk type (diet treatment), processing treatment and storage treatment was not significant for the fatty acid content of dairy products. For this reason results were reported (Table 4.4) as mean values for fatty acids from processing treatment and storage time. Pasteurization is a common feature in dairy processing. In this experiment, pasteurization of milk had no significant effect on the CLA content of milk. These results are in accordance with those of Ha et al. (1989) who previously found no differences in the CLA content of milk after pasteurization. Processing milk into various fermented and non-fermented dairy products including yogurt, cultured cream, Cheddar cheese and Queso Blanco cheese and the subsequent storage of these products also had no significant effect on the CLA content (Table 4.4). Other researchers have reported similar results when processing Mozzarella cheese (Dhiman et al., 1999) or yogurt (Boylson et al., 2001) from milk with high CLA starting levels. Such results indicate that CLA is stable upon conventional processing and storage regardless of its level present in the starting milk. In terms of human health, this is very beneficial because it indicates the potential for manufacturing high CLA dairy products from CLA-enhanced starting milk.

Interestingly, a significant increase in oleic acid (C18:1 9c) (ranging between 1.36- 2.08 %) and more importantly TVA (18:1 11t) (a CLA precursor) (ranging between 0.68- 1.24 %) were found upon processing milk into the various products

(Table 4.4). To our knowledge no one else has reported such findings. However, this may be another avenue for increasing CLA in dairy products.

Table 4.4. Effect of processing and storage on CLA and select fatty acid acids in dairy products.

	Processing		Storage		
Fatty acids	Raw	Day 1	Day 7	Day 14	SEM
	% of total fatty acids*				
Milk					
C18:0	16.51	16.45	16.89	16.62	0.29
C18:1 11t	2.06^{ab}	1.69^{b}	2.69^{a}	2.71^{a}	0.18
C18:1 9c	32.95	32.99	33.14	33.43	0.46
C18:2	1.97	1.99	2.05	2.00	0.08
C 18:3	0.36	0.39	0.38	0.37	0.02
CLA	1.05	0.98	1.04	1.04	0.05
Yogurt					
C18:0	16.51	17.47	17.47	17.30	0.31
C18:1 11t	2.06^{b}	3.30^{a}	3.23^{a}	3.12^{a}	0.19
C18:1 9c	32.95^{b}	34.65^{a}	34.67^{a}	34.39^{a}	0.37
C18:2	1.97	2.06	2.09	2.10	0.08
C 18:3	0.36	0.43	0.43	0.42	0.02
CLA	1.05	1.15	1.15	1.14	0.06
Cultured cream					
C18:0	16.63	17.65	17.61	17.04	0.33
C18:1 11t	2.10^{b}	2.78^{ab}	2.95^{ab}	3.50^{a}	0.24
C18:1 9c	33.23^{b}	35.31^{a}	35.29^{a}	34.92^{ab}	0.42
C18:2	1.93	2.00	2.02	1.95	0.09
C 18:3	0.36	0.43	0.42	0.40	0.02
CLA	1.01	1.18	1.17	1.17	0.08
Queso Blanco cheese					
C18:0	16.51	17.28	17.41	17.44	0.31
C18:1 11t	2.06^{b}	3.02^{a}	3.18^{a}	3.12^{a}	0.21
C18:1 9c	32.95^{b}	34.31^{a}	34.61^{a}	34.65^{a}	0.37
C18:2	1.97	2.05	2.08	2.06	0.07
C 18:3	0.36^{b}	0.45^{a}	0.43^{a}	0.46^{a}	0.02
CLA	1.05	1.14	1.15	1.14	0.06
Cheddar cheese			3 months	9 months	
C18:0	16.51	17.13	17.92	18.17	0.45
C18:1 11t	2.06^{b}	3.08^{a}	3.12^{a}	2.36^{b}	0.19
C18:1 9c	32.95^{b}	34.39^{a}	34.80^{a}	35.31^{a}	0.42
C18:2	1.97	2.04	2.15	2.21	0.11
C 18:3	0.36^{b}	0.42^{ab}	0.42^{ab}	0.47^{a}	0.02
CLA	1.05	1.10	1.07	1.15	0.05

*Total fatty acids = C4:0 - C20:0, % total fatty acids x 10 = mg/g fat

^{a,b,c}Means within a row without common superscripts differ ($P < 0.05$)

SEM = standard error of the mean, n = 9

Although our results demonstrated that CLA-enhanced milk could be processed and stored into various products without compromising its levels, we encountered some problems during cheese manufacturing. Cheddar cheese manufactured from the FF-solin milk oiled off during the pressing stage, leaving the cheese with a very greasy surface texture. This effect is undesirable for both economical and quality reasons. Economically, higher percent fat losses due to oiling off would consequently cause the processor to have lower cheese yields. In terms of quality, consumers may find oily cheese less appealing and may perceive it as being less healthy. This oiling off may be attributed to the higher levels of unsaturated fatty acids present in the FF-solin milk (45 %) compare with the control (40 %) (Table 4.3). Unsaturated fatty acids have low melting points and tend to be liquid at room temperature (21°C) (Hillbrick and Augustin, 2002). There were also some difficulties when processing the FF-solin milk into Queso Blanco cheese. After high heat treatment and acidification of the FF-solin milk, fine curd particles were formed as compared to control curd. In addition, the FF-solin curd particles did not mat together to produce a larger mass thus it was difficult to hoop. The FF-solin diet could have altered the protein composition of milk, which would have an impact on the protein-fat complex formation during cheesemaking, which impacts the curd formation.

The fat and moisture content of the Cheddar cheese manufactured from FF, FF-solin and control milk were marginally different from the standard requirements for this product (Table 4.5). Current Federal regulations state that cheese must contain a minimum of 32 % fat with no more than 39 % moisture to be marketed as Cheddar

cheese (Health Canada Food and Drug Act, 2000). Cheese manufactured from fresh forage (FF), FF-solin and control milk in this study contained 32 %, 30 % and 31 % fat respectively with moisture contents slightly above 39 %. The moisture content of the FF and control cheese could be changed by modifying the recipe of manufacture. The low fat content in the FF-solin cheese was probably a result of fat losses due to oiling off. However, this speculation cannot be confirmed since cheese yield was not measured in this experiment. Additional research in cheese manufacturing practices would be required to correct this problem.

Feed flavour is a known defect in milk that is commonly used as a criteria for judging the quality of dairy products, and results from feed flavours being transferred to the milk. It was of interest to determine whether feed flavour from the solin diet would be transferred into milk. Results from the duo-trio sensory testing for milk and yogurt are shown in Tables 4.6 and 4.7. Panelists did not detect significant flavour differences between milk produced from cows fed FF and control diets after either 4 or 10 days storage. Similarly, they did not detect significant differences between stored yogurts made from FF milk and control milk with the exception of yogurt stored for 2 days in period 3. However, the results from subsequent testing of the yogurt at 27 days storage showed no significant differences (Table 4.6).

The sensory results for milk and yogurt produced from cows fed FF-solin diets were inconsistent (Table 4.7). Panelists detected significant differences between FF-solin milk and control stored for 4 days in period 1 and after 10 days in period 2. The panelists also found a significant difference between FF-solin yogurt and control yogurt

stored for 27 days in period 2. However, the comments noted by panelist who detected differences among milk samples did not indicate evidence of a flavour defect. Those who found differences between the yogurt samples attributed these differences to texture.

For Cheddar cheese the general consensus amongst the expert panelist group was that there was no difference in flavour between control and FF or FF-solin cheeses. These results are in accordance with those previously reported by Lightfield et al. (1993), who found that Cheddar cheese manufactured with milk from cows fed extruded soybean or sunflower had similar flavour to that made from control milk.

Table 4.5. Composition of Cheddar cheese manufactured from control, FF and FF-solin milk.

	Cheddar cheese			
	Control	FF	FF-solin	Standard*
Fat %	31.0	32.0	30.0	≥ 32
Moisture %	40.1	40.6	41.4	≤ 39

*Values for standard of identity for Cheddar cheese were obtained from Health Canada Food and Drug Act (2000).

Table 4.6. Duo-trio sensory testing results for milk and yogurt from cows fed FF and control diets.

Period	Product	Storage time (days)	Number of panelists	Number of correct responses	Probability[a]
1	Milk	4	31	18	0.237
	Milk	10	-	-	-
	Yogurt	2	31	8	0.998
	Yogurt	27	-	-	-
2	Milk	4	24	15	0.154
	Milk	10	24	14	0.271
	Yogurt	2	28	18	0.092
	Yogurt	27	20	8	0.968
3	Milk	4	23	15	0.150
	Milk	10	19	8	0.820
	Yogurt	2	25	21	<0.002
	Yogurt	27	18	10	0.407

[a] $P > 0.05$ considered not significant

Table 4.7. Duo-trio sensory testing results for milk and yogurt from cows fed FF-solin and control diets.

Period	Product	Storage time (days)	Number of panelists	Number of correct responses	Probability[a]
1	Milk	4	31	27	<0.002
	Milk	10	-	-	-
	Yogurt	2	31	17	0.360
	Yogurt	27	-	-	-
2	Milk	4	24	15	0.154
	Milk	10	24	17	0.031
	Yogurt	2	28	13	0.714
	Yogurt	27	20	15	0.021
3	Milk	4	23	13	0.339
	Milk	10	19	12	0.180
	Yogurt	2	25	16	0.115
	Yogurt	27	18	11	0.240

[a] $P > 0.05$ considered not significant

4.2. Experiment II

For experiment II the objectives were: 1) to determine the effect of feeding a fishmeal solin supplemented diet to dairy cows on the CLA levels of milk; 2) to determine the effect of processing and storage on the CLA levels of dairy products manufactured from FM-solin and control milk; 3) to determine the effect of probiotic cultures and whey protein concentrate on the CLA levels in yogurt; 4) to evaluate and compare the flavour of dairy products made from control and CLA enhanced milk.

The composition of milk from cows fed control and FM-solin are shown in Table 4.8. The protein and SNF content were similar between the two types of milk. The fat content of FM-solin milk (2.51 %) was notably lower than that of the control (3.71 %). Similar results have been reported by others, who have fed cows fishmeal or fish oil supplemented diets as a source of PUFA (Baer et al., 2001; Abu-Ghazales et al., 2001; Cruz et al., 2002).

The CLA-enhanced 2.5 % fat milk produced in this experiment may be nutritionally more attractive to health conscious consumers but limiting for producers and some processors. The value of milk is currently determined by a multiple component pricing payment system. Canadian milk producers are paid on the basis of butterfat, protein as well as other solids (lactose and minerals) in milk and how components are used by processors (Therese Beaulieu, Dairy Farmers of Canada, Personal communication). Therefore lower fat levels in milk could lead to reduced revenues for dairy producers unless they are compensated for the higher CLA levels. For processors, there may be a limitation on the type of products they could manufacture

from 2.5 % milk. Certain cheese such as Cheddar cannot be manufactured from low fat milk without compromising its flavour and textural characteristics (Drake et al., 1995).

Milk fatty acid profiles from cows fed control and FM-solin diets are shown in Table 4.9. Overall, milk from cows fed a FM-solin diet contained significantly lower levels of short (3 %) and medium chain fatty acids (34.82 %) and higher levels of long chain fatty acids (54.71 %) compared to control milk (7.97, 53.48, 33 % respectively). These results can be explained as described in experiment 1. Supplementing fat high in long chain PUFA increases their secretion in milk and inhibits the synthesis of de novo short and medium chain fatty acids. Along with increases in long chain fatty acids there was an increase in the level of unsaturated fatty acids. The FM-solin milk contained nearly twice the amount of unsaturated fat (46.42 % of total fatty acids) compared to the control (25.69 %) (Table 4.9).

The TVC (C18:1 11t) level was 4.5 times greater in the FM-solin milk than the control. As mentioned in experiment 1, this may prove to be beneficial for increasing availability of CLA to humans. The omega-3 linolenic fatty acid (C18:3) content was significantly lower in the FM-solin milk (0.48 %) compared to the control (0.84 %) which might be expected as it's a minor component in both fishmeal and solin (Ackman, R. G., 2000; White et al., 1998).

Fishmeal or fish oils are known for their high concentrations of long chain omega-3 fatty acids DHA and EPA (Ackman, 2000) although in this study they were not quantified in milk since our focus was on CLA However, it would be interesting to

measure their levels in future studies that incorporate fishmeal to dairy cow diets as they also have many health promoting benefits.

The FM-solin milk had a significantly higher level of CLA (2.28 %) compared to the control milk (0.40 %). It contained 3.8 times more CLA than that of locally purchased commercial milk (Table 4.1.) and 4.1 times more than levels reported in the literature (Table 2.4). In addition, the CLA levels in the FM-solin milk were 1.8 times more than the levels present in the FF-solin milk in experiment 1 (Table 4.3).

Table 4.8. Composition of milk from cows fed control and FM-solin diets.

	Diet*	
Item	Control	FM-solin
Fat, %	3.71	2.51
Protein, %	3.17	3.25
Solids non fat, %	8.59	8.45

*Means are of pooled milk from eight milking times

Table 4.9. Effect of supplementing FM-solin to dairy cow diets on the fatty acid profile of milk.

	Diet		
Fatty Acid	Control	FM-solin	SEM
	% of total fatty acids*		
C4:0	1.98[a]	1.14[b]	0.04
C6:0	2.55[a]	1.11[b]	0.04
C8:0	2.11[a]	0.75[b]	0.03
C10:0	3.31[a]	1.03[b]	0.03
C12:0	3.52[a]	1.33[b]	0.02
C14:0	11.33[a]	6.32[b]	0.05
C14:1	0.84[b]	0.96[a]	0.01
C16:0	35.32[a]	25.18[b]	0.10
C16:1	1.38[b]	2.06[a]	0.01
C18:0	10.77[b]	14.07[a]	0.03
C18:1 11t	0.87[b]	3.92[a]	0.03
C18:1 9c	18.20[b]	32.08[a]	0.09
C18:1 11c	0.72[b]	1.45[a]	0.01
C18:2	2.44[b]	3.19[a]	0.03
C18:3	0.84[a]	0.48[b]	0.01
C20:0	0.28[b]	0.33[a]	0.01
CLA	0.40[b]	2.28[a]	0.01
Unsatured FA	25.69	46.42	

*Total fatty acids = C4:0 - C20:0, % total fatty acids x 10 = mg/g fat
[a,b,]Means within a row without common superscripts differ ($P < 0.05$)
SEM = standard error of the mean, n = 12

Similar to experiment I there were no significant interactions between milk type (diet treatment), processing treatment and storage treatment for the fatty acid content of dairy products. Thus, results were reported (Table 4.10 - 4.14) as mean values for fatty acids from processing treatment and storage time. As in experiment 1, pasteurization of milk had no significant effect on the CLA content of milk (Table 4.10). In addition, processing milk into cultured cream, Queso Blanco cheese and Feta cheese had no significant effect on CLA levels (Table 4.10), nor did storage of these products. Processing milk (cream) into butter significantly decreased the CLA content (1.28 %) as compared to the starting milk (1.34 %) however storage had no further effect (Table 4.10). The decrease of CLA content in butter could have been a result of churning. During the churning process, air is normally introduced into the cream and this may cause oxidation of CLA. Another possible explanation for the lower CLA level in butter is that churning could have destabilized the fat globular membrane, which resulted in a loss of unsaturated fat in the buttermilk.

Table 4.10. Effect of processing and storage on CLA and select fatty acid acids in dairy products.

	Processing		Storage		
Fatty acids	Raw	Day 1	Day 7	Day 14	SEM
	% of total fatty acids*				
Milk					
C18:0	12.38	12.42	12.46	12.43	0.048
C18:1 11t	2.41	2.38	2.42	2.36	0.041
C18:1 9c	25.10	25.09	25.21	25.16	0.122
C18:2	2.81	2.78	2.80	2.86	0.038
C 18:3	0.66	0.67	0.66	0.64	0.012
CLA	1.34	1.35	1.36	1.31	0.015
Butter					
C18:0	12.38^{a}	12.15^{b}	12.18^{b}	12.04^{b}	0.05
C18:1 11t	2.41	2.16	2.15	2.05	0.10
C18:1 9c	25.10^{a}	24.79^{ab}	24.81^{ab}	24.52^{b}	0.09
C18:2	2.81^{b}	2.99^{a}	3.00^{a}	2.98^{a}	0.01
C 18:3	0.66^{a}	0.63^{b}	0.62^{b}	0.62^{b}	0.004
CLA	1.34^{a}	1.28^{b}	1.31^{ab}	1.27^{b}	0.01
Cultured cream					
C18:0	12.38^{a}	12.43^{a}	-	12.12^{b}	0.06
C18:1 11t	2.41^{a}	2.39^{a}	-	2.00^{b}	0.04
C18:1 9c	25.10^{a}	25.04^{a}	-	24.71^{b}	0.10
C18:2	2.81^{b}	2.82^{b}	-	3.00^{a}	0.01
C 18:3	0.66^{a}	0.64^{b}	-	0.62^{c}	0.01
CLA	1.34	1.33	-	1.28	0.02
Queso Blanco cheese					
C18:0	12.38^{a}	12.22^{b}	12.28^{b}	12.23^{b}	0.03
C18:1 11t	2.41^{a}	2.27^{b}	2.28^{b}	2.27^{b}	0.02
C18:1 9c	25.10	25.01	25.08	25.94	0.06
C18:2	2.81^{b}	2.92^{ab}	3.08^{a}	2.91^{ab}	0.05
C 18:3	0.66	0.64	0.64	0.64	0.01
CLA	1.34	1.33	1.31	1.21	0.03
Feta cheese			4 months		
C18:0	12.36^{b}	12.69^{a}	12.7^{b}		0.09
C18:1 11t	2.39	2.43	2.38		0.05
C18:1 9c	25.09^{a}	25.56^{a}	24.32^{b}		0.18
C18:2	2.82	2.76	2.80		0.08
C 18:3	0.66^{a}	0.66^{a}	0.62^{b}		0.01
CLA	1.34	1.33	1.39		0.02

*Total fatty acids = C4:0 - C20:0, % total fatty acids x 10 = mg/g fat

^{a,b,c}Means within a row without common superscripts differ ($P < 0.05$)

SEM = standard error of the mean, n = 6

Certain strains of commonly used dairy starter cultures and probiotic bacteria have been reported to promote the formation of CLA in linoleic acid enriched skim milk medium (Jiang et al., 1998; Lin et al., 1999b). The FM-solin milk in this study provided a medium with a natural supply of linoleic acid that could be used as a substrate by probiotic cultures to form CLA. The ability of commercial freezed dried *L. acidophilus* and other probiotic cultures to form CLA when added into a yogurt system was examined.

Processing milk into yogurt with probiotics or without the addition of probiotic cultures had no significant effect on CLA levels (Table 4.11). There are a number of possible explanations for these results. It could be that the conditions of the yogurt system such as pH, redox potential and/or time and temperature of incubation were inadequate for cultures to form CLA. Another possible reason is that these cultures were unable to convert esterified linoleic acid to CLA. Previous researchers (Jiang et al., 1998; Lin et al., 1999b), who have found *L. acidophilus* capable of forming CLA provided free linoleic acid as a substrate for the micoorganisms in their experiments. The linoleic acid present in milk fat is esterified to glycerol in a triacylglycerol molecule. Furthermore, it is likely that not all strains of *L. acidophilus* contain enzymes capable of isomerizing linoleic acid to CLA. In this study, commercial freeze dried probiotic cultures used in the dairy industry were tested for their ability to form CLA and as a result the specific bacterial strains added to yogurt were unknown. Given the potential physiological health benefits that a yogurt containing both high CLA and probiotic cultures could offer to humans, further research in this area is warranted.

Table 4.11. Effect of adding different probiotic cultures on CLA and select fatty acids in yogurt.

		Yogurt treatments				
Fatty Acid	Milk	Yogurt	L. acidophilus	B. bifidum	L. casei	SEM
	% of total fatty acids*					
C18:0	12.38	12.25	12.36	12.20	12.13	0.08
C18:1 11t	2.41^{a}	2.29^{ab}	2.25^{ab}	2.21^{b}	2.22^{b}	0.04
C18:1 9c	25.10	24.88	24.75	24.90	24.44	0.20
C18:2	2.81^{b}	2.89^{ab}	3.15^{a}	2.86^{ab}	2.86^{ab}	0.07
C18:3	0.66^{a}	0.66^{ab}	0.65^{ab}	0.65^{ab}	0.62^{b}	0.01
CLA	1.34	1.31	1.31	1.26	1.30	0.02

*Total fatty acids = C4:0 - C20:0, % total fatty acids x 10 = mg/g fat
^{a,b,}Means within a row without common superscripts differ ($P < 0.05$)
SEM = standard error of the mean, n = 6

Table 4.12. Effect of adding WPC on CLA and select fatty acids in yogurt.

		Yogurt treatments			
Fatty Acid	milk	Yogurt	6%WPC	10%WPC	SEM
	% of total fatty acids*				
C18:0	12.38^{ab}	12.25^{abc}	11.98^{cd}	11.82^{d}	0.08
C18:1 11t	2.41^{a}	2.29^{ab}	2.16^{b}	2.18^{b}	0.04
C18:1 9c	25.10^{ab}	24.88^{ab}	24.44^{b}	24.64^{ab}	0.20
C18:2	2.81^{d}	2.89^{cd}	3.67^{b}	4.06^{a}	0.07
C18:3	0.66^{c}	0.66^{c}	0.81^{b}	0.90^{a}	0.01
CLA	1.34^{a}	1.31^{ab}	1.22^{b}	1.22^{b}	0.02

*Total fatty acids = C4:0 - C20:0, % total fatty acids x 10 = mg/g fat
^{a,b,c}Means within a row without common superscripts differ ($P < 0.05$)
SEM = standard error of the mean, n = 6

Table 4.13. Effect of storage on CLA and select fatty acids of yogurt manufactured with probiotic cultures.

Fatty acids	Storage			SEM
	Day 1	Day 7	Day 14	
	% of total fatty acids*			
Yogurt				
C18:0	12.25	12.36	12.18	0.08
C18:1 11t	2.29	2.24	2.25	0.04
C18:1 9c	24.88	24.75	24.82	0.20
C18:2	2.89	2.83	2.91	0.07
C 18:3	0.66	0.65	0.63	0.01
CLA	1.31	1.29	1.28	0.02
L. acidphilus				
C18:0	12.36[a]	12.51[a]	11.90[b]	0.08
C18:1 11t	2.25[ab]	2.38[a]	2.13[b]	0.04
C18:1 9c	24.75[ab]	25.38[a]	24.19[b]	0.20
C18:2	3.15	2.91	2.96	0.07
C 18:3	0.65	0.64	0.62	0.01
CLA	1.31[ab]	1.33[a]	1.21[b]	0.02
B. bifidum				
C18:0	12.20	12.25	12.10	0.08
C18:1 11t	2.21	2.30	2.27	0.04
C18:1 9c	24.90	24.82	24.75	0.20
C18:2	2.86	2.89	3.06	0.07
C 18:3	0.65	0.64	0.63	0.01
CLA	1.26	1.28	1.26	0.02
L. casei				
C18:0	12.13	12.26	12.05	0.08
C18:1 11t	2.22	2.34	2.29	0.04
C18:1 9c	24.44	25.23	24.71	0.20
C18:2	2.86	2.87	2.83	0.07
C 18:3	0.62	0.64	0.62	0.01
CLA	1.30	1.32	1.27	0.02

*Total fatty acids = C4:0 - C20:0, % total fatty acids x 10 = mg/g fat
[a,b,c]Means within a row without common superscripts differ ($P < 0.05$)
SEM = standard error of the mean, n = 6

Table 4.14. Effect of storage on CLA and select fatty acids of yogurt manufactured with WPC.

Fatty acids	Storage			SEM
	Day 1	Day 7	Day 14	
	% of total fatty acids*			
Yogurt + 6 % WPC				
C18:0	11.98	12.03	11.95	0.08
C18:1 11t	2.16	2.24	2.18	0.04
C18:1 9c	24.44	24.98	24.72	0.20
C18:2	3.67	3.68	3.69	0.07
C 18:3	0.81	0.79	0.80	0.01
CLA	1.22	1.29	1.25	0.02
Yogurt + 10 % WPC				
C18:0	11.82	11.95	11.87	0.08
C18:1 11t	2.18	2.24	2.19	0.04
C18:1 9c	24.64	25.07	24.82	0.20
C18:2	4.06	4.13	4.12	0.07
C 18:3	0.90	0.92	0.92	0.01
CLA	1.22	1.27	1.23	0.02

*Total fatty acids = C4:0 - C20:0, % total fatty acids x 10 = mg/g fat
[a,b,c]Means within a row without common superscripts differ ($P < 0.05$)
SEM = standard error of the mean, n = 6

In addition to investigating the effect of cultures on CLA, the effect of adding WPC to yogurt was also examined. In earlier studies some researchers (Ha et al., 1989; Shantha et al., 1992) reported that Cheddar-based processed cheeses containing WPC had higher CLA levels than natural cheeses. In contrast, this study indicated that adding either 6% or 10% WPC to yogurt significantly decreased CLA levels in yogurt (1.22%) as compared to milk (1.34%) and yogurts without WPC (1.31%). Yogurts with WPC also had significantly lower levels of stearic acid and TVA and higher levels of linoleic and linolenic acids compared to milk (Table 4.12).

There is no confirmed explanation in the literature as to why the addition of whey protein would increase CLA. However, Ha et al. (1989) postulated that whey proteins contribute to CLA formation by acting as a hydrogen donor. They suggested that high processing temperatures first convert linoleic acid to a linoleic acid free radical, then hydrogen (from whey protein) is added to form a conjugated double bond system which is characteristic of CLA. Currently, we have no plausible explanation for the decrease in CLA or other changes found in the fatty acid composition of yogurt containing WPC.

When processing FM-solin milk into Queso Blanco cheese, the same type of fine curd particles as in experiment I with FF-solin milk was obtained. Similar to the FF-solin curd, this fine curd did not mat together to produce a larger coagulum and was difficult to hoop. Based on these results it may be speculated that feeding cows FF-solin or FM-solin diets may have altered milk proteins or the coagulum complex on acidification.

The composition of Feta cheese manufactured from control and FM-solin milk was slightly different from the values typically found in Feta (Table 4.15). Feta cheese typically contains about 20 % fat and 59 % moisture, however it is a non-standardized product (Health Canada Food and Drug Act, 2000). In addition to being a non-standized product unlike Cheddar cheese, Feta is also a non-pressed cheese. Feta is not subjected to the same pressing stage that gives Cheddar cheese its characteristic tight knit texture (Kosikowsi, 1977). Feta cheese would therefore not be subjected to the same oiling off problem that was encountered during the cheddar cheese manufactured from FF-solin milk in experiment I (section 4.1).

The expert panelist group found no difference in the flavour characteristics of milk from cows fed the control and FM-solin diets. Similarly, they found no flavour difference in yogurt, Feta cheese and butter manufactured from each type of milk. In terms of texture, panelists found the FM-solin butter considerably softer and more spreadable at 5°C compared to control butter. These findings are in agreement with those from Baer et al., (2001) and Cruz et al., (2002), who also reported that butter was softer and more spreadable at refrigeration temperatures when made from milk of cows fed unsaturated dietary fat or fish oil. Butter with high CLA and increased spreadability may be very desirable to consumers. It may even help butter gain leverage from the margarine market.

Table 4.15. Composition of Feta cheese manufactured from control and FM-solin milk.

	Feta cheese		
	Control	FM-solin	Typical value*
Fat %	18.5	24.0	20.3
Moisture %	42.2	39.2	59.7

*Kosikowski (1977)

4.3 Experiment III

The objectives of this experiment were: 1) to determine the effect of feeding a FM-solin supplemented diet to dairy cows on the CLA levels of milk; 2) to determine the effect of heat treatments typically used in dairy processing on the CLA levels of FM-solin and control milk; 3) to determine the effect of probiotic cultures on the CLA levels in Kefir manufactured from FM-solin and control milk; 4) to evaluate the flavour of Kefir manufactured from FM-solin and control milk.

In an attempt to further increase CLA and the total fat content in milk, modifications were made to the formulation of the FM-solin diet fed to cows in experiment II. The changes consisted of substituting the alfalfa silage and corn silage based diet for an alfalfa silage and grass hay based diet. Table 4.16 illustrates the composition of milk from cows fed control and FM-solin diets. The protein content was the same in both types of milk. The fat and SNF content were higher in the control (3.96 % and 8.76 % respectively) compared to FM-solin milk (3.44 % and 8.50 %). However, the fat content in the FM-solin milk (3.44 %) was much higher in this experiment than it was in milk from the previous experiment (2.51 %). It was closer to the average value typically found in bovine milk (3.5%) (Jensen et al., 1991).

Table 4.17 shows the fatty acid composition of milk from cows fed control and FM-solin diets. Trends in the fatty acid profile of control and FM-solin milk were similar to those found in experiments I and II (Tables 4.3 and 4.9). There was a shift towards higher levels of long chain fatty acids and increased content of total unsaturated fatty acids in the FM-solin milk. Similar to experiment II, the FM-solin milk in this

experiment contained a significantly lower level of omega-3 linolenic acid (0.65 %) compared to the control (0.97 %). The proportion of TVA was significantly higher in FM-solin milk (5.47 %) than control (0.70 %). The CLA content in FM-solin milk (2.78 %) was significantly higher than that of control milk (0.41 %). Fishmeal solin milk contained 6.8 times more CLA (2.78 %) than the control (0.70 %) (Table 4.17). More importantly, the FM-solin milk from this experiment contained 0.5 % more CLA than the milk from experiment II and 1.5 % more than FF-solin milk from experiment I. These results indicate that this modified FM-solin diet is more efficient in increasing CLA than diets fed in experiments I or II.

Some researchers (Shantha et al. 1992; Garcia-Lopez et al., 1994) reported that thermal processing contributed to increased CLA levels in processed cheese. In this experiment the effect of heat treatment on milk CLA levels was investigated. The heat treatments applied were 72°C for 16 s, 90°C for 5 min. and 121°C for 2 s.

As in experiment I and II, the interaction of milk type (diet treatment), processing and storage treatment did not significantly affect the fatty acid content of dairy products. Therefore, results were reported (Tables 4.18 - 4.21.) as previously described in section 4.2. Heating milk had no significant effect on CLA. This indicated that CLA was stable under thermal processing temperatures routinely used by the dairy industry. Milk is pasteurized at 72°C for 16 s when marketed as fresh milk, and at 121°C for 2 s when sold as a shelf stable product. For yogurt and Queso Blanco cheese manufacture, milk is heated at 90°C for 5 min. to denature whey proteins.

Table 4.16. Composition of milk from cows fed control and FM-solin diets.

	Diet*	
Item	Control	FM-solin
Fat, %	3.97	3.44
Protein, %	3.36	3.36
Solids non fat, %	8.76	8.50

*Means are of pooled milk from four milking times

Table 4.17. Effect of supplementing FM-solin to dairy cow diets on the fatty acid profile of milk.

	Diet		
Fatty Acid	Control	FM-solin	SEM
	% of total fatty acids*		
C4:0	2.07[a]	1.80[b]	0.03
C6:0	2.73[a]	1.83[b]	0.03
C8:0	2.20[a]	1.23[b]	0.02
C10:0	3.47[a]	1.65[b]	0.02
C12:0	3.81[a]	1.75[b]	0.02
C14:0	12.03[a]	7.32[b]	0.03
C14:1	1.11[a]	0.81[b]	0.004
C16:0	39.03[a]	22.92[b]	0.08
C16:1	1.71[a]	1.49[b]	0.02
C18:0	7.87[b]	14.19[a]	0.03
C18:1 11t	0.70[b]	5.47[a]	0.03
C18:1 9c	15.78[b]	28.42[a]	0.06
C18:1 11c	0.61[b]	1.30[a]	0.01
C18:2	2.03	3.62	0.02
C18:3	0.97[a]	0.65[b]	0.003
C20:0	0.27[b]	0.35	0.02
CLA	0.41[b]	2.78[a]	0.01
Unsaturated FA	23.32	44.54	

*Total fatty acids = C4:0 - C20:0, % total fatty acids x 10 = mg/g fat
[a,b]Means within a row without common superscripts differ ($P < 0.05$)
SEM = standard error of the mean, n = 12

Processing milk into Kefir with or without the addition of *L. acidophilus* had no effect on promoting CLA formation. Possible reasons for these results could be similar to those previously discussed for yogurt manufacture (section 4.2). Furthermore, the expert panellists found no flavour differences between Kefir manufactured from milk of cows fed the FM-solin and control diet.

Ogawa et al. (2001) found that *L. acidophilus* AKU 1137 transformed free linoleic acid into CLA under microaerobic conditions. The ability of *L. acidophilus* to produce CLA in Kefir made from both control and FM-solin milk was investigated in this study. Kefir, unlike yogurt, undergoes both lactic acid and alcohol fermentation. It is manufactured from Kefir grains, which are gelatinous, whitish or yellowish irregular granules that consist of a mixture of bacteria and yeast cells. The predominant microorganisms that make up Kefir grains are *Saccharomyces Kefir, Torula Kefir, Lactobacillus caucasius, Leuconostoc spp.* and lactic acid streptococci. During alcohol fermentation yeast converts glucose into ethyl alcohol and measurable amounts of CO_2 (Kosikowski, 1977). It was postulated that a change in redox potential could influence CLA formation by *L. acidophilus*.

Table 4.18. Effect of various heat treatments on CLA and select fatty acids in milk.

Fatty Acid	Milk	Heat treatments			SEM
		72°C/16 s	90°C/5 min	121°C/2 s	
	% of total fatty acids*				
C18:0	11.02[ab]	11.19[a]	11.07[ab]	11.03[ab]	0.07
C18:1 11t	3.28[a]	2.89[b]	3.34[a]	3.32[a]	0.06
C18:1 9c	22.10[ab]	22.38[a]	22.22[ab]	22.06[b]	0.14
C18:2	2.84	2.86	2.85	2.82	0.04
C18:3	0.81	0.83	0.81	0.80	0.01
CLA	1.59[b]	1.64[a]	1.60[ab]	1.61[ab]	0.02

*Total fatty acids = C4:0 - C20:0, % total fatty acids x 10 = mg/g fat
[a,b,c]Means within a row without common superscripts differ ($P < 0.05$)
SEM = standard error of the mean, n = 6

Table 4.19. Effect of storage on CLA and select fatty acids in milk heated at various temperatures.

Fatty acids	Storage Day 1	Day 7	Day 14	SEM
	% of total fatty acids*			
72°C/16 s				
C18:0	11.19[a]	10.87[b]	11.08[ab]	0.07
C18:1 11t	2.89[b]	2.71[b]	3.15[a]	0.06
C18:1 9c	22.38[a]	21.69[b]	22.15[ab]	0.14
C18:2	2.86	2.74	2.83	0.04
C 18:3	0.83	0.82	0.81	0.01
CLA	1.64[a]	1.53[b]	1.62[ab]	0.02
90°C/5 min				
C18:0	11.07	11.01	10.99	0.07
C18:1 11t	3.34[a]	2.82[b]	3.28[a]	0.06
C18:1 9c	22.22	22.12	22.09	0.14
C18:2	2.85	2.85	2.85	0.04
C 18:3	0.81	0.81	0.81	0.01
CLA	1.60	1.60	1.59	0.02
121°C/2 s				
C18:0	11.03	11.03	11.01	0.07
C18:1 11t	3.32[a]	2.79[b]	3.30[a]	0.06
C18:1 9c	22.06	22.13	22.10	0.14
C18:2	2.82	2.79	2.79	0.04
C 18:3	0.80	0.81	0.81	0.01
CLA	1.61	1.57	1.59	0.02

*Total fatty acids = C4:0 - C20:0, % total fatty acids x 10 = mg/g fat
[a,b,c]Means within a row without common superscripts differ ($P < 0.05$)
SEM = standard error of the mean, n = 6

Table 4.20. Effect of processing milk into Kefir with and without *L. acidophilus* on CLA and select fatty acids in Kefir.

Fatty Acid	Milk	Treatments		SEM
		Kefir	+ *L. acidophilus*	
	% of total fatty acids*			
C18:0	11.13	10.94	11.03	0.09
C18:1 11t	2.82	3.08	3.30	0.11
C18:1 9c	22.3	22.12	22.35	0.18
C18:2	2.80	2.87	2.87	0.04
C18:3	0.83	0.82	0.82	0.01
CLA	1.63	1.60	1.63	0.03

*Total fatty acids = C4:0 - C20:0, % total fatty acids x 10 = mg/g fat
[a,b,c]Means within a row without common superscripts differ ($P < 0.05$)
SEM = standard error of the mean, n = 6

Table 4.21. Effect of storage on CLA and select fatty acids in Kefir.

Fatty acids	Storage			SEM
	Day 1	Day 7	Day 14	
	% of total fatty acids*			
Kefir				
C18:0	10.94	11.05	10.86	0.09
C18:1 11t	3.08	2.96	2.99	0.11
C18:1 9c	22.12	22.24	22.20	0.18
C18:2	2.87	2.82	2.86	0.04
C 18:3	0.82	0.82	0.83	0.01
CLA	1.60	1.59	1.57	0.03
Kefir (*L. acidophilus)*				
C18:0	11.03	11.02	10.81	0.09
C18:1 11t	3.30	3.31	2.92	0.11
C18:1 9c	22.35	22.28	22.07	0.18
C18:2	2.87	2.93	2.88	0.04
C 18:3	0.82	0.84	0.82	0.01
CLA	1.63	1.57	1.54	0.03

*Total fatty acids = C4:0 - C20:0, % total fatty acids x 10 = mg/g fat
[a,b,c]Means within a row without common superscripts differ ($P < 0.05$)
SEM = standard error of the mean, n = 6

5.0 SUMARY AND CONCLUSION

Results from the presented studies show that CLA-enhanced milk and dairy products can be produced by incorporating FF-solin or FM-solin to dairy cow diets. The highest levels of CLA in milk (2.78 %) were obtained from cows fed a FM-solin supplemented diet. The milk contained 6.8 times more CLA than the control milk and 4.6 times more than commercial milk locally purchased.

In addition to increasing CLA levels, there were other notable changes found in the fatty acid profile of milk when either FM-solin or FF-solin was fed to cows. The proportion of long chain fatty acids (C18:0 – C18:2) significantly increased while short and medium chain fatty acids (C4:0 – C16:0) decreased significantly. The levels of TVA (C18:1 11t) were also significantly higher in milk from cows on CLA promoting diets. In terms of human health, such modifications in the fatty acid profile of milk are regarded as very desirable. The medium chain fatty acid myristic acid (C14:0) and palmitic acid (C16:0) in milk fat are implicated in causing increased CHD (Ney, 1991). In contrast there is evidence that ingestion of TVA increases CLA levels in human serum (Salminen et al. 1998; Adlof et al. 2000) and may reduce the risk of CHD.

Processing operations or the starting level of CLA in milk had no significant effect on CLA in resulting products with the exception of butter. Conjugated linoleic acid was transferred into resulting dairy products in proportion to the starting level in raw milk. Heat treatments commonly used in the dairy industry had no effect on the CLA levels of control or CLA-enhanced milk. Similarly, the storage of products had no significant effect on the CLA levels. Processing milk into butter resulted in a

significant decrease in the level of CLA regardless of the starting level in the milk. The incorporation of air during churning of cream may have caused oxidation of CLA and consequently its decreased level in butter.

The present study also investigated the possibility that cultures and WPC would have an affect on CLA levels in dairy products since researchers speculated that both would have a positive effect. In the present study it was found that commercial freeze dried probiotic cultures *L. acidophilus*, *B. bifidum* or *L. casei* in yogurt manufacture and *L. acidophilus* in Kefir production had no effect on the CLA levels of these products. In contrast, incorporating 6 or 10 % WPC in starting milk decreased the CLA content in yogurt. Further work is required to investigate the mechanisms involved.

Results from the sensory studies indicated that the flavour of milk and dairy products manufactured from CLA-enhanced milk were comparable to those produced from control milk. However, some textural differences were detected in certain products manufactured from CLA enhanced milk. Curd formed in Queso Blanco cheese manufactured from both FF-solin and FM-solin milk (experiments I and II) was extremely fine leading to difficulty in recovery of "fines". Cheddar cheese produced from FF-solin milk (experiment I) was softer and greasier than control cheese. Similarly, butter made from FM-solin milk (experiment II) was softer, and had better spreadability at room temperature than control butter. This was due to the higher level of unsaturated fat present in FM-solin milk. In terms of consumer acceptability, the former defect in Cheddar cheese would be regarded as undesirable, whereas the latter.

may be embraced by consumers. This is an example where the deliberate alteration of milk for health also results in a manufactured product benefit.

Results of this study provide conclusive evidence that CLA enhancement in milk results in dairy products with correspondingly increased levels of CLA, and these levels are maintained during storage. However, there are some limitations with manufacture of CLA-enhanced butter, Cheddar cheese and Queso Blanco cheese. Further research is needed to determine what causes the decrease in CLA during butter manufacture and how it can be prevented. Ultimately, this type of study would also help us gain a better understanding of CLA's chemical activity and stability during processing. Additional research is also required to modify or develop a method for processing Cheddar and other cheese from milk with higher levels of unsaturated fats. In this study, when using milk with a higher level of unsaturated fat, oil leakage was observed during the pressing stage of cheese manufacture. The development of a modified processing method for cheese manufacturing could very beneficial to all processors in the near future, since many Canadian dairy farmers are already incorporating oilseeds in cow diets. It is also recommended that research be conducted to determine the effect of supplementing FM-solin or FF-solin on the protein composition and characteristics of milk. This would help us gain a better understanding of possible changes in cheese curd formation.

Further work is required to investigate the ability of probiotic cultures such as *L. acidophilus* to form CLA from linoleic acid in cultured products such as yogurt or Kefir. In this study, commercial freezed dried cultures selected based on availability were tested for their ability to enhance CLA, during product manufacture. It would be

interesting to test the ability of several different strains of *L. acidophilus* to form CLA in a yogurt or Kefir system under different conditions. As previously mentioned, more work is needed to investigate the effect of WPC on CLA levels in dairy products to define WPC activity as a protein donor. It is also recommended that future research involving the determination of CLA levels in dairy products be analyzed using both GC and Ag+-HPLC to obtain complete separation of CLA isomers.

In conclusion, it is strongly recommended that research be continued in the area of processing CLA-enhanced dairy products given that these findings would be beneficial for both the consumer and the dairy industry. Dairy products high in CLA will allow consumers to obtain CLA and its therapeutic health benefits by consuming a natural food product source. This will increase the nutritional value of dairy products, help reduce risk of disease and change the public attitude toward milk fat. Furthermore, this will position the dairy industry as an important contributor to the emerging nutraceutical/functional food industry.

REFERENCES

Abu-Ghazaleh, A. A., Schingoethe, D. J. and Hippen, A. R. 2001. Conjugated linoleic acid and other beneficial fatty acids in milk fat from cows fed soybean, fish meal or both. J. Dairy Sci. 84:1845-1850.

Ackman, R. G., Eaton, C. A., Sipos, J. C. and Crewe, N. F. 1981. Origin of cis-9, trans-11 and trans-9, trans-11-octadecadienoic acids in the depot fat of primates fed a diet rich in lard and corn oil and implications for the human diet. Can. Inst. Food Sci. Technol. J. 4:103-107.

Ackman, R. G. 2000. Fatty acids in fish and shellfish. Pages 153-173 in: Fatty Acids in Foods and their Health Implications. Chow, C. K. ed. Marcel Decker Inc. New York, N.Y.

Adlof, R. O., Dubal, S. and Emken, E. A. 2000. Biosynthesis of conjugated linoleic acid in humans. Lipids. 35:131-135.

Ashes, J. R., Gulati, S. K. and Scott, T.W. 1997. Symposium: New Approaches to Changing Milk Composition. J. Dairy Sci. 80:2204-2212.

Baer, R. J. 1991. Alteration of fatty acid content of milk fat. J. Food Prot. 54:383-386.

Baer, R. J., Ryali, J. and Wschingoethe, D. J. 2001. Composition and properties of milk and butter from cows fed fish oil. J. Dairy Sci. 84:345-353.

Baumgard, L. H., Corl, B. A., Dwyer, D. A., Saebo A. and Bauman, D. E. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Physiol. 278, R179-R184.

Beaulieu, T. 2002. Dairy Farmers of Canada. Personal Communication.

Bell, J. A. and Kennelly, J. J. 2001.Conjugated Linoleic Acid Enriched Milk: A Designer Milk with Potential. Pages 213-228. In Advances in Dairy Technology. Vol 13.

Belury, M. A., Nickel, K. P., Bird, C. E. and Wu, Y. M. 1996. Dietary conjugated linoleic acid modulation of phorbol ester skin tumor promotion. Nutr. Cancer. 26:149-157.

Bligh, E. G. and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Boylston, T. D., Beitz, D. C. and Hammond, E. G. 2000. Influence of conjugated linoleic acid and soy oil supplementation in diets of cows on the CLA and fatty acid composition of yogurt. Presented at the 2000, Annual Meeting of the Institute of Food Technologists, Dallas TX., June12-14.

Chin, S. F., Liu, W., Storkson, J. M., Ha, Y. L. and Paziza, M. W. 1992. Dietary sources of conjugated dienoic isomers of linoleic acid a recognised class of anti-carcinogens. J. Food Comp. Anal. 5:185-197.

Chin, S. F., Storkson, J. M., Albright, K.J., Cook, M. E. and Pariza, M. W. 1994. Conjugated linoleic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency. J. Nutr. 124:2344-2349.

Chouinard, P. Y., Corneau, L., Saebo, A. and Bauman, D. E. 1999. Milk yield and composition during abomasal infusion of conjugated linoleic acids in dairy cows. J. Dairy Sci. 82:2737–2745.

Chouinard, P. Y., Corneau, L., Buttler, W. R., Chillard, Y., Drackley, J. K. and Bauman, D. E. 2001. Effect of dietary lipid source on conjugated linoleic acid concentration in milk fat. J. Dairy Sci. 84:680-690.

Colbert, L. B. and Decker, E. A. 1991. Antioxidants of an ultrafiltration permeate from acid whey. J. Food Sci. 56:1248-1250.

Cook, M. E., Miller, C.C., Park, Y. and Pariza, M. W. 1993. Immune modulation by altered nutrient metabolism: Nutritional control of immune induced growth depression. Poultry Sci. 72:1301-1305.

Cook, M. E., Jerome, D. L., Crenshaw, T. D., Buenge, D. R., Pariza, M. W., Albright, K. J. Schmidt, S. P., Scimeca, J. A., Lofgren, P. A. and Hentges, E. J. 1998. Feeding conjugated linoleic acid improves feed efficiency and reduces carcass fat in pigs. FASEB J. 12: A836 abs.

Corl, B. A, Baumgard, L. H., Griinari, J. M., Delmomonte, P. Morehouse, K. M., Yurawecz, M. P., and Bauman, D. E. 2002. Trans-7, cis-9 CLA is endogenously synthesized by Δ-9-deesaturase in lactating dairy cows. In Proceedings of the 93rd AOCS Annual Meeting and Expo. Montreal, Quebec. May 5-8. p. S5.

Cruz-Hernandez, C. C., Avramis, C., Kramer, J. K. G., McBride, B. M., Wright, T. C. and Hill, A. R. 2002. Changes in the DHA, CLA and trans-18:1 content in milk fat from cows fed diets containing either fish meal or algae. In Proceedings of the 93rd AOCS Annual Meeting and Expo. Montreal, Quebec. May 5-8. p. S2.

De Voney, D., Pariza, M. W. and Cook, M. E. 1997. Conjugated linoleic acid increases blood and splenic T-cell responses post lipopolysaccharide injection. FASEB J. 9:3355.

Dhiman, T. R., Helmink E .D., McMahon, D. J., Fife, R. L. and Pariza, W. W. 1999. Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds. J. Dairy Sci. 82:412-419.

Dhiman, T. R., Satter, L. D., Pariza, M. W., Galli, M. P. Albright, K. and Tolosa, M. X. 2000. Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. J. Dairy Sci. 83:1016-1027.

Donovan, D. C., Schingoethe, D. J., Baer, R. J., Ryali, J., Hippen, A. R. and Franklin, S. T. 2000. Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. J. Dairy Sci. 83:2620-2628.

Drake, M. A., Herrett, W., Boylston, T. D. and Swanson, B. G. 1995. Sensory evaluation of reduced fat cheeses. J. Food Sci. 60:898-901.

Engle, T. E., Fellner, V. and Spears, J.W. 2000. Effect of supplemental copper on copper status, serum cholesterol and milk fatty acids in Holstein cows. J. Dairy Sci. 83:287, Suppl. 1.

Fogerty, A. C., Ford, G. L. and Svoronos, D. 1988. Octadeca-9, 11-decenoic acid in lipids of human blood and breast milk. Nutr. Rep. Int. 38:937-944.

Fox, P. F. and McSweeney, P. L. H. 1998. Dairy Chemistry and Biochemistry. Blackie Academic & Professional, London, UK. 478pp.

Garcia-Lopez, S., Echeverria, E., Tsui, I. and Balch, B. 1994. Changes in the content of conjugated linoleic acid (CLA) in processed cheese during processing. Food Res. Int. 27:61-64.

Griinari, J. M. and Bauman, D. E. 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. Pages 180-197. In Adv. In Conjugated Linoleic Acid Res. Vol. 1. Eds. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza and G. J. Nelson. AOCS Press, Campaign, IL.

Grummer, R. R. 1991. Effect of feed on the composition of milk fat. J. Dairy Sci. 74:3244-3257.

Ha, Y. L., Grimm, N. K., and Pariza, M. W. 1987. Anti-carcinogens from fried ground beef: heat altered derivatives of linoleic acid. Carcinogenesis, 8:1881-1887.

Ha, Y. L., Grimm, N. K. and Pariza, M. W. 1989. Newly recognized anticarcinogenic fatty acids: Identification and quantification in natural and processed cheeses. J. Agric. Food Chem. 37:75-81.

Ha, Y. L., Storkson, J. and Pariza, M. W. 1990. Inhibition of benz(a)pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. Cancer Res. 50:1097-1101.

Health Canada, Food and Drug Act. URL:www.hc-sc.gc.ca/food-aliment/friia-raaii/food_drugs-aliments_drogues/act-loi/e_index.html (February 18,2003)

Hillbrick, G. and Augustin, M. A. 2002. Milkfat characteristics and functionality: Opportunities for improvement. Aust. J. Dairy Technol. 57:45-51.

Ip, C., Chin, S. F., Scimeca, J. A. and Pariza, M. W. 1991. Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. Cancer Res. 51:6118-6124.

Ip, C., Singh, M., Thompson, H. J. and Scimeca, J. 1994. Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. Cancer Res. 54:1212-1215.

Ip, C., Scimeca, J. A. and Thompson, H. J. 1995. Effect of timing and duration of dietary conjugated linoleic acid on mammary cancer prevention. Nutr. Cancer. 24:241-247.

Jensen, R. G. and Clark. 1988. Lipid composition and properties. In Fundamentals of Dairy Chemistry. 3rd ed. N. P. Wong, R. Jenness, M. Keeney and E. H. Marth, ed. Van Nostrand Reinhold Co., New York, NY.

Jensen, R. G., Ferris A. M. and Lammi-Keefe., C. J. 1991. Symposium: Milk Fat Composition, Function and Potential For Change. The composition of milk fat. J. Dairy Sci. 74:3228-3243.

Jiang, J., Bjoerck, L. Fonden, R. and Emanuelson. 1996. Occurrence of conjugated cis-9, trans-11- octadecadienoic acid in bovine milk: Effects of feed and dietary regimen. J. Dairy Sci. 79:438-445.

Jiang, J., Bjoerck, L. and Fonden, R. 1998. Production of conjugated linoleic acid by dairy starter cultures. J. of Appli. Microbiol. 85.:95-102.

Jones, D. L., Weiss, W. P. and Palmquist, D. L. 2000. Influence of dietary tallow and fish oil on milk fat composition. J. Dairy Sci. 83:2024-2026.

Kaylegian, K. E., Hartel, R. W. and Lindsay, R. C. 1993. Applications of modified milk fat in food products. J. Dairy Sci. 76:1782-1796.

Kelly, M. L. and Bauman, D. E. 1996. Conjugated linoleic acid: A potent anticarcinogen found in milk fat. Proc. Cornell Nutr. Conf. pp. 68-74.

Kelper,C. R. and Tove, S. B. 1967. Biohydrogenation of unsaturated fatty acids. J. Biol. Chem. 242:5686-5692.

Kosikowski, F. V. 1977. Cheese and Fermented Milk Foods. F. V. Kosikowski and associates, New York, NY. 711pp.

Kramer, J. K. G., Fellner, V., Dugan, M. E. R., Sauer, F. D., Mossoba, M. M. and Yurawecz, M. P. 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. Lipids, 32:1219-1228.

Larmond, E. 1977. Laboratory Methods for Sensory Evaluation of Food. Canada Department of Agriculture Publication. 1637. Ottawa.

Lawless, F., Stanton, C., L'Escop, P., Devery, R., Dillion, P. and Murphy, J .J 1998. Influence of breed on bovine milk cis-9, trans-11-conjugated linoleic acid content. Livestock Prod. Sci. 62:43-49.

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

Liew, C., Schut, H. A. J., Chin, S. F., Pariza, M. W. and Dashwood, R. H. 1995. Protection of conjugated linoleic acids against 2-amino-3-methylimidazo[4,5-f)quinoline-induced colon carcinogenesis in the F344 rat:a study of inhibitory mechanisms. Carcinogenesis, 16:3037-3043.

Lightfield, K. D., Baer, R. J., Schingoethe, D. J., Kaspersons, K. M. and Brouk, M. J. 1993. Dairy Foods: Composition and flavour of milk and cheddar cheese higher in unsaturated fatty acids. J. Dairy Sci. 76:1221-1232.

Lin, H., Boylston, T. D., Chang, M. J., Luedecke, L. O. and T. D. Shultz. 1995. Survey of the conjugated linoleic acid contents of dairy products. J. Dairy Sci. 78:2359-2365.

Lin, H., Boylston T. D., Luedecke, L. O. and Shultz, T. D. 1998. Factors affecting the conjugated linoleic acid content of Cheddar cheese. J. Agric. Food Chem. 46:3 801-807.

Lin, H., Boylston T. D., Luedecke, L. O. and Shultz, T. D. 1999a. Conjugated linoleic acid content of Cheddar-type cheeses as affected by processing. J. Food Sci. 64:5 874-878.

Lin, T. Y., Lin, C. W. and Lee, C. H. 1999b. Conjugated linoleic acid concentration as affected by lactic cultures and added linoleic acid. Food Chem. 67:1-5.

Lin, T. Y. 2000. Conjugated linoleic acid concentration as affected by lactic cultures and additives. Food Chem. 69:27-31.

Macdonald, H. B. 2000. Conjugated linoleic acid and disease prevention: A review of current knowledge. J. Ameri. College Nutr. 19:111S-118S.

Morales, M. S., Palmquist, D. L. and Weiss, W.P. 2000. Effects of fat source and copper on unsaturation of blood and milk triacylglycerol fatty acids in Holstein and Jersey cows. J. Dairy Sci. 83:2105-2111.

Murphy, J. J., Connolly J. F. and McNeill, G. P. 1995. Effects on cow performance and milk fat composition of feeding full fat soybeans and rapeseeds to dairy cows at pasture. Livest. Prod. Sci. 44:13-25.

Ney, D.M. 1991. Symposium: The role of the nutritional and health benefits in the marketing of dairy products. J. Dairy Sci. 74:4002-4012.

Nicolosi, R. J. and Laitinen, L. 1996. Dietary conjugated linoleic acid reduces aortic fatty streak formation greater than linoleic acid in hypercholesterolemic hamster. FASEB Journal abstr. #2751.

Offer, N.W., Marsden, M., Dixon, J., Speake, B.K. and Thacker, F.E. 1999. Effect of dietary fat supplements on levels of n-3 poly-unsaturated fatty acids, trans acids and conjugated linoleic acid in bovine milk. Anim. Sci. 69:613-625.

Ogawa, J., Matsumura, K., Kishino, S., Omura, Y. and Simizu, S. 2001. Conjugated linoleic acid accumulation via 10-hydroxy-12-octadecaenoic acid during microaerobic tranformation of linoleic acid by *Lactobacillus acidophilus*. Appli. Environ. Microbiol. 67:1246-1252.

O'Shea, M., Lawless, F., Stanton, C. and Devery, R. 1998. Conjugated linoleic acid in bovine milk fat: A food-based approach to cancer chemoprevention. Trends Food Sci. Technol. 9:192-196.

Palmquist, D. L. and Mattos, W. 1978. Turnover of lipoproteins and transfer to milk fat of dietary (l-carbon-14) linoleic acid in lactating cows. J. Dairy Sci. 61:561-565.

Palmquist, D. L. 2001. Ruminal and endogenous synthesis of CLA in cows. Aust. J. Dairy Tech. 56 (2):134-137.

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

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

Pariza, M. W., Park, Y. and Cook, M. E. 2000. Mecanisms of action of conjugated linoleic acid: Evidence and speculation. Proc. Soc. Exp. Biol. Med. 223:8-13.

Parodi, P. W. 1994. Conjugated linoleic acid: An anticarcinogenic fatty acid present in milk fat. Aust. J. Dairy Technol. 49:93-97.

Parodi, P. W. 1999. Conjugated linoleic acid and other anticacinogenic agents of bovine milk fat. J. Dairy Sci. 82:1339-1349.

Riel, R. R. 1963. Physico-chemical characteristics of Canadian milk fat. Unsaturated fatty acids. J. Dairy Sci. 46:102-106.

Roessler, E.B., Pangborn, R.M., Sidel, J.L. and Stone H. 1978. Expanded statistical tables for estimating significance in paired-preference, paired difference, duo-trio and triangle tests. J. Food Sci. 43:940-943.

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

Schonberg, S. and Krokan, H. E. 1995. The inhibitory effect of conjugated linoleic dienoic derivatives (CLA) of linoleic acid on the growth of human tumor cell lines is in part due to increased lipid peroxidation. Anticancer Res. 15:1241-1246.

Shantha, N. C., Decker, E. A. and Ustunol, Z. 1992. Conjugated linoleic acid concentration in processed cheese. J. Amer. Oil. Chem. Soc. 69:425-428.

Shantha, N. C., Ram, L. N., O'Leary, J., Hicks, C. L. and Decker, E. A. 1995. Conjugated linoleic acid concentrations in dairy products as affected by processing and storage. J. Food Sci. 60:695-697.

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

Stanton, C., Lawless, F., Kjellmer, G., Harrigton, D., Devery, R., Connolly, J.F. and Murphy, J. 1997. Dietary influences on bovine milk cis-9, trans-11-conjugated linoleic acid content. J. Food Sci. 62:1083-1086.

Steele, R. G. D. and Torrie, J. H. 1980. Principles and Procedures of Statistics: A biometrical approach. 2nd. Ed. McGraw Hill Book Co. Inc. New York, N. Y. 665pp.

Stockdale, C. R. 2000. Differences in body condition and body size affect the responses of grazing cows to high-energy supplements in early lactation. Aust. J. Exp. Agric. 40:903-911.

Visonneau, S., Cesano, A., Tepper, S. A., Scimeca, J. A., Santoli, D. and Kritchevsky, D. 1997. Conjugated linoleic acid suppresses the growth of human breast adenocarcinoma cells in SCID mice. Anticancer Res. 17:969-973.

Walstra, P., Geurts, T. J., Noomen, A., Jellema, A. and van Boekel, M. A. J. S. 1999. Dairy Technology: Principles of Milk Properties and Processes. Marcel Decker Inc. New York, NY. 727pp.

Walker, G. P., Stockdale, C. R., Wales, W. J., Doyle, P. T. and Dellow, D. W. 2001. Effect of level of grain supplementation on milk production responses of dairy cows in mid-late lactation when grazing irrigated pastures high in paspalum (*Paspalum dilatatum* Poir.) Aust. J. Exp.Agric. 41:1-11.

Ward, A. T., Wittenberg, K. M., Froebe, H. M., Przybylski, R. and Malcolmson. (Submitted 2002) L. Fresh Forage and solin supplementation on conjugated linoleic acid levels in plasma and milk. J. Dairy Sci.

Werner, S. A., Luedecke, L.O. and Shultz., T. 1992. Determination of conjugated linoleic acid content and isomer distribution in three Cheddar-type cheeses: Effects of cheese cultures, processing and aging. J. Agric. Food Chem. 40:1817-1821.

White, N. D. G., Mills, J. T., Kenaschuk, E. O., Oomah, B. D. and Dribnenki, P. 1999. Quality changes occurring in stored solin, high linolenic acid and standard flaxseed (Linum usitatissimum L.). Can. J. Plant Sci. 79:35-42.

Yurawecz, M. P., Delmonte, P., Roach, J. A. G., Morehouse, K. M., Weisz, A., Ito, Y., Nyman, P. and Lehmann, L. 2002. Synthesis and isolation of trans-7, cis-9 octadecadienoic acid and other CLA isomers by base conjugation and partially hydrogenated octadecatrienoic fatty acids. In Proceedings of the 93rd AOCS Annual Meeting and Expo. Montreal, Quebec. May 5-8. p. S4.

Zu, H. X. and Schut, H. A. J. 1992. Inhibition of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-DNA adduct formation in CDF1 mice by heat-altered derivatives of linoleic acid. Food Chem. Toxicol. 30:9-16.

APPENDIX 1

CONSENT FORM

SENSORY EVALUATION OF DAIRY PRODUCTS

I agree to participate as a panelist in the evaluation of dairy products.

I understand that I will be tasting dairy products, which may include whole milk, plain yogurt, cheddar cheese, direct acidified cheese, butter and cream.

I understand that results generated from this study will not be reported by individuals' names nor will any names be associated with the results. All data will be kept strictly confidential and held in a locked filing cabinet for three years or after publication whichever is lesser. Any personal data will remain strictly confidential.

I also understand that I am free to withdraw from the study providing I notify the experimenter.

Name (please print)_____

Signature_____

Date_____

Daytime Telephone Number_____

E-mail Address_____

PANELIST QUESTIONNAIRE
MILK

Please check the box, which best answers, the following questions.
How often do you drink milk?

☐ at least once a day
☐ several times a week
☐ once a month
☐ rarely

What type of milk do you usually drink?

☐ whole milk
☐ 2% milk
☐ 1% milk
☐ skim milk
☐ other _____

THANK YOU FOR YOUR HELP!!!

Would you be willing to participate in future milk panels?

☐ Yes
☐ No

If yes, please complete the following:

Name_____

E-mail Address_____

Daytime Telephone number_____

Milk Evaluation

You have been given 2 sets of three samples. Each set consists of ONE LABELLED REFERENCE sample (REF) and two CODED samples of milk to evaluate for flavour.

INSTRUCTION: FOR EACH SET

1. Rinse your mouth with water. If necessary eat a piece of cracker to cleanse your palate.
2. Taste the REF sample first.
3. Rinse your mouth with water again.
4. Evaluate the first coded sample listed. Rinse again and evaluate the next coded sample.
5. Circle the coded sample, which has a DIFFERENT FLAVOUR from the REFERENCE sample.
6. Please comment about the flavour of the DIFFERENT sample.
7. You can retaste samples but remember to rinse between each one.

SET 1

REF _____ _____

COMMENTS:

SET 2

REF _____ _____

COMMENTS:

PANELIST QUESTIONNAIRE

YOGURT

Please check the box, which best answers, the following questions.

How often do you eat plain yogurt?

☐ at least once a day
☐ several times a week
☐ once a month
☐ rarely

What type of yogurt do you usually eat?

☐ plain
☐ fruit flavoured

What is the fat content of the yogurt that you usually eat?

☐ > 4%
☐ 2.1 - 4%
☐ 1 – 2%
☐ < 1%
☐ other _____

THANK YOU FOR YOUR HELP!!!

Would you be willing to participate in future yogurt panels?

☐ Yes
☐ No

If yes, please complete the following:

Name_____
E-mail Address_____
Daytime Telephone number_____

Yogurt Evaluation

You have been given 2 sets of three samples. Each set consists of ONE LABELLED REFERENCE sample (REF) and two CODED samples of yogurt to evaluate for flavour.

INSTRUCTION: FOR EACH SET

1. Rinse your mouth with water. If necessary eat a piece of cracker to cleanse your palate.
2. Taste the REF sample first.
3. Rinse your mouth with water again.
4. Evaluate the first coded sample listed. Rinse again and evaluate the next coded sample.
5. Circle the coded sample, which has a DIFFERENT FLAVOUR from the REFERENCE sample.
6. Please comment about the flavour of the DIFFERENT sample.
7. You can retaste samples but remember to rinse between each one.

SET 1

REF _____ _____

COMMENTS:

SET 2

REF _____ _____

COMMENTS:

APPENDIX 2

Cheddar Cheese Manufacturing Log 1

Date : PERIOD I	June 28, 2000	June 29, 2000	June 30, 2000
Vat	Control milk	FF- milk	FF-solin milk
L of milk	155 L	165 L	154 L
Acidity of milk (%)	0.155	0.160	0.160
Starter used	8.75g (10 units)	8.75g (10 units)	8.75 g (10 units)
Time starter added	12:10 pm	10:25 am	9:50 am
Colorant (ml)	11 ml	12 ml	11 ml
Coagulant (ml)	13 ml	14 ml	13 ml
Acidity when coagulant added	0.17 %	0.17 %	0.17 %
Time coagulant added	1:05 pm	11:30 am	10:50 am
Temperature of milk	34.5°C / 94°F	34.5°C / 94°F	34.5°C / 94°F
Minutes to set	30 min	30 min	30 min
Time of cutting	1:40 pm	12:00 pm	11:30 pm
Acidity after cutting	0.105	0.120	0.110
Time cooking started	1:55 pm	12:15 pm	11:45 pm
Time cooking completed	40 min	30 min	35 min
Final cooking temperature	37.8°C / 100°F	38.9°C / 102°F	38.1°C / 100.5°F
Acidity after cooking	0.11	0.12	0.13
Acidity at draining	0.15	0.15	0.15
Time of draining	3:40 pm	2:00 pm	1:05 pm
Acidity at salting	0.24	0.23	0.24
Salt (g)	386 g	414 g	386 g
Time of salting	4:04 pm	2:15 pm	1:45 pm

Notes

Color of FF milk was darker than control or FF-solin milk

FF whey at cooking was murky and acid developed faster than the control.

Cheddar Cheese Manufacturing Log 2

Date: PERIOD II	July 2, 2000	July 25, 2000	July 29,2000
Vat	Control milk	FF- milk	FF-solin milk
Milk (l)	137 L	127 L	165 L
Acidity of milk	0.155 %	0.150 %	0.150 %
Starter used	4.5 g (5 units)	4.5 g (5 units)	4.5 g (5 units)
Time starter added	10:05 am	10:00 am	9:30 am
Colorant (ml)	10.0 ml	9.0 ml	11.6 ml
Coagulant (ml)	11.5 ml	10.5 ml	13.5 ml
Acidity when coagulant added	0.16 %	0.16 %	0.16 %
Time coagulant added	11:05 am	11:00 am	10:25 am
Temperature of milk	34.5°C / 94°F	34.5°C / 94°F	34.5°C / 94°F
Minutes to set	45 min	40 min	40 min
Time of cutting	11:50 am	11:50 am	11:05 am
Acidity after cutting	0.10 %	0.11 %	0.10 %
Time cooking started	12:10 pm	12:05 pm	11:20 am
Time cooking completed	35 min	35 min	30 min
Final cooking temperature	38.9°C / 102°F	38.9°C / 102°F	38.9°C / 102°F
Acidity after cooking	0.11 %	0.115 %	0.11 %
Acidity at draining	0.14 %	0.14 %	0.15 %
Time of draining	2:15 pm	2:20 pm	1:45 pm
Acidity at salting	0.17 %	0.20 %	0.20 %
Salt (g)	340 g	315 g	414 g
Time of salting	3:10 pm	3:15 pm	2:15 pm

Cheddar Cheese Manufacturing Log 3

Date PERIOD III	August 16, 2000	August 17, 2000	August 18, 2000
Vat	Control milk	FF milk	FF-solin milk
Milk (L)	130 L	135 L	114 L
Acidity of milk	0.155 %	0.14 %	0.15 %
Starter used	3.9 g (5 units)	4.0 g (5 units)	2.6 g (3.4 units)
Time starter added	10:45 am	12:05 pm	9:15 am
Colorant (ml)	9.0 ml	9.5 ml	8.0 ml
Coagulant (ml)	11.0 ml	11.0 ml	9.5 ml
Acidity when coagulant added	0.160 %	0.150 %	0.155 %
Time coagulant added	11:45 am	1:10 pm	10:15 am
Temperature of milk	35.6°C / 96°F	36.1°C / 97°F	34.5°C / 94°F
Minutes to set	50 min	55 min	45 min
Time of cutting	12:50 pm	2:05 pm	11:00 am
Acidity after cutting	0.11 %	0.11 %	0.10 %
Time cooking started	1:05 pm	2:20 pm	11:15 am
Time cooking completed	35 min	35 min	35 min
Final cooking temperature	38.9°C / 102°F	38.9°C / 102°F	38.9°C / 102°F
Acidity after cooking	0.11 %	0.11 %	0.105 %
Acidity at draining	0.145 %	0.150 %	0.140 %
Time of draining	3:05 pm	4:35 pm	1:45 pm
Acidity at salting	0.23	0.24	0.22
Salt (mg)	325 g	331.5 g	285 g
Time of salting	3:30 pm	5:00 pm	2:05 pm

Notes

During the FF-solin cheese manufacturing we found fat droplets floating in whey at draining and salting cheese curds were soft not squeaky

Feta Cheese Manufacturing Log 1

Date	May 10, 2001	May 10, 2001
Vat	Control milk 1	Control milk 2
Milk (L)	150 L	152 L
Temperature of milk	34.5°C / 94°F	34.5°C / 94°F
Acidity of milk	0.185 %	0.185 %
Starter used	4.3 g (4.5 units)	4.3 g (4.5 units)
Time starter added	10:45 am	2:05 pm
Acidity of milk after ripening	0.190 %	0.188 %
Coagulant (ml)	50 ml	50 ml
Time coagulant added	11:40 am	2:55 pm
Time of cutting	12:30 pm	3:25 pm
Acidity after cutting	0.12 %	0.12 %
Acidity at draining	0.123 %	0.125 %
Time of draining	1:10 pm	4:10 pm
Time curd hooped	1:15 pm	4:15 pm
Date cheese salted	May 11, 2001	May 11, 2001
Salting method	10 % brine solution	10 % brine solution

Feta Cheese Manufacturing Log 2

Date	May 11, 2001	May 11, 2001
Vat	FM-solin milk1	FM-solin milk 2
Milk (L)	132	132
Temperature of milk	34.5°C / 94°F	34.5°C / 94°F
Acidity of milk	0.15 %	0.15 %
Starter used	4.0 g (4 units)	4.0 g (4 units)
Time starter added	10:00 am	1:00 pm
Acidity of milk after ripening	0.16 %	0.15 %
Coagulant (ml)	44 ml	44 ml
Time coagulant added	11:00 am	2:00 pm
Time of cutting	11:30 am	2:35 pm
Acidity after cutting	0.10 %	0.10 %
Acidity at draining	0.11 %	0.15 %
Time of draining	12:10 pm	3:15 pm
Time curd hooped	12:15 pm	3:20 pm
Date cheese salted	May 12, 2001	May 12, 2001
Salting method	10 % brine solution	10 % brine solution

APPENDIX 3

Table 1. Dietary treatments fed to cows in experiment I.

	Diets		
Ingredients (%)	Control	FF	FF-solin
Fresh grass forage	-	59.2	59.5
Grass hay	57.1	-	-
Concentrate	30.6	30.9	30.5
Solin	-	-	10.2
Canola meal	8.9	7.8	-
Tallow	1.1	1.1	-
Alifet[tm1]	2.9	2.1	-

[1]Alifettm is a tallow-starch based product sold as an energy source for dairy cattle (Alifet USA, Inc. 9403 Kenwood Rd., Suite C107 Cincinnati, Ohio 452442).
(Adapted from Ward et al., 2002)

APPENDIX 4

Figure1. Gas chromatogram of CLA standards.

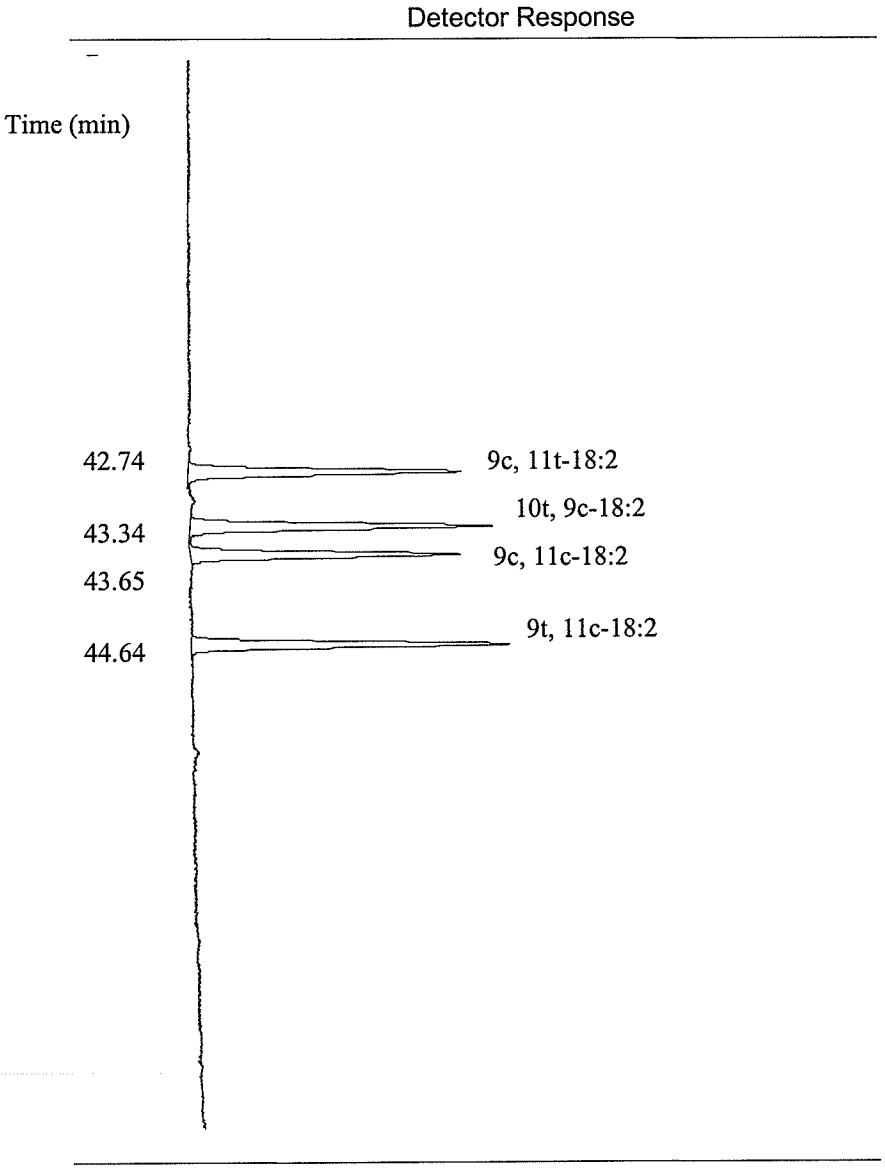

Figure 2. Gas chromatogram of control bovine milk fatty acids.