THE UNIVERSITY OF MANITOBA

USE OF FORMALDEHYDE AND LIGNOSULFONATE TREATED FLAXSEED AND LINOLA TO ALTER FATTY ACID COMPOSITION AND INCREASE OMEGA-3-FATTY ACIDS IN THE MILK FAT OF DAIRY COWS

By

JANE MARGARET GOODRIDGE

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DEDICATED TO MY HUSBAND, BRUCE GOODRIDGE, FOR ALL OF HIS LOVE AND SUPPORT AND TO THE MEMORY OF MY DEAR MOTHER, MARY MARGARET WALLIS, WHO PASSED AWAY JANUARY 24TH, 1994.

ABSTRACT

Two experiments were conducted to determine the effects of supplemental ground flaxseed or Solin (Linola), protected either with formaldehyde or lignosulfonate, on the fatty acid (FA) composition of milk. Linolenic acid (C18:3),an omega-3-fatty acid present in flaxseed (approximately 55-60% of total fatty acids) was of particular interest due to the reported associated health benefits to humans of this group of fatty acids. In the first experiment, two multiparous and two primiparous Holstein cows in midlactation were randomly assigned to four diets in a 4x4 Latin square design. Diets consisted of total mixed ration (TMR) plus i) 0% added fat-Control ii) Linola, a low linolenic (C18:3), high linoleic (C18:2) variety of Solin at 400-450 g fat per kg milk fat produced, iii) flaxseed at 200-225 g fat per kg milk fat produced (FL) and iv) flaxseed at 400-450 g fat per kg milk fat produced (FH). The high level of supplemental fat provided approximately 123 g C18:3 to the milk fat, representing a theoretical value of 10% C18:3, assuming an average production of 36 kg milk with 3.4% fat.

Feed intake; milk yield, fat, protein and SNF; rumen ammonia, volatile fatty acids (VFA); and plasma urea nitrogen (PUN) were not influenced by diet ($P>0.05$). Diet did not affect plasma C16:0 or C16:1 concentrations, however, plasma C18:0 was lowest for cows fed FH. Oleic acid (C18:1) was significantly higher in milk produced by control cows, perhaps indicating some hydrogenation in the rumen from any fat present naturally in the TMR. There were no effects ($P<.05$) in plasma C18:2 concentrations between treatments and the control, however, C18:3 was highest in the plasma of cows fed the FH supplemented diets

indicating protection of fat from rumen degradation. Medium chain fatty acid C12:0, and long chain fatty acids C14:0 to C16:0, were significantly lower in cows fed supplemental fat. Linoleic acid was increased ($P< 0.05$) and made up 10.34% of the milk fatty acids in the Linola fed cows versus 4.83%, 5.99% and 6.88% for control, FL and FH diets, respectively. Linolenic acid, C18:3, was greatest at 6.39% of the fatty acids in the milk from cows fed FH. The lower level of flaxseed inclusion in the diet still produced significantly higher C18:3 in the milk (3.70%) than either the control or the Linola, which were not significantly different (0.83 and 1.0% respectively).

In the second experiment, four Holstein primiparous cows in midlactation were randomly assigned to a 4x4 Latin square. Diets consisted of TMR top dressed with I) 0% added fat, Control, ii) formaldehyde treated flaxseed (FH) at 1.64 kg iii) low level lignosulfonate treated flaxseed at 0.57 kg (LL) and iv) high level of lignosulfonate treated flaxseed at 1.14 kg (LH). Approximately 377 g fat, 193 g fat and 386 g of fat were provided by the top dress. The flaxseed was treated with lignosulfonate by EXL Milling (Hassall, Sask.), ground and heated to 155°C and steeped for 30 minutes at a beginning temperature of 124°C and an end temperature of 118°C.

Treatment had no effect on feed intake or milk production parameters such as yield, fat, protein and SNF; rumen ammonia, VFA or PUN. Palmitic acid in blood plasma was not affected by either levels of lignosulfonate treated flaxseed in the diet, however, it was lower ($P<.05$) in the plasma of cows fed FH. Linolenic acid was present (10.64% of total fatty acids) in the plasma of cows fed FH at significantly greater levels than all other treatments. Linolenic acid was also significantly greater in the plasma of LH vs LL supplemented cows,

the latter being greater ($P<0.05$) than the control. This would suggest some ruminal bypass of the fat in the lignosulfonate treated flax.

Milk content of myristic acid (C14:0) was significantly affected by treatments with control>LL=LH>FH (9.72%, 9.19%, 9.09% and 8.39% of total milk fatty acids, respectively). Palmitic acid (C16:0) in the milk, was unaffected by the addition of either LL or LH, but was significantly decreased by the addition of the FH. Linolenic acid in milk was not significantly affected by the lignosulfonate treated flaxseed despite the increase present in the plasma. As expected, milk C18:3 was significantly greater in FH fed cows (3.51% vs 0.35% in the control). It appears that if properly protected from biohydrogenation in the rumen, flaxseed offers a unique opportunity to substantially improve the C18:3 in milk (or C18:2 in the case of Linola). This would represent a positive change from the standpoint of public perception of dairy products. The second experiment indicated that lignosulfonate and heat treatment of flaxseed was not effective in providing significant by-pass fat.

Key words: Flaxseed, Linola, formaldehyde, lignosulfonate, milk composition, fatty acids, linolenic acid.

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ABBREVIATIONS

ADF	-acid detergent fibre
ADIN	-acid detergent insoluble nitrogen
ALA	-alpha-linolenic acid
CLA	-conjugated linoleic acid
CP	-crude protein
DHA	-docosahexaenoic acid
DIM	-days in milk
DM	-dry matter
EE	-ether extract
EPA	-eicosapentaenoic acid
FA	-fatty acid
FCM	-fat corrected milk
FH	-flaxseed formaldehyde-high level
FL	-flaxseed formaldehyde-low level
GC	-gas chromatograph
HDL	-high-density lipoprotein
IDL	-intermediate-density lipoprotein
LCAT	-lecithin:cholesterol acyltransferase
LDL	-low-density lipoprotein
LH	-lignosulfonate flaxseed-high level
LL	-lignosulfonate flaxseed-low level
LPL	-lipoprotein lipase
MFGM	-milk fat globule membrane
MUFA	-monounsaturated fatty acid
N	-nitrogen
NDF	-neutral detergent fibre
NDIN	-neutral detergent insoluble nitrogen
NEFA	-non-esterified fatty acid
NH3	-ammonia
P	-probability
PUFA	-polyunsaturated fatty acid
PUN	-plasma urea nitrogen
SD	-standard deviation
SEM	-standard error of means
SFA	-saturated fatty acid
SNF	-solid non-fat
TG	-triglyceride (triacylglycerol)
TMR	-total mixed ration
VFA	-volatile fatty acid
VLDL	-very low-density lipoprotein
WMMB	-Wisconsin Milk Marketing Board

WRSB	-whole roasted soybean

INTRODUCTION

Saturated fatty acids have been implicated as a contributory factor in coronary heart disease and stroke. Any correlation between dairy products and cardiovascular disease is likely due to the effects of dietary myristic (C14:0) and palmitic (16:0), which can raise blood concentrations of low density lipoprotein (LDL) cholesterol (Noakes et al, 1996). Low density lipoprotein has been identified as a risk factor in cardiovascular disease. Milk fat, in general, is largely saturated (70%) and has, therefore, been subject to much criticism in regards to human nutritional health. The issues are far from clear as not all saturated fatty acids are equal in their hypercholesterolemic effects. Nonetheless, it remains possible to improve upon the fatty acid composition of dairy foods, including milk, by increasing proportions of polyunsaturated fatty acids such as omega-3-fatty acids (Hussein et al, 1996, Noakes et al, 1996, Lightfield et al, 1993, Kennelly and Khorasani, 1992).

Consumption of omega-3-fatty acids, as represented by ∝-linolenic acid ($C18:3_{n-3}$), have been associated with a decreased incidence of cardiovascular disease (Nash et al, 1995). Linolenic acid has been shown to reduce platelet aggregation, decrease plasma triglyceride levels, lower cholesterol and display tumorcidal and anti-thrombotic and anti-inflammatory effects (Cunnane, 1996, Simopoulos,1996, Nash et al, 1995). Flaxseed contains high levels of C18:3 and has been utilized to increase the proportions of C18:3 in meat, eggs and milk. In the case of ruminants, C18:3 must first be protected from biohydrogenation in the rumen. Polyunsaturated fatty acids (PUFA) generally undergo extensive hydrogenation by rumen microorganisms which largely results in the fat from such animals being mainly saturated.

Several methods have been proposed to offer protection of dietary fat from rumen

microorganisms. Formaldehyde has been used successfully to increase PUFA and monounsaturated fatty acids (MUFA) in milk by as much as 54%, with C18:2 and C18:3 comprising 35% and 22% of the total fatty acids respectively (Ashes et al, 1992, Grummer, 1991). The amount of alteration to the fatty acid composition of milk through feeding of formaldehyde protected supplements has been variable, likely due to variation among laboratories (Grummer, 1991). Formaldehyde treatment is, as of yet, unavailable in Canada. Lignosulfonate is derived from used sulfite liquor produced during sulfite digestion of wood and contains lignosulfonic acid or its salt, hemicellulose and sugars (Windschitl and Stern, 1988). This product represents a possible method with which to protect the fatty acids in fat supplements from hydrogenation in the rumen. Lignosulfonate binds and precipitates protein and has been used to make rumen by-pass protein supplements from treated canola or soybean meals (McAllister et al, 1993, Windschitl and Stern, 1988). Formaldehyde use on proteins had originally been developed to increase rumen escape protein. The formaldehyde products, combined with some form of protein such as casein, form a protein matrix which encapsulates the fat and protects it from hydrogenation by rumen microorganisms. If lignosulfonate could form a similar complex with the protein in a supplement, the potential then exists to provide by-pass fat as well.

The objectives of this study were to determine i) the viability and extent to which flaxseed could provide omega-3-fatty acids (linolenic acid) in milk when protected with formaldehyde, a substance known to confer protection in the rumen for fat and ii) the viability of lignosulfonate and heat to provide similar protection to the fatty acids in ground flaxseed in order to increase the levels of linolenic acid in milk fat.

LITERATURE REVIEW

Flax Overview

Flax (*Linum ustitatissimum L.*) or linseed, is an oilseed from the genus Linus and family Linaceae (Hanley, 1996). Flax originates from east of the Mediterranean Sea near India (Entz, M., Cr SC 28- Course Manual). Early North American settlers utilized flax as a fibre crop until cotton production became a more desirable source of fibre. The high content of linolenic acid (45-60% of the oil), which forms a durable film upon exposure to air continued to support the production of linseed oil from flax, ensuring a market as an industrial oil for use in paints, stains, mechanical lubricants and linoleum. There are two varieties of flax, one for oil production and the other for fibre; only the former is grown in North America. Flax production in North America rose and fell throughout the years due to drought, disease and replacement by petroleum products and synthetics in traditional markets. Environmental concerns are causing a move away from such synthetics and towards "greener" alternatives. Due to flaxseed's biodegradability, renewability and environmentally safe features, it's use as an industrial oil is being reevaluated. The unique features of flax, with it's high omega-3-fatty acids, is leading to new interest in human diets and animal feeds (Hanley, 1992).

Traditionally, flaxseed was popular as a livestock feed due to it's unique feature as a laxative or digestive regulator. The seed contains approximately 10% mucilage which is largely responsible for the laxative effect. It also is highly palatable (Hanley, 1996). Recent information on use as a protein supplement for dairy cattle is limited with insufficient data regarding the value of extracted flax as a feed for dairy cows (Khorasani et al, 1994). Linseed

meal is approximately 35% protein. Khorasani et al (1994) found that solvent extracted linseed meal was similar to canola meal in its ability to support milk production in late lactation cows.

Linseed oil content of flax ranges from 40-45% with variation due to the variety and growing conditions. Canadian flax is of superior quality and quantity due to cooler temperatures during a vital stage of growth (10 -25 days post-flowering) which promotes a higher overall oil content as well as an increased level of C18:3. Increases can be as high as 1%. This has lead to Canada being the number one exporter of flax (Hanley, 1996, Hanley, 1992). New varieties low in linolenic acid have been developed by Canada and Australia for use as an edible oil. The instability and rancidity of flax oil lead to the development of Linola, which has a very low linolenic acid content (2% or less) and a high level of linoleic acid. This new crop was eventually named Solin and defined as an oilseed developed from flax. Linola is a cultivar of Solin (Hormis and Rowland, 1996). Traditional varieties of flaxseed are being utilized in animal research due to the animal's ability to incorporate linolenic acid, an omega-3-fatty acid, into such things as eggs, meat and milk. Feeding flaxseed can increase the omega-3-fatty acids in eggs from 0.38% (of total fatty acids) in those birds fed no flaxseed, to 4.6, 8.9 and 11.5% from birds whose diets contained 10, 20 and 30% flaxseed, respectively (Caston and Leeson, 1990). Romans et al. (1995a) found increases in the alpha-linolenic acid (ALA) content of swine tissues such as backfat, kidney fat, bacon and loin chops when diets contained 15% ground flaxseed. Alpha-linolenic acid increased from a control level (no added flaxseed) of 1.2 mg/g of tissue to 6.9 mg/g when 15% flaxseed was added to the diet (uncooked bacon), 1.9 mg/g to 5.6mg/g in fried bacon, and 1.4 mg/g to 4.7 mg/g in

microwaved bacon which represents significant increases. Alteration of milk fat composition has been of interest to researchers for several years. The significant changes in C18:3 brought about by the feeding of protected linseed to dairy cows (McDonald and Scott 1977) provides solid evidence for the potential utilization of flaxseed to increase the omega-3-fatty acid level in milk and milk products. Even the inclusion of unprocessed or rolled flaxseed has shown some positive increases MUFA and PUFA. Short and medium chain fatty acids (C4-C12) and C16:0 were reduced while the concentration of C18 fatty acids were increased with inclusion of flaxseed in dairy cow diets in a study by Kennelly (1994). The changes in composition of the milk fat were proportional to the level of flaxseed included in the diet (Table 1). Concentrations of short, medium and long-chain fatty acids were 81%, 71% and 146% of the control diet when flaxseed was included at 15% of dietary dry matter (Kennelly, 1994).

Thus, if flax were to be incorporated into animal diets routinely, this could lead to increased markets and greater production of flax. Production figures for Manitoba (Honey, 1995) show flax at 403,900 tonnes total production (15.9 million bushels) which is up by 8.2% from 1994. This was attributed to a 15.7% jump in harvested area (Honey, 1995). Flax is the second most important oilseed in Western Canada. The average annual seeded area is 687 thousand hectares. In 1995, 876 thousand hectares were seeded to flax which was a 20% increase over 1994. Tonnage of flax seed equalled 1.10 million, a 14% increase from 1994. Manitoba contributed to 37% of this production, with Saskatchewan at 58% and Alberta totalling 5%. Above average yields and increased acreage contributed to this trend (Hormis and Rowland, 1996). Theoretically, for dairy alone, this tonnage could be increased by 895,260 tonnes per year if flax were to be routinely incorporated into lactating cow diets.

Table 1. The influence of feeding unextracted (ground) flaxseed on milk fatty acid composition.

Fatty Acid	Added Flaxseed (%) of Diet DM				SEM	Contrast[1]
	0	5	10	15		
C4:0	2.4	2.5	2.5	2.6	0.3	NS
C6:0	2.6	2.5	2.3	2.3	0.2	Li
C8:0	1.5	1.5	1.3	1.2	0.1	Li
C10:0	3.9	3.6	3.1	2.8	0.2	Li
C12:0	4.4	3.9	3.3	3.0	0.2	Li
C14:0	15.7	12.0	10.7	10.0	2.6	Li
C14:1	1.1	1.1	1.0	0.9	0.1	Li
C15:0	2.3	1.2	1.0	1.0	0.9	Li
C16:0	27.9	26.3	22.8	21.2	1.4	Li
C16:1	1.6	1.4	1.3	1.3	0.1	Li
C17:0	0.7	0.6	0.6	0.6	0.0	Li
C18:0	9.7	12.4	14.9	14.9	0.9	Li
C18:1	19.1	23.6	26.9	28.4	1.1	Li
C18:2	2.0	2.2	2.3	3.0	0.3	Li
C18:3	0.8	1.1	1.2	1.2	0.1	Li
C20:0	0.3	0.3	0.4	0.4	0.1	Li

1.Probabilities for contrasts: Li=linear, NS=not significant.
(Kennelly, 1994).

This figure is derived from 1,811.9 thousand head of milking cows in Canada (Dairy Farmers of Canada, 1995); using 3% added fat (Palmquist and Eastridge, 1991) as flaxseed, 305 days in milk (DIM) (Ensminger et al, 1990), and a dry matter intake of 3.1% of body weight for an average 600kg cow (Schmidt et al, 1988) during the first half of lactation.. Although this is an approximate figure only, it indicates a potential growth area for the production of flax, broadening market and crop rotation options for growers.

Principles Supporting the Desire to Alter Fatty Acid Composition of Milk Fat

There are 1,811.9 thousand head of milk cows and dairy heifers in Canada. Milk yield per cow is approximately 7.02 thousand litres (Dairy Farmers of Canada, 1995). The typical concentration of fat in milk ranges from 3.0 to 5.0% of total lipid. Fat is present in emulsified globules 2-4 μm in diameter surrounded by a membrane arising from the secreting cell. The membrane is mostly casein in the case of homogenized, or whole milk (Jensen et al, 1991). Milk fat percentage can be variable, depending on feeding management, added dietary fat and the type of fat fed (Palmquist and Eastridge, 1991). Overall, the typical composition of bovine milk includes protein at 3.2%, casein at 2.6%, fat at 3.9%, lactose at 4.6%, total solids at 12.7% and an ash content of approximately 0.7% (Jensen et al, 1991). The energy content averages 66 kcal per 100 ml. Again, other factors such as breed, diet, stage of lactation may affect these averages, but these numbers can be considered typical because of pooling, standardization of fat content and exclusion of milk containing colostrum or that which is mastitic (Jensen et al, 1991).

The interest in milk fat has largely been generated by economics, payment based on fat content in milk, processing effects from fat, the kinds and amounts of fats found in milk and lipids as a source of flavour (Jensen et al, 1991). Milk containing <2% fat has increased in demand from 20% to 60% of total fluid milk consumption in the past 20 years (Erdman and Teter, 1995). However, the price that producers are paid for milk fat has decreased by more than 60% in the last 15 years (Erdman and Teter, 1995). Current consumer perceptions regarding fat in diets in general has also contributed greatly to the interest in altering and manipulating milk fat and milk fat content. Consumers have access to hundreds of dairy products, including 400 kinds of cheese alone. In recent years, an increase in low fat products has been observed. However, present day processing, which can alter total fat content, has little effect on fatty acid composition (Jensen, 1992).

Composition of Milk Fat and It's Relationship to Human Health

Ninety-eight percent of the milk fat globule is triacylglycerol, followed by phospholipids at 0.5-1.0% and sterols at 0.2-0.5%. These latter two are found mainly in the globule membrane . Of the sterols, cholesterol represents the largest fraction at 10-20mg/dl (Jensen et al, 1991). Jensen (1992) approximates the cholesterol level in whole milk at 15mg/dl (0.46% of total lipid). Ney (1991) gives an average value for cholesterol in milk fat at 0.20-0.25% of total lipid. Average values of the various classes of lipids found in milk are indicated for cows in early lactation (Table 2).

Approximately 400 fatty acids have been found in milk lipids (Jensen et al, 1991). The

Table 2. Lipids in milk at 42 days in milk (12 cows).

Lipid Class (% of Total Lipid)	
Phospholipid	1.11
Cholesterol	0.46 (15 mg/dl of whole milk)
Triacylglycerol	95.80
1,2-Diacylglycerol	2.25
Free Fatty Acids	.28
Monoacylglycerol	.08
Cholesteryl ester	.02
Fat (g/dl)	3.25

(Jensen et al, 1991).

ten major fatty acids found in milk 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 profile of milk contains roughly 10% short chain saturated fatty acids (<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). In general, milk fat contains 5% PUFA, 70% saturated fatty acids (SFA) and 25% MUFA (Grummer, 1991). Large quantities of C18:1, 20-27%, are present in the milk fat globule, with small amounts of trans-18:1 at 3% (Jensen, 1992). Jensen et al (1991) listed trans-C18:1 a little lower at approximately 2%. C18:2 is present at about 2% of total fatty acids with only trace amounts of C18:3. In fact, milk fat is considered a poor source of C18:2 and omega-3 fatty acids such as C18:3. Milk provides only 0.6 grams per day of PUFA (from 2.5% C18:2 and <1% omega-3). The human requirement (based on 2560 kcal intake and 1.5% of total calories as C18:2 and 0.5% of total calories as omega-3 fatty acids) for adult males is 4.27 grams C18:2 and 1.42 grams C18:3 (Jensen, 1992). The structure of the triacylglycerol molecule itself is unique, with most of the C4:0 to C10:0 being found at the sn-3 end of the glycerol moeity. Table 3 gives an overall indication of the fatty acids present in the triacylglycerol portion of milk and their approximate quantities in mol/100 mol fatty acid.

Fat from dairy products has been labelled a hypercholesterolemic fat due to the content of cholesterol and saturated fats. Current recommendations in regards to human health call for a reduction in calories derived from fat (see Table 4 for current recommendations of percent of calories from fat, carbohydrates and protein). Epidemiological studies have continuously shown a clear relationship between raised serum cholesterol levels and an increased risk of death from coronary heart disease. However, it

Table 3. Quantity of fatty acids present in triacylglycerol in cow's milk under normal feeding conditions.

Fatty Acids (mol/100mol)	
C4:0	11.8
C6:0	4.6
C8:0	1.9
C10:0	3.7
C12:0	3.9
C14:0	11.2
C15:0	2.1
C16:0	23.9
C16:1	2.6
C17:0	0.8
C18:0	7.0
C18:1	24.0
C18:2	2.5
C18:3	Trace

(Adapted from Jensen et al, 1991)

Table 4. Dietary recommendations by the National Cholesterol Education Program.

Nutrient	Daily Allotment
Total fat	< 30% of total calories.
Saturated fat	< 10% of total calories.
Polyunsaturated fatty acids	Up to 10% of total calories.
Monounsaturated fatty acids	10-15% of total calories.
Carbohydrates	50-60% of total calories.
Protein	10-20% of total calories.
Cholesterol	<300mg/day.
Total Calories	To achieve and maintain desirable weight.

(Ney, 1991).

remains debatable as to the efficacy of lowering blood cholesterol utilizing dietary means. It is known that higher levels of low density lipoproteins (LDL cholesterol) is related to increased risk of coronary heart disease and that, in general, decreasing total and LDL cholesterol levels reduce the risk of coronary heart disease (Ney, 1991). The recommendations of the National Heart, Lung and Blood Institute for the National Cholesterol Education Program suggested serum cholesterol levels of less than 200mg/dl to be desirable. Levels between 200 and 239 mg/dl are considered borderline high risk and greater than 240 mg/dl as high risk (Ney, 1991). The issue, however, does not remain that simple. Milk fat and products such as butter have been implicated in a higher rate of coronary heart disease in different countries. The hypercholesterolemic consequences from milk products has been demonstrated in a number of controlled studies (Noakes et al, 1996). Again, effects of these products are generally on the plasma LDL cholesterol concentration. However, reducing dietary cholesterol has little effect on plasma levels for most people (McNamara, 1990). A decrease in dietary cholesterol resulting in "some" decrease in plasma cholesterol is applicable for only about a third of the population. Other risk factors include cigarette smoking, hypertension, glucose intolerance, obesity and lack of exercise (Jensen, 1992). With respect to hypercholesterolemia, moderate intake of dairy foods by those who are not susceptible should not be considered as contributory to coronary heart disease. Milk and milk products contribute about 38 mg/day and 60 mg/day of cholesterol for women and men, respectively. The average total daily intake of cholesterol for women is 304 mg/day and 435 mg/day for men (Ney, 1991). Thus, dairy products only contribute about 12.5% and 13.8% of the average daily intake of cholesterol for women and men respectively. Current

research also has indicated that moderate amounts of dietary cholesterol (300 mg/day) are not responsible for elevated plasma levels (Jensen, 1992). Therefore, contribution from dairy products is well below this limit and therefore is unfairly suspect. Milk fat provides far less to total fat intake in the typical American diet compared to other animal products, contributing only 15% of total cholesterol intake, according to Ney (1991).

The Bridge Project, sponsored by the Wisconsin Milk Marketing Board (WMMB), and organized and administered by the National Dairy Council , was formed to unite nutrition with milk marketing and dairy products. An independent task force of nutrition scientists identified dietary fat, cholesterol and health as the most important issues facing the dairy industry in terms of nutrition and consumer acceptance. The information generated by these committees is available as a data base for the dairy industry to plan and operate future promotion and product development efforts (O'Donnell, 1989). An ideal milk fat profile was developed by this group of scientists. Ideal milk fat would consist of 10% PUFA, 8% SFA and 82% MUFA. This is an unrealistic profile for milk fat and probably not possible to obtain by dietary manipulation. Milk fat, as previously stated, contains approximately 5% PUFA, 70% SFA and 25% MUFA . The gap between the ideal profile of milk fat as set out by the WMMB and the actual composition of milk fat is simply too large to be compensated for realistically by dietary modification (Grummer, 1991). It is therefore, critical to reexamine the effects of the actual fatty acids and their impact on human health and coronary heart disease. As in the case with cholesterol, the relationship of good and bad fatty acids is far from clear. The amount of fat consumed in the United States is approximately 37% en (en= total calories). Consumption of 30% en divided equally among saturated, MUFA and PUFA

fats is the current recommendation by the National Research Council, 1989 (Jensen, 1992). Dairy products contribute approximately 25-29% of saturated fat intake, not including butter (Noakes et al, 1996). However, it is interesting to note that not all saturated fats are equal in their hypercholesterolemic effects. Research implicates those of carbon chain length 12, 14 and 16 (Ney, 1991) to be hypercholesterolemic. Nevertheless, data for C12:0 is contradictory and the acid may or may not be hypercholesterolemic. Fatty acids of chain length C4:0 to C10:0 act like carbohydrates and thus do not affect plasma cholesterol (Jensen et al, 1991). Excess consumption of saturated fatty acids, particularly C14:0 and C16:0 is said to increase plasma cholesterol (Grundy and Denke, 1990). Hayes et al, (1991) concurs with the effect of C12 and C14 but has shown C16:0 to be less hypercholesterolemic. Noakes et al (1996), agrees with this stating that myristic acid (C14:0) seems more potent in its ability to raise blood lipids in humans than palmitic acid (C16:0). Those saturated fatty acids with less than 12 carbon atoms or saturated C18:0 and monounsaturated fats with 18 carbon atoms (C18:1) do not raise blood cholesterol relative to polyunsaturated fatty acids (Ney, 1991). In fact, when stearic acid or oleic acid replaces palmitic acid (C16:0) in the diets of men, plasma cholesterol levels are lowered (Bonanome and Grundy, 1988). Thus, C18:0, C18:1 and C18:2 are considered hypocholesterolemic. Consuming amounts of C16:0 and perhaps C12:0 and C14:0 greater than 10% of total calories can increase plasma cholesterol levels, but the presence of C18:0, C18:1 and C18:2 in milk and other products may lessen this effect (Jensen et al, 1991). Because milk fat contains approximately 10% short to medium chain saturated fatty acids (<12 carbon atoms), 12% stearic acid and 25% oleic acid, approximately 60% of the fatty acids in milk are not hypercholesterolemic (Ney, 1991). The 10% myristic

acid and 26% palmitic acid, therefore, are those left which have been implicated in coronary heart disease . The situation becomes even more clouded and uncertain regarding the importance of reducing total dietary fat vs saturated fat alone and whether or not position on the triacylglycerol molecule and it's unique structure in milk fat has any effect on lipid metabolism (Ney, 1991). The maximum recommended amount of atherogenic fatty acids (C16:0 and C14:0) is 28 grams. One quart of whole milk (3.3% fat) contains approximately 12.6 grams of these fatty acids which is well within the recommended limit (Jensen, 1992). Still, modifying dairy products by utilizing such things as protected unsaturated fatty acids in the feed results in milk and tissue lipids lower in saturated fats (Noakes et al, 1996). Thus, there is opportunity to improve upon and generate a more consumer friendly milk product. The challenge remains to at least partially replace the undesirable fatty acids in milk and dairy products with unsaturated (both poly- and mono-) fatty acids.

Significance of Omega-3-Fatty Acids, Trans Fatty Acids, Conjugated Linoleic Acids and Human Health

Omega-3-fatty acids are so called based on the location of the first double bond, counting from the methyl end of the molecule (C18:3ω3 or C18:3n3). Omega-3-fatty acids are one of the two classes of polyunsaturates, the other being omega-6 fatty acids. The former is represented by α-linolenic acid and the latter by linoleic acid (C18:2ω6) (Simopoulos, 1996, Khorasani and Kennelly, 1994). These fatty acids are essential for normal growth and development since mammals are incapable of inserting double bonds beyond C10

and the methyl end of the fatty acid (Simopoulos, 1996, Lehninger et al, 1993 and Maynard et al, 1979). Plants are capable of synthesizing both C18:2 and C18:3(Lehninger et al, 1993). In mammals, linoleic and linolenic acid play a role in the structure and function of biological membranes, particularly in the central nervous system and retina (Nash et al, 1995). Omega-3 fatty acids have been implicated in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis and other such imflammatory diseases as well as cancer (Simopoulos, 1996). Omega-3-fatty acids show a cardioprotective effect and reduced occurrence of coronary heart disease. Diets which are rich in these fatty acids, such as that consumed by Northern Eskimos, show reduced platelet aggregation and this has been linked to the low incidence of cardiovascular diseases in these peoples (Nash et al, 1995). These diets have been associated with a low incidence of heart disease, low plasma TG (triglyceride) levels and a relatively long blood coagulation time (Anderson and Sprecher, 1987). A reduction in very low density lipoprotein (VLDL), which carries a large portion of the TG in the blood, was seen in individuals who ate a diet high in omega-3-fatty acids versus those who did not (Anderson and Sprecher, 1987). According to Anderson and Sprecher (1987), low density lipoprotein (LDL) cholesterol is reduced regardless of the family of polyenoic acid consumed. This is likely due to increased catabolism when ω-6 fatty acids are consumed and also a decrease in the synthesis of apolipoprotein B, a major constituent of LDL, from omega-3-fatty acids. In diets with high intakes of ω-3, high density lipoprotein, often referred to as good cholesterol, has been reported to increase. Total cholesterol is reduced only with very high levels of ω-3, probably due to the concomitant high intake of cholesterol with the omega-3 fatty acids in the fish oil. Recommendations by the Canadian government are at 3%

omega-6-fatty acids and 0.5% omega-3-fatty acids of daily energy intake for adults (Nash et al, 1995). Deficiency of omega-3-fatty acids result in poor growth and eczematous skin (Anderson and Sprecher, 1987). The periods of neonatal and aging in the human lifespan have shown an essentiality of ω-3 fatty acids. This, and the implications of the importance of omega-3 fatty acids in the improvement of several diseases including coronary heart disease, has led to a great deal of interest in improving the proportions of these fatty acids present in mammalian tissue, meat, eggs and milk (Leskanich et al, 1997). However, susceptibility to oxidation is a concern in omega-3 fatty acid enriched products such as milk fat. Damage to biological tissues and implications in other diseases such as cancer, from lipid oxidation products is a concern and requires further investigation in this area (Aymond and Van Elswyk, 1995).

Linolenic acid is found in the chloroplasts of green leafy vegetables, canola, soybeans and walnuts (Simopoulos, 1996). It is particularly high in flaxseed oil, found in the amount of 5 times that of canola (Khorasani and Kennelly, 1994). In fact, flaxseed contains the highest levels of α-linolenic acid of all of the oilseeds, comprising approximately 18% of the total seed weight and 53% of the fatty acids on average (Romans et al, 1995a). When female volunteers were fed 50 grams of ground, raw flaxseed per day for a total of four weeks, α-linolenic acid and long chain ω-3 fatty acids were raised in both plasma and erythrocyte lipids. Serum cholesterol was decreased by 9% and low density lipoprotein cholesterol by 18% (Cunnane et al, 1993).

Omega-3-fatty acids are metabolised to eicosapentaenoic acid (EPA-C20:5_{n-3}) and docosahexaenoic acid (DHA-C22:6_{n-3}), which are longer chain fatty acids and more

unsaturated by the addition of extra double bonds to the carboxyl end of the fatty acid (Leskanich et al, 1997, Simopoulos, 1996, Romans et al, 1995a). In mammalian tissue, the omega-3-fatty acids are found in TG, cholesteryl esters and to a limited extent in phospholipids. Eicosapentaenoic acid is largely found in the cholesteryl esters, TG's and phospholipids. Docosahexaenoic is present mostly in phospholipids (Simopoulos, 1996). Both of these fatty acids may be derived from linolenic acid or obtained in the diet. Fish oils are particularly high in EPA and DHA (Simopoulos, 1996). Elderly people may require dietary EPA and DHA due to a limited capacity to elongate and desaturate C18:$3_{\omega 3}$ (Nash et al, 1995). Eicosapentaenoic acid is transformed into eicosanoids which includes prostaglandins, thromboxanes, prostacyclins and leukotrienes, which perform many regulatory functions and are found throughout the body (Anderson and Sprecher, 1987). They are also involved in the maintenance of normal growth and development in the brain (Romans et al, 1995a). The families of fatty acids, such as the ω-3 and ω-6, compete for enzyme systems. If there is an excess of ω-6 fatty acids, this will obstruct synthesis of the eicosanoids of the ω-3 family (Simopoulos, 1996, Anderson and Sprecher, 1987). Evidently, it is the EPA and DHA, from the metabolism of the omega-3-fatty acids, which also seem to lower blood TG levels, reduce the risk of blood clots and reduce the inflammation resulting from rheumatoid arthritis (Leeson and Caston, 1996). In experiments conducted with Leeson and Caston in cooperation with B. Holub (Leeson and Caston, 1996), volunteers had consumed omega-3-fatty acid rich eggs, with no effect on blood cholesterol resulting from those eating either enriched eggs, regular eggs or control (no eggs). This concurs with other studies mentioned previously where dietary cholesterol manipulation has not been found to affect blood

cholesterol in most individuals. The enriched eggs produced a slight decrease in serum TG and a significant increase in DHA in blood platelets (35% increase). Docosahexaenoic acid aids in preventing blood platelet aggregation, a condition which could lead to various cardiovascular problems (Leeson and Caston, 1996).

The addition of hydrogen to unsaturated fatty acids to make them more saturated (harder, less susceptible to oxidation) is a process known as hydrogenation, This however, results in the production of at least some trans fatty acids which have been criticized for their unhealthy nutritional effects (Kaylegian et al, 1993). Trans fatty acids in the American diet largely arise from partially hydrogenated vegetable oil products such as margarine (Ney, 1991), which in some cases make up 90-95% of the trans-fatty acids found in human tissue (Wolff, 1994). Beef and milk fat are also sources of trans-fatty acids, however, the contribution of trans fatty acids from ruminant milk and meat in the American diet is only moderate (Wolff, 1994). According to the review by Ney (1991), the main trans isomer in ruminant fat is vaccenic acid a trans-11-octadecenoic acid, formed by incomplete hydrogenation of poly-unsaturated fatty acids. These trans isomers are the metabolic intermediates in the hydrogenation of such fatty acids as C18:2 and C18:3. Further conversion to C18:0 is the rate limiting step (Grummer, 1991). The trans C18:1 in products such as margarine occur at C9 to C12. Milk fat trans isomers range from 4.3 to 7.6% depending on feeding management (Ney, 1991). The trans fatty acids from vegetable fats may act like fatty acids C12, C14 and C16 in raising blood cholesterol. Intake of trans fatty acids in the U.S. is between 2-4%. The range of intake, however, is between 1.8-20% which could represent health problems for certain individuals. When 10% of daily energy came from

trans rather than cis-oleic isomers, the level of LDL cholesterol was increased and HDL decreased in both men and women. Whether or not this is important is unclear because the average intake of trans fatty acids is only 20-45% of that used in this study (Ney, 1991). Human subjects fed a partially hydrogenated sunflower oil produced significantly higher serum cholesterol and LDL levels and decreased HDL levels compared to a diet high in cis-unsaturated fatty acid diets (Zock and Katon, 1992, Mensink and Katan, 1990). Also, from a dairy management and production perspective, trans fatty acids in either the diet the cow is consuming or from incomplete biohydrogenation in the rumen, are thought to inhibit milk fat and fatty acid synthesis if present in large enough amounts. This, however, has not been unequivocally demonstrated (Palmquist et al, 1993).

Conjugated linoleic acid (CLA) has recently gained considerable interest due to the antioxidative and anticarcinogenic properties shown by this intermediate in the biohydrogenation of linoleic acid (Jiang et al, 1996). Conjugated linoleic acid contains various positional and geometric isomers of octadecadienoic acids with conjugated double bonds (Kelly and Bauman, 1996, Jiang et al, 1996). The most biologically active form appears to be cis-9, trans-11-octadecadienoic acid (C-9, t-11-C18:2) due to the fact that it is the main isomer found in the phospholipids of cell membranes (Jiang et al, 1996). According to Jiang et al (1996), although CLA's are present in vegetables as well as in meat and milk fat, products of ruminant origin are the highest source, and in particular, c-9, t-11, C18:2 is the major isomer found in dairy products. Conjugated linoleic acids are derived by the rumen bacteria, *Butyrivibrio fibrisolvens*, and is the first intermediate in linoleic acid hydrogenation.

Manipulation of the fatty acid composition of mammalian tissues, eggs and milk, is a result of the concern consumers have regarding the saturated fatty acids in animal products and the rising awareness of the role unsaturated fatty acids play as regulators of cell function (Jenkins et al, 1996, Schingoethe et al, 1996, Lightfield et al, 1993). The response of individuals to the various saturated, unsaturated, short, medium and long chain fatty acids may be variable according to genetic expression and is not completely understood (O'Donnell, 1989). Thus, this should be kept in mind when exposed to broad statements regarding the negative effects of dairy products.

Potential Technologies for Changing Milk Fatty Acid Composition

Using nutrition as a means of altering milk fat and fatty acid profiles is a complex issue. Many factors play a role in milk fat synthesis including feed intake, meal frequency, forage to concentrate ratio, fat and type of fat in the diet and carbohydrate make-up of the concentrate (Sutton, 1989). The greatest effects seem to be from intake and type and level of lipid supplements (Sutton, 1989, Jensen et al, 1991). Nutrition as a means of altering milk fat composition was recognized in 1938 when Powell (Sutton, 1989) found a correlation between roughage intake and type with variations in milk composition. Fat concentration is the most sensitive of the major milk components to nutritional manipulation. Concentration of fat in milk can be altered over a wide range of approximately 3 percentage points. Protein may be altered by about one-fifth of this amount with little, if any, effect on lactose (Sutton, 1989). Roughage is important in maintaining milk fat concentration. The length of fibre is

also a contributing factor, with a mean length of approximately 0.6 to 0.8 cm minimum for the maintenance of milk fat. Decreasing the forage to concentrate ratio generally results in a decrease in milk fat, although this response can be variable. With the forages that are generally used in dairy diets, it seems that fat concentration is fairly stable until the forage concentration falls below 50% where changes become inconsistent. The source of carbohydrates play an important role in this variability. Soluble carbohydrates generally maintain a higher milk fat than starch (Sutton, 1989). Dietary fat, added anywhere from 6-8% (diet dry matter) usually increases milk yield but fat concentration is variable (Sutton, 1989). Feeding supplemental fat in the form of alginate treated tallow, increased milk yield from 31.7 kg/d to 32.9 kg/d but had a negative effect on milk protein concentration (Hoffman et al, 1991). Nonnutrient additives such as buffers and antibiotics offers some control of rumen fermentation patterns, which would also affect milk fat (Sutton, 1989).

According to Jensen et al (1991), fatty acid composition is not greatly affected by typical changes in diet due to biohydrogenation in the rumen and the production of short chain fatty acids in the mammary gland. Effects can and do occur when cows are underfed, fed larger quantities of fat or fed protected fats. For example, a protected oil high in C18:2 produced linoleic acid rich milk with a change from 2.5 mol/100 mol fat in normal milk to 15.3 mol/100 mol fat in the milk from cows receiving oil encapsulated in denatured casein (Jensen et al,1991). Sutton (1989) states that nutrition may offer the best means of changing milk composition rapidly in order to meet varying changes in consumer demand. The original objective of dietary manipulation was to reduce the amount of C16:0 and increase the proportion of C18:1 (Ney, 1991). This is fairly easily accomplished, particularly by utilizing

fat supplements such as roasted soybeans. Monounsaturated C18:1 and saturated C18:0 may be increased by 55-80% (Grummer, 1991). Feeding diets lower in roughage also increases the proportion of oleic acid (Grummer, 1991). Fatty acids C6 to C14 were decreased by the addition of supplemental fat (Hoffman et al, 1991).

The changes that would occur as a result of breeding and genetics is slow (Sutton, 1989). Gibson (1991) concludes that despite the fact that milk fat composition could be altered through genetics, economic incentives are not clear. Changes to the fat would be positive for some products while negative for others and the differences due to conventional genetic manipulation would be too gradual and therefore of little value to the breeder. Thus, alterations to the fatty acid composition of milk is not likely to be a part of genetic improvement programs in dairy cows.

Special technologies in milk processing have been examined as ways to improve upon the nutritional profile of milk fat. Due to the low level of cholesterol (0.20-0.25% of total lipid) in milk fat, removal requires special technology, utilizing one or more of three physical or chemical properties; solubility, adsorption or molecular conversion (Ney, 1991). However, due to the controversial evidence surrounding the contribution of dietary cholesterol to blood cholesterol and heart disease in humans, the expense of such technology has kept this type of processing from becoming commonplace. In fact, according to Ney (1991), it is more likely to be utilized to produce a specific product rather than a general milk product.

Milk fat fractionization is being offered as a means to develop designer milk fat and milk products (Ney, 1991). This technique relies on melting point, molecular weight, sensitivity to detergents and solubility properties to manipulate fatty acid make up of milk fat

fractions. This could provide a fat which contains a nutritionally improved fatty acid profile (Ney, 1991). Lai et al (1995), found that changes in the fatty acid composition and triacylglycerol of butterfat derived by the fractionization process improved it's nutritional profile and resulted in similar lipoprotein cholesterol and very low density lipoprotein concentrations relative to corn oil in rats on a high cholesterol diet.

Synthesis of Milk Fatty Acids

Adipose Tissue Mobilization

The mammary gland relies on two main sources, rumen fermentation and circulating blood lipids, for fatty acid synthesis and incorporation into milk fat (Grummer, 1991). The blood lipids arise either from the diet the animal is consuming or from the mobilization of adipose tissue stores. The adipocyte, or fat cell, has only two major functions, the synthesis of fat and the mobilization, or breakdown of fat (Bauman and Currie, 1980). Adipose tissue is in a constant state of transformation. Mobilization, transportation, combination and/or conversion to other fatty acids, degradation and reesterification with glycerol as well as transportation back to the depots is so well balanced that the fatty acid profile in the blood, organs and depots for any given species remains quantitatively and qualitatively relatively constant (Maynard et al, 1979). Ruminant adipose tissue stores fat as a neutral fat and utilizes acetate as a substrate. Water (4.5-14.4%) and some nitrogen (0.18-0.62%) are also present in adipose tissue (Maynard et al, 1979). Hydrogenation of dietary unsaturated fatty acids by the rumen microorganisms leads to an adipose tissue fat that is largely saturated in nature

(Noakes et al, 1996). Grummer (1991) states that due to the effects of ruminal microorganisms on unsaturated fatty acids (hydrolysis and hydrogenation), both tissue and milk fatty acids tend to be more saturated. Esterified long chain fatty acids stored in adipose triglycerides are derived mostly from short-chain fatty acids as a result of de nova synthesis. Approximately 90% or more of lipogenic activity takes place in the bovine adipose tissue (Dunshea and Bell, 1989). Storage of triglycerides within the adipocyte is a result of an equilibrium between fatty acid uptake, de nova synthesis, fatty acid esterification, triglyceride hydrolysis and reesterification of fatty acids released by lypolysis (Chilliard, 1993). Any glycerol released during the breakdown of TG by the adipose tissue is not reutilized by the adipocyte (Dunshea and Bell, 1989).

Plasma insulin levels decline after calving and actually begin to decline in the close up dry period (Bell and Bauman, 1996). These levels remain low for the first few weeks. Plasma insulin has an antilipolytic effect. It is possible to artificially raise insulin levels, yet the adipose tissue remains highly resistant to the anabolic effects of such insulin in the close up dry period and a few weeks post-calving (Bell and Bauman, 1996). At the same time, endogenous levels of plasma somatotropin increase and are highest during the transition period (Bell and Bauman, 1996). Lipolytic response to catecholamines is increased in the periparturient cow and β-adrenergic receptors increase on bovine adipocytes around calving as well (Bell and Bauman, 1996). Other hormones such as cortisol, prolactin and estradiol also increase at and around calving. However, further study to validate the effects of these hormones is still necessary (Bell and Bauman, 1996).

The importance of fat mobilization lies in the fact that the cow is in peak lactation

approximately 3-6 weeks post-calving yet maximum feed intake does not coincide and only reaches a maximum several weeks later. This results in the dairy cow being in a negative energy balance (Dunshea and Bell, 1989). Body fat is thus mobilized to compensate for the negative energy state that the cow is in. However, the rate of lypolysis (triglyceride hydrolysis) is not equal to fat mobilization. The fatty acids coming from mobilized adipose stores are actually equal to the net difference between the intracellular rates of esterification of fatty acids and TG lypolysis. Furthermore, fatty acids esterified to make TG may result from several sources including uptake of preformed fatty acids from plasma, de nova synthesis or recycling of non esterified fatty acids (NEFA) within the adipocyte. Any changes in any one of these processes can affect the net rate of fat mobilization (Dunshea and Bell, 1989). Still, these fat stores become extremely important for high yielding cows in early lactation. Genetically speaking, in order for a dairy cow to be a high producer, she must have the ability to lay down and mobilize body stores at this stage in time to fully reach her milk production potential (Dunshea and Bell, 1989). For most dairy breeds (not including the British Friesen), the internal stores of body fat, or that which is associated with the internal organs rather than muscle or subcutaneous fat, tend to play a larger role in fat storage and mobilization for early lactation (Dunshea and Bell, 1989).

Non-esterified fatty acids comprise the main transport form of mobilized body fat (Dunshea and Bell, 1989). Short chain fatty acids do not occur in blood plasma (Maynard et al, 1979). Non-esterified fatty acids are bound to plasma albumin for transport in the blood. In fact, one molecule of serum albumin can carry up to ten molecules of free fatty acid (Lehninger et al, 1993). The mammary gland, plus a variety of other tissues, utilize these

fatty acids mobilized from body adipose tissue. The majority, however are taken up by the hepatic system. In fact, approximately 80% of the mobilized lipid will be oxidized in non-mammary tissues such as the liver and muscle (Bell and Bauman, 1996). Uptake by the hepatic system increases as the blood concentration of NEFA becomes greater. If the energy balance of the diet is low, blood lipid mobilization increases. Thus, mobilization of adipose tissue fatty acids leads to an increase in the NEFA (long chain) which in turn increases metabolism of these fatty acids in the liver (Grum et al, 1996). The liver may then either oxidize or esterify the fatty acids, generally to glycerol forming triglycerides. These may then be stored or removed from the liver as very low density lipoproteins. The removal of VLDL from the liver is not believed to occur at a very high rate in ruminants (Grummer, 1991). Dunshea and Bell (1989) state that the hepatic uptake of NEFA increases as energy balance decreases and body mobilization rises. As the plasma NEFA concentration increases, non-mammary utilization of NEFA's also increases, especially in those tissues such as the heart, skeletal muscle and kidneys which have a high capacity for long chain fatty acid oxidation. This oxidation in non-mammary tissues contributes to the overall catabolism of NEFA, though not directly. However, the liver has a limited capacity to synthesize and secrete lipoproteins, which results in fat accumulating in the liver (Bell and Bauman, 1996). Fatty acids of dietary origin generally bypass the liver. Chylomicrons enter the lymphatic system for one, and the liver itself does not contain appreciable quantities of lipoprotein lipase and hepatic lipase. Adding fat to the diet may increase the role that the liver plays in the metabolism of dietary fatty acids. This may be a result of an increase in NEFA in the blood or increased uptake of high density lipoproteins and/or chylomicron remnants (Grum et al,

1996).

The adipose tissue contributes to the milk fat both directly and indirectly. By sparing lactogenic nutrients due to increased metabolism of fatty acids in non-mammary tissue, there is an indirect contribution to milk fat resulting from the mobilization of body tissue (Dunshea and Bell, 1989). The mobilized fatty acids undergo oxidation in other tissues which spares glucose and produces energy in support of mammary lactose secretion (Chilliard, 1993). In early lactation, the mammary gland places a direct demand on adipose tissue stores for incorporation into milk fat. Fatty acids in milk containing more than 16 carbon atoms are preformed. Most of these will come from the adipose stores when the cow is in a negative energy balance (Dunshea and Bell, 1989). Utilization of preformed long-chain fatty acids increases as the negative energy balance becomes larger and body mobilization of fat increases (Dunshea and Bell, 1989). Thus, adipose tissue provides a greater contribution to milk fat early in lactation than it does in later lactation. Overall, of the blood fatty acids, only 12% is of animal origin, while 88% is derived from the diet (Palmquist and Mattos, 1978). The mammary gland usually only utilizes TG from blood plasma and not NEFA except when those concentrations of NEFA are at higher levels (300 μ-equiv. per litre) such as the case during mobilization of adipose tissue stores (Moore and Christie, 1979). Adrenegenic stimulation of lypolysis and increased sympathetic nervous activity at day of calving or soon after, results in the highest level of plasma NEFA's (Bell and Bauman, 1996). Lipogenesis is suppressed at this time. It must be noted though, that the values derived from Palmquist and Mattos (1978), were from low producing cows and the contributions of dietary versus endogenous fatty acids to the milk fat may be affected by other things such as milk production, stage of

lactation and plane of nutrition (Grummer, 1991). Estimates of milk fat synthesized from the blood lipids is approximately 50% (Grummer, 1991). Therefore, it may be assumed that the adipose tissue contributes to 6% of the milk fat. Bell and Bauman (1996), however, predict that a cow producing 29.5 kg of milk at 4.7% fat and four days postpartum, will utilize NEFA for only 40% of the milk fat synthesized which accounts for 20% of the mobilized lipid. The rest undergoes oxidation in non-mammary tissues as previously discussed. According to Bauman and Currie (1980), examining data from past studies, the body reserves being utilized for lactation accounted, energetically speaking, for 33% of the milk produced.

Long chain fatty acids which are incorporated into the milk fat from the adipose tissue inhibit de nova synthesis of the short chain fatty acids by the mammary gland. All short chain fatty acids, with the exception of C4:0, are low in early lactation (Palmquist et al, 1993). By eight weeks, these short chain fatty acids will be at 90% of their maximum. This coincides with the inhibition of the mobilization of adipose tissue, which is generally finished by the fourth to sixth week of lactation (Palmquist et al, 1993). In the case of C4:0, two separate pathways independent of the acetyl-coenzyme A(CoA) carboxylase pathway result in there being little effect from adipose tissue mobilization on this fatty acid. Typically, fatty acid synthesis involves a source of substrates and related enzymes to undertake conversion to acetyl CoA. NADPH is also produced and further conversion to malonyl CoA takes place which is subsequently added to a "primer" (acetyl CoA and/or butyryl-CoA) until the fatty acid is released from the fatty acid synthetase complex. Acetate and β-hydroxybutyrate are the main carbon sources in the ruminant animal and not glucose as in the case of the monogastric (Maynard et al, 1979). The principle pathways are as follows:

1. $CH_3COSCoA + HCO_3^- + ATP \rightarrow HOOC{\cdot}CH_2{\cdot}COSCoA + ADP + Pi$
2. $CH_3COSCoA + 7HOOC{\cdot}CH_2{\cdot}COSCoA + 14NADPH \rightarrow$
$CH_3{\cdot}CH_2(CH_2(CH_2{\cdot}CH_2)_6{\cdot}CH_2{\cdot}COOH + 7CO_2 + 14NADP + 8CoA + 6H_2O$

In the first reaction, acetyl CoA undergoes carboxylation to malonyl CoA. The reaction is catabolized by acetyl-CoA carboxylase. In the second step, a group of enzymes referred to as fatty acid synthetase catalyze the reaction. These are the main reactions responsible for the de nova synthesis of fatty acids in the mammary gland (Moore and Christie, 1979). Previous ^{14}C labelling studies have shown that carbon atoms one and two of 4:0 arise from malonyl CoA and carbons three and four come from acetyl CoA. Fatty acids C6:0 and C8:0 utilized malonyl CoA for subsequent additions of two carbon units (Moore and Christie, 1979). However, it was also discovered that an intact four carbon unit is utilized for fatty acid synthesis in the mammary gland (Moore and Christie, 1979). Preformed 4-carbon units, specifically β-OH-butyric acid, gives rise to approximately one half of the C4:0. Butyric acid (C4:0), also comes from the condensation of acetyl units in a β-reduction pathway which is independent of the malonyl-CoA pathway. Inhibition of chain length increases as chain length becomes greater (Palmquist et al, 1993). Fewer acetyl units are required to combine with the four carbon primer on shorter chain fatty acids and none are required on preformed four carbon fatty acids thus inhibition resulting from NEFA has a lesser effect the shorter the chain length and none on C4:0. The more acetyl unit additions required from the acetyl-coenzyme A carboxylase pathway, which can be inhibited, the greater the effect of inhibition (Palmquist et al, 1993). Both lipoprotein lipase and acetyl CoA carboxylase, however, will decrease in activity during late pregnancy and lactation (Bauman and Currie, 1980).

Mammary Gland Synthesis of Milk Fat

Acetate and 3-OH butyrate, arising from ruminal fermentation, are the main carbon sources for denova synthesis of fatty acids within the mammary gland (Grummer, 1991). Most, if not all, of the short chain fatty acids from C4:0 to one half of C16:0 are synthesized in the mammary gland whereas the other half of C16:0 and all of the longer chain fatty acids arise from the circulating blood lipids. Milk fat itself is composed of approximately 97% TG containing fatty acids of C4-C10 carbon length (short), C12 carbon length (medium) or C14-C22 carbons (long chain). In general, about 50% of fatty acids arise from de nova synthesis and 50% from the blood (Linn and Otterby, 1986). Palmquist and Jenkins (1980) support these figures. Bitman and Wood (1990) found that TG, at about 96-97% of total lipids, remained relatively constant during lactation. Wallenius and Whitchurch (1975) found significant changes with stage of lactation in percent total lipid and triglyceride fatty acid in plasma. Palmitic (C16:0), palmitoleic (C16:1) and oleic (C18:1) were found in the greatest concentrations earlier on in lactation (range of day 20-57), subsequently decreasing from day 52 -157. Stearic acid (C18:0) showed an increase in days 52-129 as well as in days 129-157. Myristic (C14:0) and linoleic (C18:2) were essentially unchanged. The higher percentages of C16:0 and C18:1 in early lactation were attributed to mobilized adipose tissue stores. Of the circulating blood lipids, 40-45% result from the diet and less than 10% is attributed to mobilized adipose tissue (Palmquist and Jenkins, 1980). The glycerol portion of milk fat results largely from the hydrolysis of TG from the blood. Some glycerol, however, may be synthesized from glucose within the mammary gland (Banks et al.,1982). It must be noted

however, that although most fatty acids less than C16:0 come from de nova synthesis, it is still possible for absorption of C12:0 and C14:0 from the blood. The scarcity of C12-C14 in most typical dairy diets leads to the assumption that most, if not all, arises from mammary gland synthesis (Banks et al, 1982). Ultimately, transfer efficiencies of fatty acids from the diet to the milk are difficult to ascertain. Basal diet, stage of lactation and feed intake may all have an influential effect (Grummer, 1991).

Long chain preformed fatty acids act within the mammary gland as inhibitors of fatty acid synthesis. High concentrations of long chain fatty acyl-CoA limits de nova synthesis in the mammary gland by inhibition of acetyl-CoA carboxylase (Palmquist, 1975). Fats arising from the diet which are high in C18 fatty acids, to a large extent, become saturated in the rumen by rumen microorganisms. Most of this is absorbed as stearic (C18:0) acid which can then be desaturated to oleic (C18:1) acid, in both the intestinal and mammary tissue. After the fatty acids leave the rumen, approximately 10% of the stearic acid is desaturated to oleic acid in the enterocyte of the intestine (Chilliard, 1993). These C18 fatty acids substitute for C16:0 and shorter chain fatty acids in milk (Palmquist and Eastridge, 1991). The desaturation of C18:0 to C18:1 occurs in the mircosomes (Moore and Christie, 1979) and an enzyme specific for the reaction of C18:0 to cis 9-18:1 is present in the mammary gland (Banks et al, 1982). Non-lactating cows show very low desaturase activity in the mammary mircosomes, which is to be expected (Moore and Christie, 1979). In general, milk fat long chain fatty acid content reveals what may have been the relative amounts of the 16:0 and 18 carbon fatty acids which were present in the diet (Palmquist and Eastridge, 1991). The desaturation of C16:0 to C16:1 occurs at 20% of the activity of C18:0 to C18:1 in lactating goats (Moore

and Christie, 1979). There was no evidence of more than one double bond being inserted per fatty acid. Those fatty acids of C14 and C12 did not show desaturation (Moore and Christie, 1979). For cattle, it has been shown in vitro that desaturation of C16:0 to C16:1 and C18:0 and C18:1 occur equally but does not seem to be the case in vivo (Moore and Christie, 1979).

Cholesterol contributes about 0.5% of total lipids in the milk fat while cholesteryl esters are usually less than .05%. These may be found in both the fat globule and skim milk fraction, generally in the membranes due to membrane structure and function (Moore and Christie, 1979, Jensen et al, 1991). These components may arise from contributions from the diet, synthesis within the mammary gland itself or from elsewhere in the animals body and subsequently transported to the mammary gland by lipoproteins. Acetate is also the primary precursor in this case (Moore and Christie, 1979). Phospholipids comprise approximately 1% of total lipids, and function as important constituents of the milk fat globule membrane (MFGM). As opposed to the TG, which remain relatively constant, the cholesterol and phospholipids decline as lactation progresses as do fatty acids of C18, 20 and 22 (PUFA) chain length. C10:0 to C16:0 rose approximately 50% from day 7 to 42 in lactation while C18:1 decreased. The animals utilized in this experiment were offered a TMR based on production level with a forage to concentrate ratio of 60:40. The diet consisted of alfalfa and corn silage, shelled corn and concentrate. Palmitic acid remained relatively constant and C18:2 and C18:3 proportions showed little change in relation to stage of lactation (Bitman and Wood, 1990).

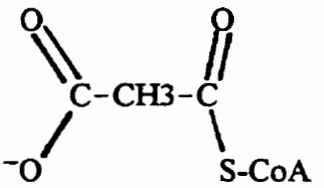
The mammary gland utilizes, in general, hydrolysed fatty acids from blood plasma very low density lipoproteins (VLDL) or chylomicrons. Lipoproteins, either chylomicrons or

VLDL, with a density less than 1.006 (mg TG/dl serum) are the major transport vehicles of fatty acids for milk fat synthesis (Palmquist, 1975, Grummer, 1991). Lipoprotein lipase hydrolyses these TG at the capillary endothelium where a large proportion is taken up by the mammary gland (Grummer, 1991). Cant and McBride (1995) have shown that increased blood flow to the mammary gland may not always enhance the uptake of nutrients from the blood. The arterio-venous differences into the mammary gland of metabolites may rise or fall as blood flow increases. This rate depends on the mechanism of blood flow regulation. Thus, a change in A-V difference may not necessarily reflect changes in uptake. The transport capacity of cells, as well as blood flow rate may affect extraction percentages. It has been proposed that precapilliary sphincters may be the major site of vasodilatory control in the lactating mammary gland (Cant and McBride, 1995). As stated previously, NEFA are also taken up by the mammary gland, but this is typically low except in early lactation and mobilization of adipose tissue stores.

The mammary gland synthesizes fatty acids via the malyonyl-CoA pathway. Chain length is increased two carbons at a time. Acetate, as previously stated, is the major carbon donor. Acetate is converted to acetyl-CoA which then enters the Krebs cycle (Ensminger et al, 1990). Malonyl-CoA (Figure 1) is a three carbon intermediate formed from acetyl-CoA and catabolized by acetyl-Co A carboxylase (Lehninger et al, 1993). Carbon dioxide is added to acetyl CoA with biotin as a cofactor to form malonyl-CoA (Figure 2) (Maynard et al, 1979). Synthesis of C18:2 or C18:3 does not occur in mammals due to the fact that the enzymes responsible for inserting a double bond beyond $\Delta 9$ are absent (Maynard et al, 1979).

Fatty acyl-CoA may join a glycerol moiety, diacylglycerol or a monoacylglycerol to

Figure 1: Malonyl-CoA.



Malonyl-CoA (Lehninger et al, 1993)

Figure 2: Condensed pathway of VFA to fatty acids.

Absorbed Acetate, Butyrate
↓
Acetyl CoA
↓ ($NADPH_2$)
Fatty Acids

(Maynard et al, 1979)

form TG. This is not a random event (Linn and Otterby, 1986). Most of the short chain FA are found in the number 3 position while C18 acids are on either the one or the three position (Linn and Otterby, 1986). This synthesis takes place in the endoplasmic reticulum (Sutton, 1989, Linn and Otterby, 1986). The TG then move to the apical membrane of the secretory cell , enlarging as they go, where they are enveloped, pinched and released into the alveoli as milk fat globules (Jensen et al, 1991, Sutton, 1989, Linn and Otterby, 1986). During the release into the alveoli, part of the cell membrane envelopes the globule, becoming it's membrane, or MFGM (Jensen et al, 1991). The milk fat globule membrane is not likely homogeneous and decreases in amount as lactation progresses (Jensen et al, 1991). The milk fat secreted from the apical cell membrane is largely composed of a TG core and a rim of cytoplasm (<50nm thick). The MFGM then surrounds this structure. Cholesteryl esters, retinyl esters and others also make up part of the core of the milk fat globule (Jensen et al, 1991). Prolactin, a hormone, initiates secretion. Maximum secretion is dependent on ACTH or adrenocorticotropin (Maynard et al, 1979). Past research has revealed differences in fatty acid makeup from small to large globules in processed milk, large generally found in cream and small present in skim milk. In general, the smaller globules contained fewer C4:0 to C10:0 and C18:0, while more C18:1 was present (Jensen et al, 1991). In underfed cows, small globules (1μm) made up only 5% of the total TG in the milk fat globule vs 80% found in adequately fed cows (Jensen et al, 1991).

Dietary Fatty Acid Metabolism and Origin

The digestion of lipids in the ruminant animal begins in the reticulorumen, or forestomach. In the rumen, the fat obtained from the diet undergoes acute lypolysis and biohydrogenation as well as lipid cellular synthesis by microorganisms (Bauchart, 1993, Jenkins, 1993). However, very little absorption of fatty acids occurs across the epithelium of the rumen or to any great extent in the abomasum (Bauchart, 1993). The VFA's are absorbed through the rumen wall. Fatty acids of long chain length are absorbed through the intestinal wall and are transported through the lymphatic system into the blood (see following section). Depending on the chain length and degree of saturation, approximately 90% of fatty acids are absorbed under normal conditions (Barks et al, 1982). Lypolysis serves to release the fatty acids from esterified plant lipids, while biohydrogenation by the microorganisms results in a reduction of the number of double bonds (Figure 3). Biohydrogenation proceeds after an initial isomerization reaction which changes the cis-12 double bond present in unsaturated fatty acids to a trans-11 isomer (Jenkins, 1993). This requires a free carboxyl group which confirms lypolysis as a necessity for biohydrogenation.

The fat which flows out of the rumen tends to be more saturated in nature. Diets which produce higher levels of the VFA's acetic and butyric acid result in the production of higher fat milk (Banks et al, 1982). Decreasing these VFA may cause milk fat to decline. This is particularly the case in diets which are supplemented with fat which is high in unprotected PUFA. The effect of unprotected PUFA on milk fat is more likely due to an upset in biohydrogenation in the rumen rather than VFA production because changes in VFA

Figure 3: Lypolysis and biohydrogenation in the rumen.[1]

Esterified Plant Lipid
↓lipases, galactosidases, phospholipases
Unsaturated Free Fatty Acids
(eg. cis-9, cis-12, C18:2)
↓isomerase
cis-9, trans-11 C18:2
↓reductase
trans-11 C18:1
↓reductase
C18:0

[1]Outline of the important steps in the conversion to saturated fatty acids from plant lipid by ruminal biohydrogenation and lypolysis adapted from Jenkins, 1993.

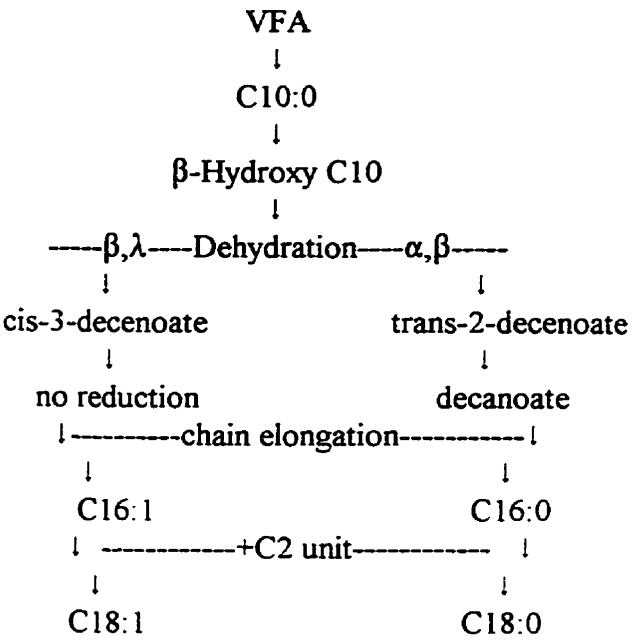
production on these diets tends to be modest (Banks et al, 1982). A trend towards a higher proportion of propionic acid and an overall decrease in VFA production , is observed with diets which are high in unprotected fat (Banks et al, 1982). In a study by Tackett et al (1996), supplemental fat caused a decrease in acetate while propionate increased. Unsaturated fatty acids undergo extensive biohydrogenation with estimates summarized from the literature ranging from 60-90% (Grummer, 1991). Thus, it is obvious that a difficulty arises when trying to increase unsaturated fatty acid flow out from the rumen. Biohydrogenation may also lead to an unnaturally high total tract digestibility of PUFA's while the digestibility of C18:0 could appear negative (Grummer, 1991).

Microorganisms utilize carbohydrates as precursors for de nova synthesis of microbial lipid in the rumen (Jenkins, 1993). The review by Jenkins (1993) suggests bacterial lipid may arise either from the diet (uptake of long chain fatty acids) or from manufacture within the cell itself. Contribution from each source depends both on the dietary makeup and the particular species of bacteria. Adding more fat to the diet will result in some species increasing their uptake from this source. Stearic acid and C16:0 are the main fatty acids produced by the bacteria generally in a ratio of 2:1. Acetate and glucose result in straight chain, even numbered fatty acids while propionate or valerate result in odd numbered long-chain fatty acids. Isobutyrate, isovalerate and 2-methylbutyrate serve as primers for branch-chain fatty acids. The total lipid component found in bacterial dry mass falls in the range of 10-15%. Monounsaturated fatty acids synthesized by the rumen bacteria make-up approximately 15-20% of bacterial fatty acids. Wu et al (1991) found that fatty acids were selectively synthesized by the ruminal microorganisms. The VFA's undergo an anaerobic pathway

forming C10 which is then converted to β-hydroxy C10. A dehydration step then follows. In the case of MUFA formation, which make up 15-20% of fatty acids synthesized by bacteria, β-hydroxy C10 is dehydrated in the β,λ position forming cis-3 decenoate rather than trans-2-decenoate, as in the case with the formation of saturated fatty acids (Jenkins, 1993). No reduction occurs in the next step by C10-enoyl reductase due to this position of the double bond. Thus, this bond remains through elongation resulting in C16:1 and C18:1 (Figure 4). MUFA's may also be formed by anaerobic desaturase activity found in ruminal bacteria acting to convert stearic acid to oleic acid. Little, if any, PUFA's are formed from bacterial synthesis except in cyanobacteria. Exogenous uptake of preformed PUFA is largely responsible, therefore, for most of the fatty acids of this type found in the microbes. The fat which then ends up in the milk can be said to be both of dietary and microbial origin (Jenkins, 1993).

Rumen metabolism of fats including lypolysis, microbial fatty acid synthesis and biohydrogenation have been extensively covered, in whole and in part, in several excellent review papers and books (Jenkins, 1993, Grummer, 1991, Banks et al, 1982, Palmquist and Jenkins, 1980, Maynard et al, 1979, Viviani, 1970). A brief summary, according to Jenkins (1993) is as follows. After hydrolysis of esterified plant lipids, free fatty acids are released. Specifically, *Anaerovibrio lipolytica*, a species of bacteria found in the rumen, manufactures a lipase and cell bound esterase. Membranous substances made up of protein, lipid and nucleic acid surround the extracellular lipase which results in complete hydrolysis of acylglycerols to free fatty acids and glycerol. Few mono- or diglycerides remain. The glycerol then undergoes rapid fermentation with propionic acid as the main end product. The

Figure 4: Synthesis of monounsaturated fatty acids by rumen microbes (anaerobic). Adapted from Jenkins (1993).



esterase activity of this microbial species is lower than other, non-lipolytic bacteria. At least seventy-four bacterial strains have been identified which were capable of hydrolysing the ester bond in p-nitrophenylpalmitate. Not all bacteria producing esterases are able to hydrolyze lipid esters. Only a few bacteria have the ability to hydrolyze long chain fatty acids. Galactolipids and phospholipids are also subject to hydrolysis releasing fatty acids. Enzymes such as phospholipase A, C, lysophospholipase and phosphodiesterase are some of the phospholipases produced by rumen bacteria which also produce galactosidases.

The rate at which the biohydrogenation of unsaturated fatty acids to saturated fatty acids by the rumen microbes takes place is relatively quick (Grummer, 1991). The protection of fat, such as encapsulation of lipids to escape microbial metabolism, reduces hydrogenation of fatty acids in the rumen. In the absence of such protection, the cis-12 double bond in unsaturated fatty acids is converted to a trans-11 isomer by an isomerization reaction (Jenkins, 1993). The fatty acid must have a free carboxyl group in order for the isomerase to operate. Those fatty acids such as C18:2 and other PUFA also contain a cis-9 double bond. Hydrogenation of this bond occurs after the trans-11 bond is formed from the cis-12 diene double bond by a microbial reductase. Conversion of C18:1 to C18:0 depends upon conditions in the rumen. Cell free ruminal fluid and the presence of feed particles promotes complete hydrogenation. Substantial amounts of linoleic acid, on the other hand, cause irreversible inhibition (Jenkins, 1993).

Fat must first be broken down into glycerol and free fatty acids before it can be metabolized by bacteria. The glycerol is then converted to propionic acid which can be absorbed through the rumen wall (Banks et al, 1982). Also, not all unsaturated fatty acids

undergo complete hydrogenation. A few of the intermediates formed from PUFA are stable enough to pass out of the rumen before complete reduction. These are then absorbed and ultimately transported to the mammary gland. The most common of these intermediates is trans-11 C18:1 or vaccenic acid (Banks et al, 1982). Large amounts of unprotected, unsaturated fatty acids can also over exceed the capacity for biohydrogenation (Grummer, 1994).

Dietary sources of fatty acids in the dairy cow's diet arise mainly from supplemental fat. Forages, and other typical dairy feed ingredients, are inherently low in fat. It must also be noted that up to 50% of forage and 20% of grain ether extract material may not be fatty acids (Palmquist and Jenkins, 1980). Substances such as cuticular waxes and various plant pigments (such as chlorophyll) as well as other unsaponifiable material are also present. However, 40 g of ether extract/kg DM is an approximation for ruminants. Forages contain approximately 40% of this as fatty acids, while grains fall in the range of about 70% (Jenkins, 1993). The fatty acids found in the highest concentration in seeds is generally C18:2 whereas C18:3 usually predominates in forages (Palmquist and Jenkins, 1980). There are exceptions such as flaxseed, which is very high in C18:3, and soybean oil, which also contains significant amounts of C18:3. Fatty acids make up 90-95% of the lipids in most fat supplements (Grummer, 1991). They are usually greater than 14 carbons in length and 75% consist of C18 fatty acids. The amount of unsaturation varies (Grummer, 1991). Cottonseed, soybean and sunflower seed contain greater than 50% PUFA. Canola oil, tallow, oleic acid rich sunflower and safflower are relatively high in monounsaturates (Grummer, 1991). Animal fats are the main sources of saturated fats (Hutjens, 1993). Fat supplements include oilseeds, tallow,

rumen protected fats such as calcium soaps of fatty acids, prilled fatty acids, encapsulated fat (joined to a protein matrix), and heat or chemically treated fats (such as formaldehyde) (Pires et al, 1996, Jenkins, 1995, Salfer et al, 1995, Grummer, 1994, Kennelly and Khorasani, 1992, Scott et al, 1991, Linn and Otterby, 1986). Sources of fatty acids include TG from cereal grains, oilseeds, and animal fats; glycolipids (2 fatty acids plus 1 sugar on glycerol) found in forages and phospholipids, which are only a minor part of most feedstuffs but make up a major portion of some supplements. Although diets today are as high as 8% fat (of total DM intake), past diets usually comprised no more than 5% (Palmquist and Jenkins, 1980). Diets which are too high in unprotected levels of unsaturated, non-esterified fatty acids should be avoided due to interference with microbial fibre digestion (Grummer, 1994) and hydrogenation (Banks et al, 1982). When fat added to the diet contains a large proportion of double bonds in the fatty acids on the TG, low fat milk syndrome occurs. However, the alteration of the VFA pattern is modest. Therefore, it appears that some upset in ruminal hydrogenation is also occurring, rather than simply an alteration in VFA pattern (Banks et al, 1982). Rumen methane production, and hence biohydrogenation, may also be disrupted by long-chain saturated fatty acids in the diet (Banks et al, 1982). Polyunsaturated fatty acids result in the greater interference on ruminal hydrogenation (Banks et al, 1982). Levels above 8% total fat (DM basis) can lead to several difficulties. Ruminants require sources of energy which can be converted to glucose and lactose and fat cannot be used in these conversions (Palmquist and Jenkins, 1980). Bovines depend more on non-glucose metabolites for energy metabolism than monogastrics. Fatty acid output in milk generally exceeds intake from the diet (Palmquist and Jenkins, 1980). Therefore, lipid metabolism is very important to the

energy reserves of the lactating cow.

Post-Ruminal Fatty Acid Metabolism, Absorption and Transport

The digesta leaving the rumen contains largely saturated NEFA's arising from the diet and microbes (70% of NEFA leaving the rumen) with approximately 10-20% microbial phospholipids as well (Bauchart, 1993, Palmquist and Jenkins, 1980). These are usually insoluble and complexed ionically with particulate matter (Bauchart, 1993, Palmquist and Jenkins, 1980). Triglycerides from any protected fats which may have been fed would make-up part of the digesta, again along with the solid matter. The abomasum and duodenal digesta range in pH from 2.0 to 2.5 which fully protonates the NEFA, further maintaining adherence to the solid portion of the digesta (Bauchart, 1993).

The form, level of intake and amount of saturation are all critical factors influencing post-ruminal digestion. When cows were fed full fat crushed rapeseed, fatty acid digestibility decreased from 91.4% to 76.4% for 2 kgs added supplement and to 84.2% when fed only 1 kg (Murphy et al, 1987). Pantoja et al (1996), found that cows which were fed supplemental fat (saturated tallow, tallow or animal-vegetable fat) tended to show a greater disappearance of total fatty acids in the rumen than those fed diets with no added fat. Apparent digestibility was greater in the small intestine for total fatty acids in those cows fed the control diet (no added fat). As the fat saturation increased, particularly saturated tallow, apparent digestibility was reduced. In the study by Pantoja et al (1996), the diets supplemented with the animal-vegetable blend were also examined with three different levels of fibre, 40% forage, 40%

forage plus 20% soyhulls and 60% forage. This had no effect on the apparent digestibility of the fatty acids. Ruminant animals are capable of digesting moderate levels of fat efficiently and fatty acid digestion may be even more efficient in ruminants than in monogastrics (Grummer, 1991). However, in general, ruminants show lower digestibilities for unsaturated fatty acids than non-ruminants whereas the opposite effect is shown for saturated fatty acids (Palmquist and Jenkins, 1980). Wu et al (1991) found that 75% of fatty acids were recovered at the duodenum when five diets, fed in a 5x5 latin square, contained either no added fat, 3 or 6% added Ca-soap (largely saturated) or 3 or 6% animal-vegetable blend. Rumen microorganisms produced 106 g/d irrespective of diet. Odd or branched chain fatty acids were synthesized most often while those of less than 14 carbon atoms disappeared to the extent of 90%. The Ca-soap protected fat showed 57% biohydrogenation while the animal-vegetable blend was hydrogenated to the extent of 87%. Wu et al (1991) also found that the fatty acids in the calcium soap were 80% digestible whereas the animal-vegetable blend was 75.7% digestible. This difference was attributed to the greater amount of unsaturation in fat reaching the small intestine. Thus degree of fatty acid saturation can affect postruminal digestibility, with C18:0 less digestible than C16:0 which is less digestible than C18:1 (Maynard et al, 1979). However, it is likely due to the distinctive interaction of bile acids, lysolecithin, oleic acid, particulate matter and the acidic pH in the upper small intestine that saturated fatty acids show a higher degree of digestibility in ruminants (Palmquist and Jenkins, 1980) than non-ruminants. For example, stearic acid is better digested and utilized in ruminants vs non-ruminants (Maynard et al, 1979). The digestibility of saturated fatty acids, when fed as part of an oil, led to the conclusion that the monomeric dispersion of SFA may

increase small micelle formation and more efficient absorption (Steel, 1983). Hydrogenated tallow, however, has a low digestibility which is attributed to it's hardness or high melting point (Palmquist and Jenkins, 1980, Grummer, 1991). When tallow was melted and applied to the concentrate, the digestibility was increased from 30% (tallow fed as a solid flake) to 40% (Macleod and Buchanan-Smith, 1972). Also, as chain length increases, saturated fatty acid digestibility is significantly lower (Grummer, 1991). Data generated by Jenkins and Jenny (1989) showed that C14:0 and C16:0 were 36.5% and 22.3% more digestible when unhydrogenated yellow grease vs hydrogenated yellow grease were fed. This lead to the conclusion by Grummer (1991) that the presence of PUFA's may help in the digestion of SFA's. Upon reaching the small intestine, a biphasic medium has formed containing an insoluble particulate phase with attached fatty acids and a soluble micellar phase wherein the fatty acids are dissolved (Bauchart, 1993). The insoluble phase slowly undergoes a transfer of fatty acids to the soluble phase along the intestinal tract. Approximately 5% occurs in the duodenum , 20% in the upper jejunum, 25% mid to lower jejunum and finally 50% in the ileum (Bauchart, 1993). Bile is secreted in the duodenum which leads to an interaction of FA with the bile phospholipids and water. A liquid crystalline phase forms and as pH increases, dispersion in the presence of bile salts occurs and the micellar phase is formed (Bauchart, 1993). Fatty acid absorption through the unstirred water layer covering the microvilli and subsequently the mucosal cells of the intestine is aided by the alteration of the bile phospholipid to lysophospholipids. This in turn stimulates micellar solubulization of the FA. Passage through the aqueous layer is thus improved (Bauchart, 1993). However, Bauchart (1993) suggests that the cellular structure of feeds may impair the availability of fatty acids

for micelle formation. In the lower pH of the duodenum, the fatty acids are emulsified by taurocholic acid, phosphatidyl choline and phosphatidyl ethanolamine according to the review by Palmquist and Jenkins (1980). Dispersion into micelles then follows. Pancreatic lipase and phospholipase activity increase the oleic acid and lysolecithin content as the pH increases. This further promotes the formation of micelles and absorption of the fatty acids. One-acly lysolecithin, fatty acid and some 2-monoglyceride are thus absorbed by the mucosa of the intestine. Pancreatic lipase activity is dependent upon adequate emulsification of fat (Grummer, 1991).

Summarizing the review by Bauchart (1993), the majority of the fatty acids (55-65%) in traditional dairy diets are absorbed by the middle and lower jejunum. The pH in the mid to lower jejunum ranges from 4.2 to 7.6. Approximately 15-25% are absorbed in the upper jejunum (pH 2.8 to 4.2). With diets containing protected lipids, the situation differs slightly. A biphasic system forms as usual, with an oil phase and a micellar phase. The TG are transformed into 2-monoacylglycerols and free fatty acids which is a significant factor in the micellar solubilization of free fatty acids. Pancreatic lipase and colipase are the enzymes responsible for these conversions. Under these dietary circumstances, and an optimal pH of 7.5 for lipase activity, the hydrolysis of TG and therefore fatty acid absorption, does not occur before the mid-jejunum. In diets that are low in fat (2-3% DM), the range of intestinal absorption coefficient is from 80% for individual saturated fatty acids to 92% for PUFA. Ruminant animals have a tremendous ability to solubilize FA through the bile salt and lysophospholipid micellar systems. Low concentrations of pancreatic bicarbonate lead to a lower pH and more acidic conditions in the duodenum and jejunum (pH 3-6). The formation

of insoluble Ca-soaps with the saturated fatty acids is thus largely restricted. Therefore, the higher efficiency demonstrated by ruminants to absorb saturated fatty acids is defined. Apparent intestinal fatty acid digestibility is high due to the dilution of bile FA and bacterial FA produced in the large intestine. True digestibility appears to decrease from 95 to 78% when intake of FA increases from 200g/d (1%DM) to 1400g/d (8%DM). A decreased activity and presence of biliary lipids such as bile salts and phospholipid and pancreatic lipase are indicated. This is believed to affect fatty acid absorption when the intake of dietary fat is high. With the absence of 2-monoglycerides, phospholipid micelle formation is more critical in ruminants vs. monogastrics. If, however, oil is infused in the proximal duodenum, similar FA digestibilities are obtained even when using high levels of fat, with or without lecithin.

Fatty acids less than fourteen carbons are absorbed directly into the blood, where they are transported in non-esterified form and undergo rapid oxidation (Palmquist and Jenkins, 1980). One-acyl-lysolecithin is easily absorbed and shows preferential esterification with linoleic acid thus ensuring availability of this essential fatty acid for the ruminant (Leat and Harrison, 1974). Linoleic acid in lymph makes up 50% of the phospholipid which is 20% of the intestinal lymph lipid (Leat and Harrison, 1974). Higher fat diets can cause difficulty with micellar solubilization and absorption of fatty acids (Bauchart, 1993). Even though Wu et al (1991) found that the digestibility was better with the Ca-soap supplement than the animal/vegetable blend, neither diet surpassed that of the control with no added fat. These results were found when cows were fed a diet containing emulsified fat which had a higher digestibility (86% vs 80%) than a low fat, control diet (Bauchart et al, 1987). The intestines

(intestinal epithelium) also have the capability to desaturate saturated fatty acids. Stearate is converted to oleate and ensures the fluidity of milk fat for efficient secretion by the mammary gland (Grummer, 1991). However, since milk contains fair amounts of C18:1, and considering the extensive biohydrogenation by the rumen, most of the conversion takes place in the mammary gland as little is attributed to the intestinal desaturase activity (Grummer, 1991). The proportions of C18:1 to C18:0 in milk is generally between 2:1 to 3:1 . Conversely, the ratio in TG-rich lipoproteins in plasma in approximately 1:2 (Grummer, 1991).

Chylomicrons synthesized from the intestines incorporate fat derived from the diet (Grummer, 1991). Plasma lipoproteins function mainly to transport lipids from intestine and liver to the peripheral tissues, including the mammary gland (Bauchart, 1993). The chemical composition and rate of secretion of these compounds are extremely important in the control of lipid utilization and therefore, the quantitative and qualitative properties of milk (Bauchart, 1993). Lipoprotein molecules can be equated with pseudomicelles due to it's hydrophilic component of phospholipid, free cholesterol and apo-lipoprotein present on the surface of the particles and their hydrophobic core containing lipids (TG and cholesteryl esters) (Bauchart, 1993). Thus they are soluble in plasma, lymph and the intestinal or follicular fluids. According to the review by Bauchart (1993), the plasma lipoproteins are divided into five main density classes, each reflecting the lipid-protein content of the particle. These categories are chylomicrons, VLDL, IDL (intermediate density lipoproteins), LDL and HDL.

Chylomicrons are the largest and have the lowest density (Lapland et al, 1990). TG is removed from this fraction (as well as from VLDL) very rapidly, ranging from 2 to 11

minute half-lives (Palmquist and Jenkins, 1980). The intestine produces and secretes chylomicrons after the consumption of a diet containing fat. These chylomicrons function to transport dietary TG to adipose tissue and the mammary gland for production of fat (Bauchart, 1993). They may also undergo oxidation if a requirement for energy is present (Bauchart, 1993). Dietary PUFA and increased fat in the diet stimulate chylomicron synthesis whereas saturated fatty acids are more often associated with VLDL. Harrison et al (1974) found after 24 hour infusion of corn oil into the duodenum of sheep, 38.5% of the lipids in the lymph were in VLDL and 61.5% were found in chylomicrons. Table 5 gives a comparison of the various components in chylomicrons and VLDL in the plasma and lymph TG in the bovine. According to Bauchart's review (1993), VLDL is also responsible for the transportation of TG utilizing the portal vein as the main pathway from the intestines. High fat diets increase VLDL concentration, however, these are generally low (0.5% of total lipoproteins), likely due to the quick turnover of the VLDL pool. A large amount of TG, increased phospholipid, but little cholesteryl ester are incorporated by the VLDL. The TG fatty acid composition is not altered by dietary fat to any great degree, largely as a result of biohydrogenation in the rumen. Hydrolysis of TG from chylomicrons and VLDL compounds by lipoprotein lipase generates large amounts of free FA's for tissues such as the mammary gland, heart, skeletal tissue and adipose tissue (Bauchart, 1993). This takes place at the capillary epithelium. Degradation of VLDL results in the IDL, an intermediate between VLDL and LDL. IDL is not found in any great concentration in the plasma or lymph. LDL is the end product of the breakdown of VLDL, not being found in very large concentration in plasma or lymph either. In general, 48% of total lipids found in this class are cholesteryl

Table 5. Percent of various components in the chylomicrons and very low density lipoprotein in lymph and plasma of bovines.

	Chylomicrons		VLDL	
Component (%)	Lymph	Plasma	Lymph	Plasma
TG	67-88	72-87	75-80	45-63
Free cholesterol	1-2	4-6	1-2	3-9
Cholesterol ester	1-8	1-4	1-2	5-15
Phospholipid	8-20	4-5	10-12	12-17
Protein	2-3	2-3	6-8	8-16

Adapted from Bauchart (1993).

Table 6. Differences in lipid composition of low density lipoprotein between plasma and lymph.

	LDL	
Component (%)	Lymph	Plasma
Free cholesterol	3-7	6-8
Cholesteryl esters	16-32	31-36
TG	5-35	4-21
Phospholipid	22-33	18-22

Adapted from Bauchart (1993).

esters, 27% phospholipids, 10% free cholesterol and 15% TG. Some differences between the lymph and the plasma were found with LDL (Table 6). Plasma HDL plays a major role in the transport of cholesteryl esters and with the exception of the intestinal lymph, HDL are the main plasma lipoproteins. The liver and intestine produce and secrete HDL. The HDL functions to return cholesterol from peripheral cells to the liver for excretion with bile and the production of new VLDL. Plasma HDL plays a significant role in cholesteryl ester transport in ruminants. Two forms, light and heavy are present, differing in size, density and lipid composition (Table 7). Also present in bovine plasma and lymph are very light HDL, with a density ranging from 1.039-1.060 g/ml (Bauchart, 1993). During lactation, light HDL concentration is high compared to heavy HDL (Bauchart, 1993). High fat diets increase TG-rich lipoprotein secretion. This is also the case with the liver when NEFA content is high in early lactation. This results in major lypolysis of these particles and quick turnover in the vascular system. Utilizing a curve analysis of FA radioactivity disappearance in milk fat, results indicate that the contribution to milk fat from TG-rich lipoproteins ranges from 44-47% (Palmquist and Mattos, 1978, Glascock and V.A. Welch, 1974). Triglyceride, some mono and diglycerides, phospholipid and cholesterol are united with specific apolipoproteins and leave the intestine via the absorptive cells into the lamina propria (Sterzing et al, 1971, Tytgat et al, 1971) to the lymph lacteals (Palmquist and Jenkins, 1980). Apolipoprotein's have many different classes (for example A-1 or B-100) and are involved in many functions including particle formation, receptor binding, LCAT (lecithin:cholesterol acyltransferase) activation, LPL (lipoprotein lipase) inhibitor and/or lipid binding depending upon which class the apolipoprotein is. They are synthesized in the liver and/or intestines and are distributed

Table 7. Density,size and lipid composition of high density lipoprotein in plasma.

	Light	Heavy
Density (g/ml)	1.060-1.091	1.091-1.180
Size (Å)	120-150	93-120
Free cholesterol (%)	4-6	1-4
Cholesteryl esters (%)	29-33	13-29
TG (%)	1-3	1-6

Adapted from Bauchart (1993).

in the plasma in chylomicrons, VLDL, LDL and HDL or combinations thereof (Bauchart, 1993).

Post-ruminal hydrolysis of TG by mammalian enzymes is probably limiting (Grummer, 1991). Diarrhea was induced with post-ruminal infusion of soy oil whereas this does not occur when it is fed (Grummer, 1991, Grummer et al, 1987). Thus, Grummer (1991) indicates that in order to increase efficiency of milk fat alteration, limitations in the digestibility of TG's and fatty acids post-ruminally should be addressed.

Potential to Alter Milk Fatty Acid Composition

There exist certain biological barriers, such as the biohydrogenation of dietary fatty acids in the rumen, which must be overcome in order to achieve a more desirable, less saturated, milk fat. Several different feeding strategies have been explored, some with mixed results. Heat treatment, extrusion, whole oilseeds, chemical treatments such as butylsoyamide or formaldehyde, prilled fatty acids and Ca-salts of long chain fatty acids are some of the techniques which have been examined to determine their effectiveness in providing protection from ruminal hydrogenation. Polyunsaturated fatty acids may undergo hydrogenation to the extent of 60-90% in the rumen, as stated previously (Grummer, 1991). Thus, it remains crucial to provide some sort of protection if they are to be passed into the milk fat.

The effect of feed and feeding management has been covered in several excellent reviews (Palmquist et al, 1993, Grummer, 1991, Sutton, 1989, Banks et al, 1982) and thus will not be covered extensively here. In general, short, medium and long chain fatty acids

C12:0 to C16:0, are reduced when fat is supplemented to the diet while C18 fatty acids show some increase, regardless of form of fat fed (ground, whole, roasted etc). Oleic acid is often the fatty acid found in larger proportions and is often not reported as levels of cis or the trans isomers. Processing oilseeds may increase the effect of the amount of unsaturated fatty acids in milk (rolling, heating etc), however, unless the supplement is truly protected from the rumen, levels of trans-fatty acids are likely to increase (Khorasani and Kennelly, 1994) and the magnitude of change in PUFA is unlikely to be as great. Linoleic and C18:3, show little, if any, increases unless the fat is protected. Thus, if the objective is to increase the proportion of these fatty acids in the milk, then it remains prudent to focus on those studies which examine methodologies designed to protect the fat either through physical form (oilseed vs whole seed vs ground) or processing (heat, encapsulation in a protein matrix etc), from biohydrogenation in the rumen. Briefly, however, the aforementioned feeding strategies such as forage:concentrate ratio, feeding frequency, intake and level of supplementation will be addressed as to their effects on the fatty acid composition of milk.

The opportunity does exist to alter the fatty acid composition of milk simply by manipulating the forage portion of the dairy cow's diet (Fredeen, 1996). The feeding of low roughage diets tends to decrease yields and percentages of C6:0 to C14:0, whereas there is some increase in C18:2 (Grummer, 1991). Although the yield (g/d) of C18:1 is reduced on a low roughage diet, the proportions of C18:1 and C18:2 are increased (Table 8) (Grummer, 1991). This is likely due to the negative effects a low roughage diet has on milk fat yield, faster rumen turnover time and lower ruminal pH (Palmquist et al, 1993) seen on high concentrate, low roughage diets. This would allow more escape of C18:2. Manipulation of

Table 8. Effect of roughage level on milk fatty acid composition and yield.

Fatty Acid	Yield (g/d)		Profile (%)	
	High Roughage	Low Roughage	High Roughage	Low Roughage
C6:0 to C14:0	178	76	24	20
C16:0	262	96	34	25
C18:0	61	22	8.2	5.6
C18:1	126	108	17	29
C18:2	14	18	2	5

1. Adapted from Grummer (1991).

the fatty acid composition would be most drastically affected if the level of fibre present in the diet is enough to maintain the percentage of milk fat (Grummer, 1991). Only half of the fatty acids (20%) were transferred to the milk fat when a low roughage diet was fed compared to previous studies utilizing high fibre diets (40%) (Storry et al, 1974). However, in order to maximize C18:1 increases, it may be more desirable to utilize a combination of supplemental fat and low roughage diets (Grummer, 1991). This is supported by Hussein et al (1996) whose results suggested that treated whole canola seed was more beneficial in altering milk fatty acid composition when fed with low roughage diets. Nevertheless, this would not be desirable if the majority of the C18:1 ended up in the trans form due to hydrogenation in the rumen.

Increasing the frequency of meals from 2 per day to six had an effect on reducing the depression of milk fat caused by high concentrate, low roughage diets (Sutton et al, 1985, Sutton et al, 1986). This strategy may be utilized in conjunction with the benefits to milk fatty acid composition seen above on low roughage diets. Increasing the number of feedings from 2 per day to 24 increased the milk fat percentage obtained with only 2 feedings. The 2x a day feeding resulted in a decrease in milk fat. Although the long-chain fatty acids had increased with the added oil, little effect on fatty acid composition was seen (Banks et al, 1980). Grummer (1991) concludes that feeding frequency will affect milk fat yield but does little to alter milk fatty acid composition. According to the review by Sutton (1989), increasing the intake while maintaining the forage:concentrate ratio results in decreases of milk fat ranging from -0.003% unit/MJ NE_l to 0-.1% unit/MJ NE_l. Palmquist et al (1993) state that at higher grain intakes (>50% of DM), milk fat percent decreases. This also results

in the alteration of fatty acid composition, with short chain fatty acids decreasing and increases shown by C18 fatty acids, depending on grain source and fat. This is in keeping with the results obtained on low roughage diets. Barley tends to result in little change of short chain fatty acids while yellow corn caused marked increases in C18:1 and C18:2. The depression in short chain fatty acids was likely due to decreased acetate or the large amount of trans unsaturated fatty acids (present likely in the C18:1 fraction). Higher levels of C18:2 likely resulted from a decrease in lipolyis due to lower ruminal pH and therefore some escape of C18:2 (Palmquist et al, 1993).

Grummer (1991) summarizes several studies concluding that the levels of C4:0 to C14:0 fatty acids decreased as lipid supplementation increased, relative to the other fatty acids. When the level of saturated fatty acids in the supplements were greater, a trend towards a less extreme reduction in C4:0 to C14:0 was seen. The decrease in C16:0 showed more variability depending on the supplement and the amount of depression that occurred appeared to be negatively correlated to the amount of C16:0 present in the diet. Feeding high levels of palm oil may reverse the effects of C16:0 depression, for example. This is not necessarily desirable from a human health standpoint. As the amount of supplementation increased, proportions of C18:0 to C18:1 increased, particularly with soy oil or tallow. Supplementation of oil fed as part of a whole oilseed beyond 3% of the diet resulted in less spectacular increases of these two fatty acids. Pantoja et al (1996), found increasing levels of C18:0 and C18:1 in milk fat as fat saturation decreased indicating higher unsaturated C18 intake and hydrogenation in the rumen. Higher fat supplementation resulted in decreased C8:0 to C15:0, with variable effects on C18:n, those fatty acids 18 carbons in length regardless of

degree of saturation (Palmquist et al, 1993). When attempting to alter milk fatty acid composition, utilizing a supplement with a desirable fatty acid profile from a human health perspective would be of most interest if the capability exists to pass some of those positive aspects into the milk. The physical form (free oil vs whole oilseed) and/or processing (chemical, heat etc.) of the supplement becomes crucial. In order to pass a desirable fatty acid profile into milk fat, in terms of polyunsaturated fatty acids and omega-3 fatty acids, protection from hydrogenation in the rumen must be accomplished. Although the inclusion of unprocessed or rolled flaxseed reduced short-chain and saturated fatty acids as reported by Kennelly (1994), the effect is likely a result of decreased acetate and butyrate in the rumen, inhibition of mammary synthesis by long chain fatty acids and increased digestion in the lower gut due to the rolling. Adequate protection from the rumen was unlikely as there was little change in PUFA such as C18:3. Though the reduction of the saturated fatty acids may be deemed positive, the magnitude of the changes were not as great as when fat is protected from hydrogenation in the rumen or infused directly into the abomasum (Kennelly, 1994). The gap between the ideal milk fat (as stated previously) and typical milk fat would likely be too great to be accomplished by even the most extreme dietary modifications (Schingoethe et al, 1996, Grummer, 1991). Potential exists, nonetheless, to significantly alter and improve the fatty acid profile of milk from a consumer perspective and thus deserves attention.

Oilseeds, such as sunflower seed, offer some protection from hydrogenation in the rumen via their seedcoat. Ekeren et al (1992) found that when high oleate sunflower seed (80% oleate in oil) was used, either partially crushed, serum coated or heat treated and serum coated, that the seed/serum/heat treatment offered limited ruminal bypass. Duodenal digesta

contained 1,073 ug/g C18:1 and 216.9 ug/g C18:2 with the seed/serum/heat treated supplement vs that of 27 ug/g C18:1 and 115.6 ug/g C18:2 in the control. This was not, however, found to be significant. McGuffey and Schingoethe (1982) found that the milk fat from cows fed whole sunflower seeds (whole, rolled or extruded whole) contained more C18:0, C18:1, C18:2 and C18:3 fatty acids than did the corn-soybean meal control (with no added sunflower seeds). The extruded and rolled sunflower seeds did not significantly differ in their effects on milk fatty acid composition except for C18:2, where the milk fat from cows fed the rolled sunflower seeds had 2.47% C18:2 and the extruded contained 4.19%. Rafalowski and Park (1982) fed complete rations containing either 0, 10, 20 or 30% whole sunflower seeds in the concentrate portion of the diet. Oleic acid was increased by feeding whole sunflower seeds while fatty acids of chain length C6:0 to C16:0 were depressed. In this case, C18:2 was present in the greatest quantity in the control diet which was significantly different ($P<.05$) for this particular fatty acid.

The feeding of whole flaxseed resulted in a linear increase of C18:0, C18:1, C18:2 and C18:3 when 5, 10 and 15% whole flaxseed was added to diets vs that of no added flaxseed (Kennelly and Khorasani, 1992). Whole cottonseeds fed at 0, 10, 15, or 20% of a TMR also resulted in decreased proportions of C6:0 to C16:0 and increased C18:0 and C18:1 fatty acids in the milk (DePeters et al, 1985). However, in this case, feeding whole cottonseed did not offer much protection to the oil contained in the seed from the rumen microorganisms and subsequent hydrogenation. Feeding 10, 15 or 20% of the mixed diet as whole cottonseed actually lowered the proportions of C18:2 and C18:3 from the control diet. The result was not significant. The use of whole oilseeds seems to maintain milk fat, and in some cases,

increase the yield of milk fat. Therefore, changes to the milk fatty acid profile may be sought without compromising milk fat percent (Grummer, 1991).

Several authors have documented that free oil results in milk fat percentage decreases. In an experiment by Mohamed et al (1988), whole seed or whole roasted seed did not significantly affect milk fat percent, while free oil resulted in a decrease. When soybeans were used, milk fat percent was 3.59% for both whole and roasted seeds. The free oil resulted in a milk fat percent of 2.75 which was significantly different from the control of 3.53% ($P<.05$). Cottonseeds, whole or roasted, resulted in milk containing 3.7% and 3.56% fat which was not significantly different ($P<.01$) from the control of 3.54%. Free oil, however, once again resulted in a significant decrease in milk fat (2.99%) from the control (Mohamed et al, 1988). This was likely due to interference with rumen metabolism as proprionate was increased and butyrate decreased with the free oil diets. The feeding of fat as part of a whole oilseed may also reduce the proportion of trans-11-C18:1 in the rumen, and subsequently the milk fat, due to the slower release of the oil from such supplements (Grummer, 1991). From a human health perspective, the trans fatty acids behave like saturated fatty acids and are thus undesirable. Thus, the importance of developing and examining methodologies which would help protect PUFA's from biohydrogenation in the rumen becomes even more clear.

Roasting and heat treatments have been successfully applied to various supplements such as canola and soybeans to protect the protein from degradation by rumen microorganisms. However, it does not seem to offer much protection to the fat (Pires et al, 1996, Schingoethe et al, 1996). The milk fatty acid composition in the study by Mohamed et al (1988) was similar whether raw or roasted seeds were fed. In an experiment by Pires

et al (1996), ground roasted soybeans showed the greatest decline in milk fat percentage compared to whole roasted soybeans and two other diets containing blood meal and blood meal/tallow. Although the result was not significant, it was felt that the effect of the ground roasted soybeans was likely due to the rapid availability of the oil and it's effect on digestibility. Thus it does not appear that the heat treatment offered significant protection. However, analysis of fat from milk of cows fed extruded soybeans or rolled sunflower seeds showed an increase in the concentration of unsaturated fatty acids and long-chain fatty acids over that of cows fed a control diet which did not contain added fat (Schingoethe et al, 1996). In this case, extruded soybeans increased the PUFA in milk more than the rolled sunflower seeds. Heat treatment likely has less of an effect on the rate of oil delivered from extruded supplements than roasted supplements due to the release of oil during extrusion (Grummer, 1991). Feeding whole, roasted soybeans (WRSB) at 0, 12, 18, or 24% of diet DM did not effect milk production or milk fat percent negatively but did depress milk protein. The fact that the oil was encapsulated within the seed, and milk fat was not depressed, may lend further credence to the theory that feeding whole seeds offers some protection from the rumen to the fat contained in the seed. Supplementation with 12, 18 and 24% WRSB significantly increased milk fat percent from the control ($P<.05$) (Knapp et al, 1991). Grinding may cause the release of the oil or increase the surface area allowing greater access by ruminal microorganisms (Grummer, 1991). Heat offers little protection to the fat as indicated by the results from ground, heat treated supplements which do not provide the same results as whole, heat treated supplements (Pires et al, 1996). Pires et al (1996), attributed the greater concentration of C18:2 in the milk fat to the fact that biohydrogenation in the

rumen is not always complete and not due to any protection offered as a result of the heat treatment. The roasted soybean diet had 28.41 mg/g of DM as C18:2 vs 11.65 mg/g DM in the control, thus it seems likely that not all of this was hydrogenated and therefore passed into the milk. The utilization of Jet-Sploded Canola seed, a form of heat treatment, did not result in much, if any, change in C18:2 or C18:3 in the milk indicating these fatty acids were likely hydrogenated in the rumen (Kennelly, 1994). However, C16:0 was substantially reduced while C18:1 was elevated, thus it was interpreted as an improvement in the nutritional quality of milk for humans.

By far, the most dramatic results which have been seen in the alteration of milk fatty acid composition has been with supplements which utilize some form of chemical treatment, particularly formaldehyde. In a study by Ashes et al (1992), emulsification and encapsulation of canola seed in an aldehyde treated protein matrix significantly decreased the amounts of C16:0, C14:0 and C12:0 with increases in the proportions of C18:0, C18:1, C18:2 and C18:3. The increase of C18-mono- plus polyunsaturated fatty acids was 54% or 143 g/d. Plowman et al (1972) fed cows a diet with a formaldehyde treated safflower oil with casein as the protein matrix. Linoleic acid was protected from biohydrogenation in the rumen and increased from 3% to 35% of total fatty acids in milk. Linolenic acid content in milk has been increased to 22% (from an average of 2.5%) with efficiencies of transfer from the diet to the milk fat of both linoleic and linolenic being as high as 35-42% with formaldehyde protection to less than 5% when not protected (Grummer, 1991). Treating soybean meal with formaldehyde did not result in any effect on the level of formaldehyde in milk (Atwal and Mahadevan, 1994). Formaldehyde is a natural product of intermediary metabolism in animals

and is present in levels up to 0.027 mg kg^{-1} in milk of cows on diets with no added formaldehyde (Atwal and Mahadevan, 1994). The treatment of whole canola seed with alkaline H_2O_2 to weaken the seed coat yet protect the PUFA resulted in increased amounts of C18:1, C18:2 and C18:3 flowing to the duodenum and post-ruminal digestion from that of crushed, unprotected seed (Hussein et al, 1996). Although this experiment utilized steers, it appears that some protection from the rumen was offered by the PUFA treatment and would thus be available to the mammary gland. Protected fats such as Ca-salts of palm fatty acids and prilled fatty acids are high in C16:0 and C18:1 and C16:0 and C18:0 respectively (Wu et al, 1993). Therefore, from the standpoint of increasing PUFA in milk fat, these types of supplements do not provide much interest. In fact, in a study by Wu et al (1993), although C18:1 was increased using Ca-salts and prilled fatty acids, it was not significant from the control and tallow had a greater effect. Proportions of C18:2 were decreased by all supplemental fat (tallow, Ca-salts and prilled fatty acids), although again, this was not significant. Reactions of unsaturated fatty acids with primary amines produces fatty acyl amides which are thus protected from ruminal hydrogenation (Fotouhi and Jenkins, 1992a,b). In a study by Jenkins et al (1996), butylsoyamide, a combination of butylamine and soybean oil was fed to lactating dairy cows to examine its effect on the fatty acids of plasma and milk fat. The treatment protected the unsaturated fatty acids from biohydrogenation in the rumen and led to an increase in milk fat of C18:2 from 3.6% in the control to 6.28% for the butylsoyamide fed cows. Ekeren et al (1992) treated high oleate sunflower seed in calcium alginate. This treatment did not protect the fat from biohydrogenation in the rumen or modify the adipose tissue fatty acid composition of heifers. Lignosulfonate has been utilized

successfully in several studies to protect protein from ruminal degradation (McAllister et al, 1993, Windschitl and Stern, 1988, Stern, 1984). Although no attempts have been made to determine the usefulness of this methodology to protect fat, it may theoretically provide the benefit of a rumen undegradable protein matrix which could encapsulate the fat thus providing protection from hydrogenation by rumen microorganisms. The formation of a coat of cross linked protein which encapsulates the fat, providing protection from rumen lypolysis and thus hydrogenation, is what enables formaldehyde treated supplements to work so well (Banks et al, 1982).

Stage of Lactation

Due to the effects of mobilized adipose tissue in early lactation, it has been theorized that this would be the most difficult time to impose changes to the fatty acid composition of milk (Grummer, 1991). Several studies did indeed utilize cows in mid to late lactation (Elliott et al, 1996, Wu et al, 1993, Scott et al, 1991, Plowman et al, 1972). However, in a review by Grummer (1991), stage of lactation and milk fatty acid manipulation have shown various responses. In one case, a better response in mid relative to early lactation was shown while in another, greater responses were found in early lactation. As the animal progresses to late lactation, nutrients are partitioned towards adipose tissue and this may effect results of fatty acids in milk arising from the diet. Interestingly, in a study by Stegeman et al (1992a), adding BST did not affect the amount of C18:2 in the milk fat although decreases in short, medium and long chain saturated fatty acids and increases in long chain fatty acids was seen. Further

research is required, however, perhaps BST offers the potential to increase the window of opportunity to alter milk fat and may be especially beneficial with protected supplements. DePeters et al (1985), found little effect on milk fatty acid composition when comparing cows in early first lactation or older cows in early or late lactation. Bitman and Wood (1990), found that as lactation progressed, medium chain fatty acids increased and C18, C20 and C22 chain length decreased. This seems to indicate a lesser effect of diet on milk fat composition and synthesis. Triglyceride were fairly constant throughout lactation and the changes in milk total fatty acids were manifested in the phosphatidyl and phospholipid fraction. These milk phospholipids are thought to be synthesized entirely in the mammary gland. Salfer et al (1995) did not find any effects on milk composition (protein, fat, unsaturated, saturated, short, medium and long chain fatty acids) during the first 151 days postpartum when partially hydrogenated tallow was fed. They suggest delaying the feeding of such tallow until after 35d which did result in a greater persistency of lactation. Feeding whole sunflower seeds in early lactation depressed the secretion of short-chain fatty acids while the amount of C18:1 was increased (Rafalowski and Park, 1982). Several other studies have found similar results often demonstrating effects on milk fatty acid composition from added dietary fat in early or mid-lactation (Schingoethe et al, 1996, Jenkins et al, 1996, Lightfield et al, 1993, Boila et al, 1993, Mohamed et al, 1988, McGuffey and Schingoethe et al, 1982). This appears to be an area requiring further investigation.

Flaxseed and Milk Fatty Acid Composition

Flax oil utilization in dairy diets has not received much attention to date. As of 1994, linseed was implemented as a protein supplement only to a limited extent and data on the value of flaxseed as a protein supplement is not extensive (Khorasani et al, 1994). Nonetheless, the fatty acid profile of flaxseed makes it an ideal supplement to alter milk fatty acid composition if it does not undergo extensive hydrogenation in the rumen (Table 9). Flaxseed has been utilized in other species such as swine and poultry to successfully alter meat and egg products (Romans et al, 1995a,b, Aymond and Van Elswyk, 1995, Caston and Leeson, 1990). In 1994, Broudiscou et al, examined the effects of linseed oil on the degradation of feed and microbial synthesis in the rumen of ciliate-free and refaunated sheep. Linseed oil was supplemented at 6% of the diet and was found to decrease protozoal numbers, result in a higher propionate/acetate ratio and reduce hemicellulose digestion by direct inhibition of bacterial activity. Apparent organic matter digestibility was decreased in the rumen, however, the flow of microbial nitrogen at the duodenum was increased which resulted in an increase in the efficiency of microbial protein synthesis. Due to the importance of a higher acetate/propionate ratio and fibre digestion in high producing dairy cows in relation to milk fat, the above effects, in general, would not be desirable. Also, unless protected from the rumen, the high amounts of PUFA's present in flax would likely undergo extensive hydrogenation in the rumen, thus negating the potential benefits which could be passed to the milk fat. In a study by Kennelly and Khorasani (1992), the feeding of ground, unextracted flaxseed in the diet decreased fatty acids C4:0 to C17:0, while the concentration

Table 9. Fatty acid composition of several varieties of flaxseed.

Variety	Fatty Acid Composition (%)*				
	C16:0	C18:0	C18:1	C18:2	C18:3
Flanders	4.8	3.9	16.0	14.9	59.6
McGregr	5.5	3.4	17.0	15.5	57.8
Norlin	5.1	2.9	20.0	13.3	57.9
Norman	5.2	2.8	19.1	14.3	57.7
Somme	5.6	2.9	16.0	14.4	60.3
Vimy	5.4	3.0	14.0	14.8	62.1

*Canadian Grain Commission, Oilseeds Laboratory.

of long chain fatty acids C18:0, C18:1, C18:2, C18:3 and C20:0 increased (Table 1). There were no negative effects on feed intake, daily yield and fat, protein and lactose% although percent protein did decrease linearly with increasing levels of flaxseed.

When flaxseed was added to the diet of cows in early lactation, either whole, rolled or rolled and mixed with rolled canola seed, the rolled flaxseed resulted in the greatest increase in monounsaturated and polyunsaturated fatty acids (Khorasani and Kennelly, 1994). However, it must be noted that the increases were insignificant relative to that when protected fat is supplemented to the diet and the concentration of trans-C18:1 was also highest in the rolled seed supplemented diets, indicating more extensive biohydrogenation in the rumen (Khorasani and Kennelly, 1994, Knapp et al, 1991, Palmquist and Jenkins, 1980). These studies clearly indicate flaxseed could play a positive role in increasing the fatty acids in milk which are considered beneficial from a human health perspective. The consumption of flaxseed has been positively associated with important aspects of human health including heart disease and cancer (Cunnane, 1996). This is likely due to the high levels of omega-3-fatty acids. However, a need for some kind of viable treatment to protect the polyunsaturated fatty acids in flax would be necessary. The stability of such products thus produced from oxidation would also have to be addressed.

The intent of this study was to determine 1) the viability of formaldehyde protected ground flaxseed and Linola as a source of fat to greatly improve the level of linolenic and linoleic fatty acids in milk for human consumption and 2) the viability of lignosulfonate, a treatment available in Canada, as a method of offering cows a rumen protected fat from flaxseed in order to increase the proportion of linolenic acid in milk.

EXPERIMENT 1

Effect of Formaldehyde Treated Flaxseed and Linola in Lactation Diets on the Fatty Acid Composition of Milk

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ABSTRACT

Two primiparous and multiparous Holstein cows were randomly assigned to four treatments in a 4x4 Latin square design. The four test diets consisted of a TMR plus i) Control-no added fat, ii) Linola, a low linolenic (C18:3), high linoleic (C18:2) Solin, added at 1.6 kg per kg milk fat produced, iii) high linolenic flaxseed added at 0.8 kg supplement per kg milk fat produced (FL) and iv) high linolenic flaxseed added at 1.6 kg supplement per kg milk fat produced (FH). Supplements were added as a top dress to provide 200-250 g (FL) or 400-450 g of fat (Linola and FH). Top dress was adjusted weekly according to actual fat production. The top dress contained either 70.8% ground, formaldehyde treated flaxseed or Linola, 19% soybean meal and 10% casein.

Treatments had no effect on feed intake, milk yield or milk composition (fat, protein and SNF%). Rumen metabolism was not disrupted by treatments as volatile fatty acids (VFA), ammonia and pH were unaffected by diet. Stearic acid (C18:0) was significantly lower ($p<0.05$) in the plasma of cows fed FH while oleic acid (C18:1) was significantly greater in the control cows. The plasma level of linoleic acid was unaffected by treatments. Linolenic acid was significantly higher in the plasma of cows fed flaxseed. Medium chain fatty acids, C12:0 to C16:0, were significantly lower ($p< 0.05$) in the milk of cows fed supplemental fat. The C12:0 fatty acid was lowest in the milk fat of cows fed the Linola diet (3.19%) followed by FH (3.55%) and then FL (3.69%). Palmitic acid (C16:0) was lowest in milk of cows receiving the Linola and FH (20% and 19.8% respectively) vs the control (24.5%). Cows fed the lower level of flaxseed also had a significantly lower milk level of C16:0 (21.7%) than the control cows. The control and Linola were significantly different for

C18:0 (9.7% vs 12.1%, respectively) with no difference among the fat supplemented treatments. Milk oleic acid was significantly greater in the milk fat from cows fed Linola but was unaffected by other dietary treatments. Milk linoleic acid (C18:2) was significantly higher at 10.3% in the milk of cows fed the Linola vs the control (4.8%). Linoleic acid was also significantly greater in the milk of cows fed FL (6.0%) and FH (6.9%) diets. Linolenic acid (C18:3) was not affected by feeding Linola, but was significantly greater in the milk of cows fed the high linolenic acid flaxseed (6.4% vs 0.8% in the control). This represents a 8 fold increase, while FL increased C18:3 by 3.9 fold.

Flaxseed offers an opportunity to significantly improve the fatty acid profile of milk from a human nutritional health perspective when properly protected from biohydrogenation in the rumen. Essential fatty acids, in particular C18:3, is greatly increased with the addition of formaldehyde treated flaxseed to the diet of dairy cows. Combined with the decrease in undesirable medium chain fatty acids (C12-C16), these changes to the milk fatty acids could lead to greater consumer acceptance of dairy products.

Key words: Flaxseed, Linola, formaldehyde, milk, fatty acids, linoleic acid, linolenic acid.

INTRODUCTION

Milk and milk products have been criticized over the past several years due to the saturated nature of the fat contained in such products. While not all saturated fats are equal in their hypercholesterolemic effects, research suggests that fatty acids C12:0, C14:0 and C16:0 are indeed hypercholesterolemic and raise dietary LDL cholesterol (Noakes et al, 1996, Ney, 1991). Myristic acid (C14:0) is reputed to be more potent in raising plasma cholesterol in humans and dairy products are a major source of this fatty acid. The intake of milk and dairy fat has been associated with higher rates of cardiovascular disease in various countries (Noakes et al, 1996). However, evidence also suggests that C18:0 and C18:1 lower plasma cholesterol when replacing C16:0 in the diet of men (Ney, 1991). At the same time, omega-3-fatty acids, as represented by ∝-linolenic acid (C18:3ω_3) have shown several cardio-protective effects and have been associated with reduced incidences of cardiovascular diseases such as coronary heart disease and stroke (Leeson and Caston, 1996, Nash et al, 1995). Research has shown that diets rich in omega-3-fatty acids reduce platelet aggregation, lower blood TG levels, reduce the occurrence of blood clots, lower cholesterol and show both antithrombotic and anti-inflammatory effects (Simopoulos, 1996, Leeson and Caston, 1996, Nash et al, 1995). Reducing the levels of C12:0 to C16:0, and replacing these with mono and polyunsaturated fatty acids, particularly C18:3, could be beneficial for consumer acceptance of milk fat..

General decreases in short and medium chain saturated fatty acids with subsequent increases in long-chain fatty acids, such as C18:0 and C18:1, are often seen in the milk of cows fed supplemental fat (Schingoethe et al , 1996, Bitman et al, 1996, Beaulieu and

Palmquist, 1995, Klusmeyer and Clark, 1991, Grummer, 1991, Mohamed et al, 1988, DePeters et al 1985, McGuffey and Schingoethe, 1982). Unsaturated fatty acids may be increased in milk using oilseeds such as flax, soybeans, sunflower seeds or cottonseeds (Schingoethe et al, 1996, Khorasani and Kennelly, 1994, Stegeman et al, 1992a,b, Mohamed et al, 1988). However, in order to substantially alter the fatty acid composition of milk with respect to polyunsaturated fatty acids, the fat must first be protected from biohydrogenation in the rumen. Treatments of oilseeds such as formaldehyde (mixed with casein, zein or the seeds natural protein), forms a protective protein matrix which encapsulates the fat, preserving it from hydrogenation by rumen microorganisms (Grummer, 1991, Plowman et al, 1972). Treatments such as this result in substantial increases of C18 mono- and polyunsaturated fatty acids (54%) in milk (Ashes et al, 1992). The content of C18:2 and C18:3 fatty acids in milk fat have been as high as 35% and 22% respectively when formaldehyde protected sunflower or linseed oil were fed (Grummer, 1991). In a study by Plowman et al (1972), when formaldehyde treated safflower oil was fed to two lactating Holsteins, linoleic acid content went from 3 to 35% of total milk fatty acids.

Choosing a supplement such as flaxseed, which contains very high levels of linolenic acid (55-60% of total fat), or Solin, which contains high levels of linoleic acid, offers the opportunity to significantly improve the fatty acid composition of milk, particularly when protected from rumen hydrogenation. Solin is an oilseed which was developed from flax to contain less than 5% linolenic acid. A lighter oil, suitable for cooking, results (Hanley, 1996). Linola is a variety of Solin and contains approximately 72% linoleic acid (Fitch-Haumann, 1990). Linoleic acid is an omega-6-fatty acid which is typically considered beneficial and

important in cardiovascular disease and in lowering blood cholesterol (Simopoulos, 1996).

Formaldehyde protected supplements show the most dramatic alterations to the milk fat (Grummer, 1991), therefore, the full potential of flaxseed and Linola would best be seen utilizing this method. The objective of this experiment was to determine the effect of formaldehyde treated flaxseed and Linola on the fatty acid composition of milk and the extent of change in C18:3 in particular.

MATERIALS AND METHODS

Four Holsteins, two primiparous and two multiparous cows, in midlactation, were randomly assigned to four diets in a 4x4 Latin square design. Dietary treatments were i) control-no added fat, ii) Linola, a low linolenic (C18:3), high linoleic (C18:2) variety of Solin added at 1.6 kg per kg milk fat produced, iii) high C18:3 flaxseed added at .8 kg per kg milk fat produced (FL) and iv) high C18:3 flaxseed added at 1.6 kg per kg milk fat produced (FH) (Table 10). Supplements contained either 70.8% flaxseed or Linola, 10% casein and 19% soybean meal. They were then ground and treated with formaldehyde by Dr. J. Ashes, CSIRO Division of Animal Production (Australia). Cows were fed a TMR containing 44% dairy concentrate, 5.4% distillers dried grains, 20% corn silage and 32% alfalfa silage (Table 11). Flaxseed and Linola supplements were added to the diets as a top dress. The diets were formulated to meet the nutritional requirements of high producing dairy cows weighing an average of 625 kg and producing 40.6 kg fat corrected milk (FCM) at 3.6% butterfat (National Research Council, 1989). Animals were housed in a tie stall barn and fed once per day for ad libitum intake.

Top dress was adjusted weekly according to the kg of fat produced by each cow. The levels of supplement to be used were calculated as follows. An average production of 36 kg of milk at 3.4% fat was determined for the cows. The supplements were to provide approximately 123 g of C18:3 to the milk fat which would result, theoretically, in 10% of the fatty acids being α-linolenic acid. The top dress was calculated to have an average of 23% fat with 60% of the fatty acids C18:3, based on contributions of fat from the components. The digestibility of C18:3 was assumed to be 80% with 70% of absorbed C18:3 utilized by

Table 10. Ingredient composition (% DM) of the total mixed rations fed to cows and amount of flaxseed or Linola top dress added to TMR.

Total Mixed Ration	
Ingredient (%)	
Dairy Concentrate	43.6
Distiller's dried grains	5.4
Corn silage*	19.7
Alfalfa silage*	31.4
Top Dress (kg/d):	
Control	0.0
Linola	1.6
FL	0.8
FH	1.6

*Corn silage and alfalfa silage were 36.9 and 44.1% DM on average.

Table 11. Ingredient composition of dairy concentrate used in total mixed ration.

Ingredient	% (as fed)
Steam rolled barley	46.6
Steam rolled corn	11.00
Soybean meal	5.60
Canola meal	4.50
Wheat shorts	13.00
Corn distiller's grains	2.00
Meat meal	2.50
Blood meal	1.20
Limestone	0.70
Wheat	5.00
Barley	5.00
Dynamate (potassium)	0.70
Salt	0.65
Dicalcium phosphate	0.40
Mineral-Vitamin Premix	0.15
Luprosil salt (mold inhibitor)	0.15

*provided per kg of concentrate: 13,929 IU vitamin A, 3000 IU vitamin D, 18.5 IU vitamin E, 42.5 mg/kg copper, 145.8 mg/kg zinc, 75.4 mg/kg manganese, .29% magnesium , .16 mg/kg selenium, .70% calcium, .60% phosphorus.

the mammary gland (Palmquist and Eastridge, 1991, Dr. J.R. Ingalls, personal communication). Therefore, 1.6 kg of flaxseed or Linola supplement per kg milk fat was required. The lower level of flax was half of this figure, or 0.8 kg supplement per kg of milk fat produced. Supplements provided 454 g fat (Linola), 187 g fat (FL) and 410 g fat (FH).

Periods were three weeks, with cows allowed to adjust to the diets for the first two weeks. Dry matter intake and milk yield were recorded daily. Milk samples were taken once per week (day 4-p.m and day 5-a.m milkings), preserved with Brotab-"10" (30% 2-bromo-2-nitropropane-1,3-diol and 1.4% pimaricin) and sent to the Manitoba Milk Recording Corporation (MMRC) laboratory for infra-red analysis. The MMRC analysed milk protein by method 33.2.11 (AOAC, 1995) as well as fat and solid-non-fat (FOSS MS300 Infra-red Spectroscopy Analyser, Milk-O-Scan, Model 203B Type 17920 (Fosselectric, Cornwall, Ont.). A second milk sample, without Brotab, was retained for fatty acid analysis. Milk was combined in equal proportions from the pm/am samples to give one composite per cow. Fat from these samples were immediately separated and frozen for future analysis on the gas chromatograph (GC). In the third week, milk samples were collected on two consecutive days (day 4-pm, day 5-am; day 5-pm, day 6-am milkings) for a total of four samples which were composited for fat separation and fatty acid analysis. Equal amounts of milk were combined from d 4 and 5, am/pm, and from day 5 and 6, pm/am, to form the composites for a total of 2 samples/cow in week 3. Samples of feed were taken once a week and frozen to be composited for the period. Feedstuffs were composited for the period by taking 200 g of well mixed sample from each week and combining to make one 600 g sample. Two sub-samples of 100-200 g were taken from the period composite and dried in a forced air oven

at 60°C for 48 hr (dry matter to be used for feed intake calculations) and ground. Silage was dried in a forced air oven at 60°C for 48 hr every week to adjust diets for variable moisture content. Silages were then ground and kept in an air-tight container to be composited for the period. All feedstuffs were analysed for dry matter (DM), nitrogen (N), ether extract (EE), acid detergent fibre (ADF), neutral detergent fibre (NDF) (Table 12) and fatty acid. Results reported were averages for the four periods. Samples of feedstuff for each period were also taken and composited for a trial composite. These were composited by taking equal amounts of ground, well mixed feed from each period, combining the periods, mixing thoroughly again, and taking a final 100 g sample for the trial. These were analysed for acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN). Weigh backs were taken in the third week, dried at 60°C for 48 hr, ground and analysed for DM. Dairy concentrate was ground using a one mm screen in a Tecator Cyclotec 1093 Sample Mill (Hoganas, Sweden), while the silages and weigh backs were ground using a one mm screen in a Wiley Hammer Mill (Model 3, Thomas Scientific, Swedesboro, New Jersey).

Body condition scores were taken once at the beginning of the experiment and then once per period on d 20 of each period. Cows were weighed twice on d 6 and 7 of the week preceding commencement of the experiment, d 20 of each period and on d 20 and 21 in period 4. Blood and rumen samples were taken once in week three of each period, three hours post-feeding. The rumen fluid was collected via an esophageal tube (Ingalls et al, 1980). The pH was recorded at time of sampling using a Model 5985-50 pH metre (Cole-Parmer Instrument Co., Chicago, Illinois). Samples were heavily contaminated with saliva, therefore, pH results are not reported. Blood was drawn from the tail vein. Both rumen and

Table 12. Nutrient composition of forages, total mixed rations (dry matter basis) and kilograms added fat.

Nutrient (%)	Diet:	Corn Silage:	Alfalfa Silage:
Fat	3.6	2.2	3.2
Crude Protein	17.6	8.6	21.0
ADF	19.3	24.5	29.3
NDF	34.1	45.7	35.3
ADIN (% of CP)	7.5		
NDIN (% of CP)	11.0		

	Added Fat (kilograms):			
Diet:	Control	Linola	FL	FH
	0	0.454±.074*	0.187±.031*	0.410±.133*

* standard error.

blood samples were centrifuged and the supernatant was extracted and frozen (-20°C) for later analysis.

Chemical Analysis

Feed sample and weigh back DM was determined (Association of Official Analytical Chemists, AOAC, method 934.01, 1990). Protein content of feed samples and weigh backs was determined through analysis for N by the Kjeldahl method (method 954.01, AOAC, 1990) using a Tecator Kjeltec Auto 1030 Analyser (Tecator, Hoganas, Sweden). Neutral detergent fibre was determined according to the method developed by Goering and van Soest (1970). ADF was determined by AOAC, 973.18, 1990. Ether extract was analysed on all feed samples and weigh backs (except flaxseed and Linola) by AOAC, method 920.39,1990. The flaxseed and Linola supplement samples were sent to Norwest Laboratory (Winnipeg, Manitoba) for crude fat analysis by AOAC, 954.02, 1990). Results obtained for top dress were erratic and inconsistent and were not utilized. These methods did not give reasonable data for the formaldehyde protected flaxseed or Linola. Values obtained before processing by Dr. J. Ashes (CSIRO, Australia) were used as crude fat for all calculations. Trial composites for feedstuffs were analysed for ADIN and NDIN. Acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN) analyses were performed on ADF and NDF residues using the Micro-Kjeldahl (method 960.52, AOAC, 1990).

Fatty acid content was obtained on all feedstuffs as well as on blood and milk samples. Feed and blood samples were prepared as described by Outen et al (1976) and Sukhja and Palmquist (1988). Milk fat was extracted using the method of Lambert (1964). Fatty acids were determined as methyl esters using gas-liquid chromatography with a Varian Star 3400

and 8100 autosampler coupled with a Varian Star Workstation software for instrument control and data processing (peak integration) (Varian Canada, Inc. Georgetown, Ont.). The column for gas-liquid chromatography was 8' by 2mm ID packed with 3% SP2310 and 2% SP2300 on Chromosorb WAW (Supelco Canada, Ltd., Oakville, Ontario). A reference standard (GLC-60, NuChek Prep, Inc., Elysian, Minnesota) was used to quantitate the fatty acid content of the samples. Results were reported as a molar percentage of the total fatty acids detected. Milk samples were analysed for trans-C18:1 and conjugated-linoleic acid (CLA) as well, by Dr. D. Palmquist, Ohio State University (Ohio Agricultural Research and Development Centre, Wooster, Ohio) using the method of Sukhija and Palmquist (1988). The only modification of this method was that a computerized data system was used to collect the GC output (Hewlett-Packard, Chemstation). Samples were prepared by spinning 10-15 ml of cold (4°) milk, at 10,000 rpm for 20 minutes. The fat pad formed was then removed, placed in a small vial and flushed with nitrogen and frozen until shipment by dry ice to the Ohio Agricultural Research and Development Centre.

Rumen liquor samples were analysed for volatile fatty acids (VFA) by gas chromatography (Erwin et al, 1961). Rumen ammonia was measured with an ammonia electrode kit (model 95-10, Orion Research Inc., Cambridge, MA.). Ten ml of rumen fluid and 0.2 ml of NH^3 pH adjusting ISA (5M NaOH, 10% methanol, colour indicator and 0.05 M disodium EDTA) were used. Blood plasma samples were analysed for urea nitrogen concentration using Sigma Diagnostics Kit (Procedure No. 535) from Sigma Diagnostics (St. Louis, MO.).

Statistical Analysis

Data were analysed using the general lineral models (GLM) procedure of SAS (Statistical Analysis System Institute, Inc., 1988). Data was analysed as a 4X4 Latin square design with the model being:

$y_{ij}=\mu + a_i + b_j + t_{(k)} + e_{ij(k)}$

where u = mean

a_i = cow effect, i = 1 to 4.

b_j = period effect, j = 1 to 4.

$t_{(k)}$ = treatment effect, k = 1 to 4.

$e_{ij(k)}$ = error

Student-Neuman-Keuls test ($p<0.05$) was used to detect significant differences between treatment means (Steele and Torrie, 1980).

RESULTS AND DISCUSSION

Dietary treatments had no effect on dry matter intake (Table 13). Cow weight and body condition scores were unaffected by the addition to the diets of supplemental fat. Weight is sometimes a poor indicator of the actual condition status of the animal. Loss of weight resulting from body tissue is often masked by an increase in intake and gut fill in early to midlactation (Dunshea and Bell, 1989). Body condition score gives a good indication of body fatness (Pedron et al, 1993, Dunshea and Bell, 1989). The addition of the fat to the diet did not ($P>0.05$) effect the condition of the animals. Milk production parameters such as yield and composition (fat, protein and SNF%) were also unaffected by diet. The P-value for protein content was significant ($P<.05$) but the SNK test did not indicate a difference among treatments. The addition of the protected fats (Table 14) did not appear to disrupt rumen metabolism as there were no significant differences between the control and treatment cows for rumen ammonia or VFA (Table 14). This is in accordance with Jenkins et al (1996), who found that butylsoyamide, a form of rumen protected soybean oil, did not affect milk fat percent or VFA, suggesting that the product was indeed ruminally inert. The addition of unprotected fat, particularly vegetable or free oil, has been reported to interfere with normal rumen metabolism (Banks et al, 1982, Palmquist and Jenkins, 1980). Lipids added to the diets of ruminant animals result in decreased fibre digestibility, which is often accompanied by a reduced production of methane, hydrogen and VFA, in particular, a lower acetate to propionate ratio (Jenkins, 1993). Fat supplements also interfere with protein metabolism in the rumen, decreasing protein digestion and reducing ammonia concentration (Jenkins, 1993). Mohamed et al (1988), found that the addition of free oil to the diet of Holstein cows

Table 13. Effect of formaldehyde† treated flaxseed and Linola top dress on feed intake, milk composition, milk yield, and body weight change and condition scores.

	Treatment:				
Parameter:	Control:	Linola:	FL	FH	SEM
Intake (kg/d)	21.5	23.1	22.9	22.6	0.6
Milk:					
Yield (kg/d)	32.4	32.5	32.1	31.7	0.6
Fat (%)	2.7	3.2	3.0	3.1	0.1
Protein (%)	3.5	3.4	3.5	3.5	0.0
SNF (%)	9.0	9.0	9.1	9.1	0.1
Weight:					
Change (kg)	19.4	18.0	9.1	5.0	6.5
BCS* (change)	0.3	0.2	0.0	0.2	0.1

P>0.05.

† formaldehyde protected product courtesy J.R. Ashes. New South Wales. Australia.

*BCS=Body Condition Score.

Table 14. Effect of formaldehyde† treated flaxseed and Linola on rumen ammonia, volatile fatty acids (VFA) and plasma urea nitrogen (PUN).

	Treatment:				
Parameter:	Control	Linola	FL	FH	SEM
NH3 (mg/100ml)	10.7	10.4	9.5	13.2	1.5
PUN (mg/100ml)	21.7	21.6	24.5	24.6	1.1
VFA (%):					
Acetic	60.1	58.5	57.9	60.0	13.2
Butyric	12.8	11.6	12.0	11.6	3.3
Iso-butyric	1.0	0.9	0.8	1.4	0.2
Iso-valeric	1.8	1.5	1.4	2.4	0.5
Propionic	22.0	25.2	25.6	22.2	4.7
Valeric	2.4	2.4	2.2	2.4	0.7
A:P*	2.7:1	2.3:1	2.3:1	2.4:1	-

P>0.05.

† formaldehyde protected product courtesy J.R. Ashes, New South Wales, Australia

* Acetate:Proprionate ratio

increased propionate and decreased butyrate in ruminal VFA. Essential amino acids in the plasma of the above cows was also reduced, suggesting a reduction in microbial protein synthesis with the diets containing oil. Plasma urea nitrogen (PUN) was unaffected by dietary treatments (Table 14). This is to be expected since concentrations of serum urea tend to follow concentrations of rumen ammonia (Mielke and Schingoethe, 1981).

Protected Linola and flaxseed had no effect on plasma fatty acids, C16:0 or C16:1 (Table 15). Stearic acid (C18:0) was significantly lower in the plasma of cows fed FH than the control, but was not different from the other treatments. Jenkins et al (1996), found similar results when butylsoyamide was fed to lactating Holstein cows. Plasma oleic acid (C18:1) was decreased with treatments Linola, FL and FH compared to control. Linola and flax supplements were similar in C18:1 content (Table 16). This is in agreement with other studies (Jenkins et al, 1996, Jenkins, 1995) which show decreased plasma C18:1 when fat supplements are adequately protected from biohydrogenation in the rumen. The main end products of biohydrogenation in the rumen are stearic acid at 80% and trans-C18:1 at 12% (Palmquist and Jenkins, 1980). Free oil, or unprotected fat supplements often raise levels of C18:0 and C18:1 in plasma due to the hydrogenation of unsaturated fatty acids from the diet. Mohamed et al (1988) found significant increases in C18:1 when whole soybeans were fed and in particular, when free oil was added to rations. Similar results were not found with cottonseed or cottonseed oil. Jenkins et al (1996) found that the concentrations of C18:0 and C18:1 (both cis and trans) were highest in cows fed unprotected soybean oil. Additional C18:1 (especially trans-C18:1) may increase dramatically if the amount of unsaturated fatty acids fed is excessive due to inhibition of the conversion of trans-C18:1 to C18:0 by the

Table 15. Effect of formaldehyde† treated flaxseed and Linola on blood plasma fatty acids (least square means).

	Treatment				
Fatty Acid (%)	Control	Linola	FL	FH	SEM
C14:1	0.0[b]	0.7[a]	1.1[a]	0.6[a]	0.2
C16:0	14.9	13.7	13.4	9.3	1.4
C16:1	2.1	1.8	1.2	4.4	1.1
C18:0	24.5[a]	22.8[a,b]	22.8[a,b]	21.9[b]	0.5
C18:1	9.6[a]	6.1[b]	7.5[b]	7.2[b]	0.3
C18:2	44.5	48.7	42.2	41.0	2.4
C18:3	2.6[b]	0.8[b]	9.3[a]	11.3[a]	0.7

a-b Means not followed by the same letter differ significantly at $P< 0.05$ within fatty acid groups
† formaldehyde protected product courtesy J.R. Ashes, New South Wales, Australia.

Table 16. Fatty acid profile of supplemental formaldehyde treated flaxseed† and Linola† fed as a top dress.

	Treatments:			
Fatty Acid (%):	Linola	S.D.	FlaxForm.	S.D.
C4:0	0.0	n/a	0.0	n/a
C6:0	2.0	0.7	0.4	0.1
C8:0	0.3	0.0	0.2	0.0
C10:0	0.1	0.0	0.1	0.0
C12:0	0.2	0.2	0.5	0.3
C14:0	0.2	0.0	0.2	0.0
C14:1	0.3	0.1	0.1	0.0
C16:0	13.1	2.0	11.3	0.8
C16:1	0.1	0.0	0.1	0.0
C18:0	5.6	0.5	6.0	0.2
C18:1	23.3	0.9	25.2	0.9
C18:2	**44.6**	4.1	16.6	0.5
C18:3	2.1	0.2	**35.3**	1.4
C20:0	0.6	0.2	0.8	0.0

† formaldehyde protected product courtesy J.R. Ashes, New South Wales, Australia

rumen bacteria (Jenkins et al, 1996). This would result in high levels of C18:1 and lower levels of C18:0. The fact that treatments did not raise or effect C18:0 (with the exception of a decrease from FH diets) and decreased C18:1, indicates that the supplements were fairly well protected in the rumen. Although C18:0 was lower in the plasma of the FH fed cows, this was not true of the other treatments and C18:1 was significantly lower in the plasma of the cows fed supplemental fat. This gives a good indication that the unsaturated fatty acids did not undergo extensive hydrogenation nor impair conversion of C18:1 to C18:0 in the rumen. Protection of the fatty acids is further supported by the significant increase in C18:3 in the plasma of cows fed either FL or FH. The control diet resulted in a plasma C18:3 level of 2.60% of total plasma lipid, while levels in FL and FH were 9.33 and 11.33% respectively.

The dietary treatments had no effect on fatty acids C4:0 to C8:0 in the milk as a percentage of total milk fat (Table 17). This was similar to results reported by Jenkins et al (1996). Yield of C4:0 (appendix 1), however, was significantly greater ($p<0.05$) in Linola and FH (14.25 g each) than that of the control (11.75 grams). They were not significantly different from FL (12.5 grams) which was not significantly different from the control. Fatty acid C10:0 was significantly lower in the milk of cows fed the Linola than those fed either the control or the other treatments. Medium and long chain fatty acids, C12:0 to C16:0, were, in general, reduced by the feeding of the supplemental fat (Table 17). This is in accordance with several studies and reviews which note a decrease in these fatty acids when supplemental fat (protected or not) is fed (Schingoethe et al, 1996, Ashes et al, 1992, Grummer, 1991, Mohamed et al, 1988, DePeters et al, 1985, Rafalowski and Park, 1982, Mielke and Schingoethe, 1981, Palmquist and Jenkins, 1980, Plowman et al, 1972). This could provide

Table 17. Effect of formaldehyde treated flaxseed[†] and Linola[†] on milk fatty acids as a percentage of milk fat (least square means and standard errors).

	Treatment				
Fatty Acid (%)	Control	Linola	FL	FH	SEM
C4:0	1.36	1.39	1.39	1.41	0.03
C6:0	1.53	1.52	1.56	1.62	0.26
C8:0	0.88	0.90	0.90	0.96	0.03
C10:0	3.14 [a]	2.79[b]	3.13 [a]	3.11[a]	0.04
C12:0	3.89[a]	3.19[d]	3.69[b]	3.55[c]	0.04
C14:0	11.71[a]	9.78[b]	10.44[b]	9.91[b]	0.19
C14:1	1.96[a]	1.36[b]	1.51[b]	1.40[b]	0.05
C16:0	24.54[a]	20.00[c]	21.70[b]	19.83[c]	0.23
C16:1	3.19[a]	2.36[b]	2.52[b]	2.29[b]	0.07
C18:0	9.70[b]	12.13[a]	11.06[a,b]	11.21[a,b]	0.38
C18:1	26.26	27.08	26.80	26.24	0.22
C18:2	4.83[d]	10.34[a]	5.99[c]	6.88[b]	0.21
C18:3	.83[c]	1.00[c]	3.70[b]	6.39[a]	0.28
C20:0	0.78	0.89	0.86	0.82	0.09
C18:1-trans*.	2.04[b]	2.40[a]	2.07[b]	2.18[a,b]	0.06
CLA*	0.87	0.91	0.84	0.88	0.03
Total Saturated**	40.15[a]	32.97[c]	35.84[b]	33.29[c]	0.42
Total Unsaturated**	31.92[c]	38.42[a]	36.48[b]	39.50[a]	0.34

a-d Means not followed by the same letter differ significantly at $P< 0.05$ within fatty acid groups.
[†] formaldehyde protected product courtesy J.R. Ashes, New South Wales, Australia.
* analysis by Palmquist. CLA= conjugated linoleic acid.
** Saturated=C12:0-C16:0, Unsaturated=C18:1-C18:3

a nutritional benefit from a human health perspective as research suggests that the effect of saturated fat in human diets is largely due to fatty acids C12:0, C14:0 and C16:0 (Ney, 1991). Stearic acid (C18:0) was significantly higher in the milk fat of cows fed the protected Linola (12.13%) than that of control cows (9.70%) but was not different from other treatments. FL and FH were not significantly different from the control for C18:0. As previously stated, C18:0 often increases in the milk when unprotected fat is fed to dairy cows. The Linola and flaxseed used in this trial was approximately 70% and 80% resistant to hydrogenation in-vitro, respectively (J.R. Ashes, personal communication). Thus, the Linola, although fairly well protected, would still undergo more hydrogenation in the rumen than flax. This would result in a greater difference from the control than that of the flaxseed for stearic acid, as was the case in this experiment. Trans fatty acid, C18:1t, was similarly affected by the Linola which was likely due to the fact that the Linola was not as well protected. Cows fed supplemental FL or FH did not show a significant ($P<0.05$) difference in trans C18:1 from the control diet (2.07 and 2.18%, FL and FH respectively, vs 2.04% in control) Therefore, even though large amounts of unsaturated fatty acids were fed in these diets, they were adequately protected from the rumen. A buildup of trans-C18:1 is indicative of inhibition from large amounts of polyunsaturated fatty acids on the conversion from trans-C18:1 to C18:0 (Jenkins et al, 1996) and is also one of the primary indicators of hydrogenation in the rumen (Palmquist and Jenkins, 1980). Linoleic acid (C18:2) was significantly greater in the Linola fed cows (10.34%), followed by FH at 6.88% and FL at 5.99%. Linola contains a greater proportion of C18:2 (flaxseed contains approximately one-third of the level found in Linola for C18:2) while having very little C18:3. Flaxseed (high) and FL were also significantly different from

each other for these fatty acids representing the two different levels fed.

The absence of a significant increase in CLA, particularly in the milk from cows fed the Linola, is further evidence of the protection from the rumen microorganisms offered by the formaldehyde treated products. Conjugated linoleic acid is an intermediate in the biohydrogenation of linoleic acid by rumen microorganisms (Kelly and Bauman, 1996) and as it was unaffected by dietary treatments, suggests C18:2 was well protected. The pattern of C18:3 in milk fat was as expected. Flaxseed (high) (6.39%) was significantly greater than FL (3.70%) which were both significantly greater than either the control or Linola (0.83 and 1.0% respectively).

The average daily intake of fat from the higher level of flax (FH) was 410 grams (Table 12). Of this, 35% or 143.5 grams was C18:3. The digestibility of this was assumed to be 80% (Ashes et al, 1992, Palmquist and Eastridge, 1991). Therefore, assuming 70% utilization by the mammary gland and 80% rumen escape, 64 grams of C18:3 should end up in the milk. In this experiment, a total of 63 grams of C18:3 were present in the milk (Table 18) for an increase of 56 g with the FH treatment over the control. The calculations on amount of top dress to be added to the diet was based on an assumed 60% of the fat as C18:3 in the supplement. In fact, the flaxseed supplement contained only 35% C18:3 as a result of the blend of soybean and perhaps some oxidation. The flaxseed used may have also been lower than expected from book values. Nonetheless, 88% of the theoretical value of 64 grams (adjusted for actual levels of fat intake and content of C18:3) was achieved.

If the extra 56 grams of C18:3 in milk from cows receiving FH was derived from the supplemental fat, then this would represent a gross efficiency of transfer of 39% (56 ÷ 143.5).

Table 18. Dietary consumption (C) vs. secretion (S) of fatty acids in milk by cows receiving top dressed formaldehyde treated flaxseed (FL or FH) or Linola

	Treatment			
Fatty Acid (g d^{-1})	Control	Linola	FL	FH
C4:0				
C	–	–	–	–
S	12.0	14.0	13.0	14.0
C6:0				
C	8.1	17.0	9.0	9.0
S	13.0	16.0	14.0	16.0
C8:0				
C	1.3	3.0	2.0	2.0
S	8.0	9.0	8.0	10.0
C10:0				
C	8.9	9.0	9.0	9.0
S	27.0	29.0	29.0	31.0
C12:0				
C	9.5	10.0	11.0	11.0
S	34.0	33.0	34.0	35.0
C14:0				
C	4.7	6.0	5.0	5.0
S	101.0	102.0	97.0	96.0
C14:1				
C	3.5	5.0	4.0	4.0
S	17.0	14.0	14.0	13.0
C16:0				
C	169.0	220.0	190.0	207.0
S	211.0	207.0	202.0	192.0
C16:1				
C	7.0	7.0	7.0	7.0
S	27.0	24.0	24.0	22.0
C18:0				
C	34.1	58.0	45.0	57.0
S	83.0	124.0	103.0	109.0
C18:1				
C	180.8	277.0	225.0	275.0
S	225.0	278.0	250.0	252.0
C18:2				
C	197.1	387.0	226.0	254.0
S	42.0	107.0	56.0	67.0
C18:3				
C	59.5	64.0	124.0	200.0
S	7.0	10.0	34.0	63.0
C20:0				
C	9.8	12.0	11.0	13.0
S	7.0	9.0	8.0	8.0

The supplement provides approximately 80% protection from hydrogenation in the rumen and digestibility is assumed to be 80%. Therefore, adjusting for these factors, (Ashes et al, 1992), the efficiency of transfer of C18:3 at the mammary gland at this level of supplemental linolenic acid would be 61% as per the following equation:

$$56/(143.5 \times 0.8 \times 0.8).$$

Linoleic acid consumed was 69.7 g (17% C18:2 in FH x 410 g) and there were 42 g secreted in the control, thus 25 g would be a result of the supplemental fat. This represents a gross efficiency of transfer of 36%, or adjusting for protection and digestibility, a 56% efficiency. The transfer is much greater than that reported by Ashes et al (1992) or summarized by Grummer (1991). Success from feeding formaldehyde treated supplements has been noted to be variable, likely due to differences in protection vs digestibility achieved by different processors and laboratories (Grummer,1991). The efficiencies of transfer of C18:2 and C18:3 fatty acids from diet to milk fat is fairly consistent in this trial, with 57% and 56% transfer of C18:2 and C18:3 respectively, from Linola and 69% C18:2 and 64% C18:3 from FL. Linolenic content of milk fat increased 8.00 fold from the control, which represents a significant change. The lower level of flaxseed resulted in a 3.86 fold increase in C18:3, or 48% of that achieved with the higher level. This is about one-half of the effect seen from the FH, as expected.

The changes in fatty acids from week 1 to 2 and week 2 to 3 were not significantly effected by diet (Table 19). The changes in fatty acid levels appear to happen relatively quickly with treatments resulting in similar increases or decreases in week 1 to 2 vs week 2 to 3. Differences ($p<0.05$) in the change in fatty acids were also not observed in the time

Table 19. Change in fatty acids from weeks 1-2, 2-3 and 1-3 (percentage points)

Fatty Acid	Treatment														
(%)	Control			Linola			FL			FH			SEM		
	Week														
	1-2	2-3	1-3	1-2	2-3	1-3	1-2	2-3	1-3	1-2	2-3	1-3	1-2	2-3	1-3
C4:0	0.00	0.23	0.2.2	0.16	0.16	0.32	0.29	0.15	0.43	0.08	0.19	0.27	0.1	0.1	0.1
C6:0	0.01	0.07	0.08	0.11	0.02	0.02	0.01	0.00	0.01	0.07	0.10	0.17	0.07	0.1	0.1
C8:0	0.01	0.16	0.17	0.03	0.17	0.12	0.02	0.16	0.18	0.03	0.22	0.24	0.0	0.1	0.1
C10:0	0.12	0.14	0.26	0.12	0.00	0.19	0.02	0.02	0.02	0.13	0.11	0.24	0.1	0.1	0.2
C12:0	0.17	0.19	0.36	0.07	-0.05	0.13	0.03	-0.01	0.02	0.10	0.06	0.16	0.1	0.7	0.2
C14:0	0.48	0.24	0.72	0.42	-0.42	0.00	-0.32	-0.30	-0.62	0.00	-0.09	-0.09	0.3	0.2	0.3
C14:1	0.08	0.01	-0.06	-0.06	-0.13	-0.19	-0.05	-0.17	-0.21	-0.02	-0.10	-0.12	0.1	0.1	0.1
C16:0	0.83	-0.49	0.34	-0.12	-0.62	-0.73	-0.43	-0.77	-1.19	-0.11	-0.85	-0.97	0.5	0.3	0.6
C16:1	-0.16	-0.12	-0.29	-0.12	-0.09	-0.21	-0.03	-0.28	-0.32	-0.07	-0.15	-0.22	0.1	0.1	0.2
C18:0	-0.22	0.03	-0.25	0.53	0.49	1.02	0.33	0.44	0.78	0.29	-0.03	0.26	0.6	0.5	0.3
C18:1	-0.95	-0.52	-1.47	-0.62	-0.35	-0.98	-0.41	0.25	-0.16	-0.38	-0.45	-0.83	0.2	0.6	0.6
C18:2	-0.16	0.04	0.20	-0.24	0.08	-0.17	0.01	0.11	0.12	-0.27	0.24	-0.03	0.4	0.4	0.4
C18:3	-0.11	0.03	-0.15	-0.22	-0.09	-0.31	-0.06	0.26	0.19	0.20	0.55	0.75	0.1	0.3	0.3
C20:0	0.01	-0.13	-0.13	-0.08	-0.15	-0.23	-0.12	0.05	-0.08	-0.16	0.03	0.13	0.0	0.1	0.1

frame between weeks 1-3, further indicating that the effect from feeding the flaxseed was fairly immediate.

The inclusion of formaldehyde protected flaxseed or Linola did not appear to disrupt the normal metabolism found in the rumen nor did the treatments have any deleterious effects on any of the production parameters measured. The proportion of medium and long chain saturated fatty acids were decreased, while fatty acids C18:2 and C18:3 were increased in the milk fat produced from cows fed Linola and flaxseed respectively. Formaldehyde protected flaxseed and Linola provide sufficient by-pass fat to significantly increase the levels of desirable fatty acids such as C18:2 and C18:3 in the milk fat of dairy cows.

EXPERIMENT 2

Effect of Lignosulfonate Treated Flaxseed

on the

Fatty Acid Composition of Milk

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ABSTRACT

The following study was designed to determine whether lignosulfonate treated ground flaxseed can provide protection to polyunsaturated fatty acids from biohydrogenation in the rumen with the result of increasing levels of C18:3 in milk fat. Four primiparous Holstein cows in midlactation were randomly assigned to four dietary treatments in a 4x4 Latin square. Treatments consisted of a TMR plus i) 0% added fat (control), ii) formaldehyde treated flaxseed at 1.64 kg (FH), iii) lignosulfonate treated flaxseed at 0.57 kg (LL) and iv) lignosulfonate treated flaxseed at 1.14 kg (LH). The supplements were added as a top dress to provide approximately 386 g, 193 g and 377 g of fat from the LH, LL and FH diets, respectively. Average production for the four cows prior to the start of the experiment was 32 kgs of 4% FCM. The amount of fat required to raise C18:3 to 10% of total milk fatty acids was based on this production level.

Dietary treatments had no effect ($P>0.05$) on feed intake or milk production parameters such as yield or composition. Rumen metabolism was not affected by diet as there were no significant differences ($p>0.05$) in ammonia, VFA or pH. Palmitic acid (C16:0) was significantly lower in the plasma of cows fed FH, but was unaffected by other dietary treatments. Stearic acid (C18:0) did not show any significant differences among treatments or control while oleic (C18:1) acid was significantly lower in the plasma of FH (7.56%) fed cows versus that of LL, LH or the control (11.85, 11.67 and 11.95% respectively). Linolenic acid was significantly greater in the plasma of FH fed cows (10.64%) followed by LH (4.83%) and LL (3.48%) all of which are significantly different ($p<0.05$) from each other and the control (0.94%).

Flax-formaldehyde and LL had no effect on milk fat content of C12:0 (2.60% and 2.53% respectively vs 2.77% in the control) whereas LH was significantly lower at 2.41%. Myristic acid was significantly lower in the milk fat of all cows fed top dress with FH (8.39%)<LL (9.19%) = LH (9.09%)<control (9.72%). Palmitic acid was unaffected by LL or LH, but was significantly lower in milk from cows receiving FH. Fatty acids C18:0, C18:1 (both cis and trans) and C18:2 were not affected by diet. FH resulted in a significant increase in C18:3 (3.51% versus the control at .35%) as expected. Lignosulfonate treated flax did not significantly alter the concentration of C18:3 in the milk, suggesting inadequate protection. It appears that lignosulfonate offers some protection to the supplement from biohydrogenation in the rumen. However, it is likely that this is minor and not sufficient to adequately alter fatty acid composition in milk, particularly PUFA such as α-linolenic acid.

Key words: Flaxseed, formaldehyde, lignosulfonate, milk, linolenic acid, linoleic acid.

INTRODUCTION

Lignosulfonate has been successfully utilized to decrease rumen degradability of protein (McAllister et al, 1993, Windschitl and Stern, 1988, Stern, 1984). Other treatments, such as formaldehyde, which also protect protein from degradation in the rumen, have been employed to provide rumen by-pass fat (Ashes et al, 1992). Fat which is adequately protected from biohydrogenation in the rumen offers a source of fatty acids which may be passed into milk, thus altering the fatty acid composition of the milk fat. This may be beneficial from several standpoints. A milk fat which is higher in unsaturated fatty acids would produce butter that is softer and more spreadable at refrigeration temperatures (Stegeman et al, 1992b). Secondly, increasing the concentration of unsaturated fatty acids, while decreasing proportions of medium and long chain saturated fatty acids (C12:0 to C16:0), would be considered beneficial from a human nutritional health perspective.

Milk fat has been labelled as hypercholesterolemic due to its largely saturated nature and levels of cholesterol (Ney, 1991). Shorter chain saturated fatty acids (C4:0 to C10:0) have been shown not to be hypercholesterolemic (Jensen, 1992, Ney, 1991, Grundy and Denke, 1990), and moderate dietary cholesterol seems to have little effect on plasma cholesterol levels in non-sensitive individuals (Jensen, 1992). However, medium and long chain saturated fatty acids C12:0 to C16:0, have been implicated in raising levels of LDL cholesterol and total cholesterol and identified as a risk factor in coronary heart disease (Noakes et al, 1996, Jensen, 1992, Grundy and Denke, 1990).

Reduction of these medium and long (C12:0 to C16:0) chain fatty acids in milk is a common occurrence when fat is supplemented in the dairy cow's diet (Schingoethe et al,

1996, Beaulieu and Palmquist, 1995, Grummer, 1991). The most dramatic alterations to milk fatty acid composition have been seen with formaldehyde protected fats (Goodridge and Ingalls, manuscript I, Grummer, 1991). A method such as this also offers the greatest potential to increase the concentration of polyunsaturated fatty acids, and in particular, omega-3-fatty acids. Omega-3-fatty acids have been associated with a reduced incidence of cardiovascular disease (Nash et al, 1995) and are considered highly beneficial in terms of human health. Formaldehyde treated products, however, have not been approved for use in the U.S., largely due to concerns with formaldehyde safety and any residues left in milk (Stegeman et al, 1992b). However, Atwal and Mahadevan (1994) found no effect on milk formaldehyde level from feeding formaldehyde treated zein-coated soybean meal in lactating dairy cows. Nonetheless, finding alternative methods of protecting fat from hydrogenation in the rumen, that are commercially available in Canada, would be advantageous.

Lignosulfonate treatment of unextracted seed could be an alternative to formaldehyde for increasing rumen escape of unsaturated fatty acids. Lignosulfonate is derived from the spent sulfite liquor leftover after the sulfite digestion of wood and contains lignosulfonic acid or its salt, hemicellulose and sugars (Windschitl and Stern, 1988). It serves to bind and precipitate protein and is used as a feed pellet binder, in packing houses to remove protein from waste water and in leather tanning (Windschitl and Stern, 1988). The utilization of lignosulfonate to provide rumen undegradable protein has led to an interest in examining its effect on the fat. The objective of this study was to determine if lignosulfonate plus heat treated flaxseed could increase C18:3 in milk fat.

MATERIALS AND METHODS

Four primiparous Holstein cows in midlactation were randomly allotted to four diets in a 4 x 4 Latin square design. Periods were 21 days with data collection taking place in the third week. The first two weeks were to allow the animals time to adjust to the diet. Dietary treatments consisted of a TMR and i) a control diet with 0% added fat, ii) formaldehyde treated flaxseed at 1.64 kgs (FH), iii) low level of lignosulfonate treated flaxseed at 0.57 kgs (LL) and iv) high level of lignosulfonate treated flaxseed at 1.14 kgs (LH). The supplements were added as a top dress to the TMR which was fed once per day in the morning for ad-libitum intake.

Lignosulfonate supplements consisted of 80% flaxseed and 20% barley. Barley was added to the mix to facilitate grinding. Blending and grinding was conducted by EXL Milling (Hassell, Sask.) who then roasted the mixture at a temperature of 155°C using Jet-Pro equipment (Jet-Pro Company, Springfield, OH) modified for meal treatment. Lignosulfonate (65% DM, 70% reducing sugars with 40% of said reducing sugar being xylose) was added at 3% of the mix before a 30 minute steeping process. Steeping temperature began at 124°C and declined to 118°C by the end of the 30 minutes. Formaldehyde treated flaxseed was utilized as a comparison. The TMR was a blend of 29% corn silage, 12% chopped hay, 34% dairy concentrate (no added fat) and 26% of a protein/calcium supplement (Table 20). Diets were formulated to meet the nutritional requirements of high producing dairy cows weighing an average of 625 kgs and producing 40.6 kgs FCM at 3.6% butterfat (National Research Council, 1989).

The amount of supplemental fat added to the diets was calculated at the start of the

Table 20. Ingredient composition of the total mixed ration (% DM) and kilograms of top dress added to TMR.

Total Mixed Ration:				
Ingredient:	% of TMR:			
Dairy Concentrate* (16% crude protein)	33.4			
Protein/Calcium Supp.**	26.0			
Corn silage***	28.6			
Chopped Hay	12.1			
Top Dress (kg/d):	Control:	FH	LL	LH
FlaxFormaldehyde	—	1.64	—	—
FlaxLignosulfonate	—	—	0.57	1.36

* see Table 12 (Goodridge and Ingalls, 1997) for ingredient composition.
** protein/calcium supplement contained 44% canola meal, 48% distiller's dried grains, 38% limestone and 40% molasses.
*** corn silage was 39.4% DM on average.

experiment and was maintained throughout the trial. Milk yield and percent fat prior to the start of the study was pooled for the heifers and determined to be 32±0.83 kgs of 4.0% FCM. The goal was to provide 10% C18:3 in the milk fat based on assumptions of a digestibility of 80% with 70% of absorbed utilized in the mammary gland for milk fat synthesis. The supplement was calculated to have 34% fat, based on contribution from the flax and barley components. This would provide 386 g of fat in the milk from cows fed LH, 193 g from those receiving LL and 377 g from the FH diets. Flaxseed was assumed to be approximately 60% C18:3.

Animals were housed in a tie stall barn. Weights and body condition scores of the animals were taken the week prior to the start of the experiment and then on d 20 of each period. In the fourth period, cows were weighed twice in the third week. Blood and rumen samples were taken once in week three, three hours post-feeding. Rumen samples were collected via an esophageal tube (Ingalls et al, 1980). Heparinized tail blood samples were taken at this time also. Blood was centrifuged at 2200 rpm for 25 minutes and rumen samples at 12,500 rpm for 20 minutes to collect the supernatants, which were frozen (-20°C) and stored for future analysis. The pH of the rumen fluid was recorded at the time of sampling using a model 5985-50 pH metre from Cole-Parmer Instrument Co. (Chicago, Illinois).

Dry matter intake and milk yield were recorded daily. Milk samples were taken in the pm of day 4 and the am of day 5 for analysis of fat, protein and SNF by the Manitoba Milk Recording Corp. as in manuscript 1 (Goodridge and Ingalls, 1997). Milk samples taken on day 5 pm and day 6 am were composited (am/pm) for fatty acid analysis. The fat was immediately separated from these samples using the method of Lambert (1964), frozen, and

stored for future analysis on the gas chromatograph. Four milk samples (instead of two) for fatty acid analysis were taken on days 5,6 and 7 (pm/am-pm/am) in week three only.

Feed samples were taken once per week, kept frozen, and composited (as per manuscript 1, Goodridge and Ingalls, 1997) for the period for future analysis. Dry matter was obtained on feed and weigh back samples using a forced air oven at 60°C for 48 hr for feed intake calculations. Weigh back samples were taken in the third week of each period comprising a composite of days one to five. Silage samples were dried weekly in a forced air oven at 60°C for 48 hrs. to make any necessary adjustments to the diet due to changes in moisture content. Silages were then ground using a Wiley hammermill (Model3, Thomas Scientific, Swedesboro, New Jersey) and a one mm screen, and kept in an air tight container to be composited for each period. Period samples of weigh backs were ground using a one mm screen and a Wiley hammermill. The dairy concentrate and protein/calcium supplement composites were ground using a one mm screen and a Tecator Cyclotec 1093 Sample Mill (Hoganas, Sweden). Flax supplements were previously ground. A sample was also taken from each of the feedstuffs for each period and composited for a trial composite.

Chemical Analysis

Period feedstuff composites were analysed for DM, N, EE, ADF, NDF and fatty acids. The same analysis was conducted on the trial composites plus ADIN and NDIN. Weigh backs were analysed for DM, N, EE, ADF and NDF. Blood was analysed for plasma urea nitrogen and fatty acids. Rumen VFA and ammonia levels were determined on the rumen liquor samples.

All samples (milk, blood, rumen, feed and weigh backs) were prepared and analysed

as in the previous manuscript (Goodridge and Ingalls, 1997).

Statistical Analysis

Data were analysed using the GLM (general lineral models) procedure of SAS (Statistical Analysis System Institute Inc., (1988) as a 4 x 4 Latin square design. The model was:

$y_{ij} = \mu + a_i + b_j + t_{(k)} + e_{ij(k)}$

where μ = mean

a_i = cow effect, $_i$ = 1 to 4.

b_j = period effect, $_j$ = 1 to 4.

$t_{(k)}$= treatment effect, $_{(k)}$ = 1 to 4.

$e_{ij(k)}$ = error.

Student - Neuman - Keuls test was used to detect significant differences between treatment means (Steele and Torrie, 1980).

RESULTS AND DISCUSSION

Feed intake was not influenced by diet (Table 22). The additional fat supplied by FH, LL and LH did not affect body condition scores or weight (Table 23). Actual intake of supplemental fat from FH, LL and LH was 354 g, 170 g and 327 g (Table 21). Production parameters such as milk yield, fat, protein and SNF% were unaffected by treatments (Table 22). Rumen metabolism (Table 23) was not affected by the addition of fat to the diet as some have reported when unprotected fats (particularly polyunsaturated fatty acids) or free oils are fed (Grummer, 1991, Palmquist and Jenkins, 1980).

Addition of lipids to the diet of dairy cows may disrupt rumen function resulting in a decreased acetate:propionate ratio, and increased levels of ammonia due to a disruption in protein digestion (Jenkins, 1993). This was not the case in this experiment. Thus, superficially at least, there appears to be some protection offered to the fat in the lignosulfonate treated flaxseed or fat levels were not high enough to cause changes. When feeding supplemental oil as part of a whole oilseed, the effects on rumen function are markedly less than that seen with free oil, thus indicating a protective effect offered by the inclusion of the whole seed (Knapp et al, 1991). However, this does not necessarily result in any increases of polyunsaturated fats such as C18:2 and C18:3 in the milk (Mohamed et al, 1988). Previous studies utilizing lignosulfonate treated soybean meal to protect the protein showed decreased ruminal NH_3-N and total VFA with one study showing an increase in acetate with a subsequent decrease in propionate (Windschitl and Stern, 1988, Stern, 1984). This was attributed to a disruption in carbohydrate metabolism as insufficient N was available for the rumen microorganisms. In the present study, rumen ammonia was not significantly

Table 21. Nutrient composition of the total mixed rations (excluding top dress), forages and kg added fat (DM basis).

Nutrient	% Diet (total)	Corn Silage (%)	Chopped Hay (%)
Fat	4.4	2.6	2.6
Crude Protein	18.5	9.0	10.6
ADF	20.1	25.8	41.8
NDF	36	45.2	63.9
ADIN (% of CP)	5.4		
NDIN (% of CP)	9.5		

Added Fat (kg/d):

Diet:	Control	FH	LL	LH
	0	0.354±.012	0.170±.005	0.327±.016

Table 22. Effect of formaldehyde† and lignosulfonate†† treated flaxseed top dress on feed intake, milk composition, milk yield, and body weight change and condition scores.

	Treatment:				
Parameter:	Control:	FH	LL	LH	SEM
Intake (kg/d)	22.1	22.5	21.9	22.6	0.7
Milk:					
Yield (kg/d)	33.7	32.0	33.6	33.5	0.7
Fat (%)	2.9	3.0	2.5	2.6	0.2
Protein (%)	3.4	3.3	3.3	3.4	0.0
SNF (%)	9.2	9.2	9.2	9.2	0.0
Weight (kg):					
Change	12.3	12.9	13.5	10.5	2.9
BCS**(change)	0.1	0.1	0.1	0.0	0.1

P>0.05.

† formaldehyde protected product courtesy J.R. Ashes, New South Wales, Australia.

†† lignosulfonate treated flaxseed courtesy EXL Milling Ltd., Hassall, Saskatchewan

**BCS=Body Condition Score.

Table 23. Effect of formaldehyde† and lignosulfonate ‡‡ treated flaxseed on rumen ammonia, volatile fatty acids (VFA) and plasma urea nitrogen (PUN).

	Treatment:				
Parameter:	Control	FH	LL	LH	SEM
NH_3 (mg/100ml)	15	19.5	11.2	14.8	3.97
PUN (mg/100ml)	19.4±1.2	20.9±1.5	22.0±1.2	23.8±1.2	
VFA (%):					
Acetic	56.5	55.2	53.2	54.5	15.5
Butyric	12.5	12.8	10.7	13.1	3.9
Iso-butyric	0.5	0.6	0.5	0.1	0.2
Iso-valeric	0.9	0.7	0.8	1.1	0.2
Propionic	27.4	28.7	32.4	28.9	8.6
Valeric	2.1	2.0	2.4	1.7	0.7
A:P*	2.1:1	1.9:1	1.6:1	1.9:1	

P>0.05.

† formaldehyde protected product courtesy J.R. Ashes, New South Wales, Australia.

‡‡ lignosulfonate treated flaxseed courtesy EXL Milling Ltd., Hassall, Saskatchewan.

* acetate to propionate ratio.

affected nor was the same pattern of effect on VFA observed. Therefore, it is unlikely that the explanation provided by experiments of Windschitl and Stern (1988) and Stern (1984) are applicable in this case. Rumen NH_3 levels and PUN levels would suggest soluble N was not a limiting factor for bacteria (Windschitl and Stern, 1988, Stern, 1984).

Further support for at least partial protection of the fat in the rumen offered by the unextracted lignosulfonate treated flaxseed is seen in the blood plasma fatty acids, however, this may or may not be related to lignosulfonate treatment (Table 24). In both LL and LH fed cows, the proportion of C18:3 in the plasma is significantly greater (3.48 and 4.83% respectively) than that of the control (.94%). The elevation of C18:3 indicates some escape of this fatty acid from ruminal biohydrogenation, as was the case with C18:2 in a study conducted by Jenkins et al (1996). However, it is not nearly as large a change as that seen with FH (10.64%) indicating less protection offered by the lignosulfonate treatment. C18:1 and C18:0, main end-products of hydrogenation in the rumen, were unaffected by diet except in the case of FH, where C18:1 was significantly lower than the other treatments and control. Palmitic acid was also significantly lower in the plasma from cows fed FH, but was not affected by the other treatments. This was not seen in the previous experiment (Goodridge and Ingalls, 1997), where there were no significant differences in plasma C16:0. Jenkins et al (1996) observed C16:0 to be lowest in the plasma of cows fed soybean oil (11.72%) versus that of a control (12.39%) or protected butylsoyamide (12.75%). Cook and Scott, (1972), observed a decrease in plasma C16:0 when formaldehyde and untreated safflower oil was fed to dairy cows.

Milk fatty acid C4:0 was not affected by dietary treatments (Table 25). Short,

Table 24. Effect of formaldehyde† and ligonosulfonate †† treated flaxseed on blood plasma fatty acids of lactating dairy cows (least square means and SEM).

	Treatment			
Fatty Acid (%)	Control	FH	LL	LH
C14:1	0.50±.23	0.42±.23	0.28±.30	0.00±.23
C16:0	14.94±.35[a]	12.79±.35[b]	14.67±.45[a]	14.26±.35[a]
C16:1	1.26±.46	1.94±.46	1.74±.60	1.01±.46
C18:0	23.24±.52	22.43±.52	22.71±.68	22.92±.52
C18:1	11.95±.64[a]	7.56±.64[b]	11.85±.82[a]	11.67±.64[a]
C18:2	47.11±.72	44.45±.72	45.90±.94	44.99±.72
C18:3	0.94±.34[d]	10.64±.34[a]	3.48±.34[c]	4.83±.34[b]

a-b Means not followed by the same letter differ significantly at $P< 0.05$ within fatty acid groups.
† formaldehyde protected product courtesy J.R. Ashes, New South Wales, Australia.
†† lignosulfonate treated flaxseed courtesy EXL Milling Ltd., Hassall, Saskatchewan.

Table 25. Effect of formaldehyde treated flaxseed[†] and lignosulfonate treated flaxseed[††] on milk fatty acids as a percentage of milk fat (least square means and standard errors).

	Treatment				
Fatty Acid (%)	Control	FH	LL	LH	SEM
C4:0	1.45	1.56	1.35	1.35	0.10
C6:0	1.23^{a,b}	1.38^{a}	1.10^{b}	1.10^{b}	0.10
C8:0	0.86^{a,b}	0.96^{a}	0.76 ^{b}	0.75^{b}	0.00
C10:0	2.18^{a}	2.29^{a}	1.94^{b}	1.86^{b}	0.10
C12:0	2.77^{a}	2.60^{a,b}	2.53^{a,b}	2.41^{b}	0.10
C14:0	9.72^{a}	8.39^{c}	9.19^{b}	9.09^{b}	0.13
C14:1	1.69	1.17	1.67	1.58	0.11
C16:0	22.52^{a}	19.01^{b}	21.15^{a,b}	21.15^{a,b}	0.60
C16:1	3.15^{a}	1.81^{b}	3.23^{a}	2.96^{a}	0.21
C18:0	11.59	14.34	11.66	12.73	0.68
C18:1	31.35	30.46	32.5	33.14	0.67
C18:2	4.85	6.00	5.49	5.18	0.33
C18:3	0.35^{b}	3.51^{a}	0.64^{b}	0.71^{b}	0.20
C20:0	1.15	1.15	1.10	1.06	0.10
C18:1-trans.*	3.84	3.35	3.83	3.47	.19
CLA*	1.47	1.22	1.39	1.32	0.08
Total Saturated**	35.02^{a}	30.01^{b}	32.88^{a,b}	32.65^{a,b}	0.68
Total Unsaturated**	36.55^{b}	39.97^{a}	38.63^{a}	39.03^{a}	0.51

a-d Means not followed by the same letter differ significantly at P< 0.05 within fatty acid groups.
[†] formaldehyde protected product courtesy J.R. Ashes, New South Wales, Australia.
[††] lignosulfonate treated flaxseed courtesy EXL Milling Ltd., Hassall, Saskatchewan.
* Analysis by Palmquist. CLA= conjugated linoleic acid.
** Saturated=C12:0-C14:0, Unsaturated=C18:1-C18:3

medium and long chain fatty acids, with the exception of palmitic acid in LL and LH and C12:0 in LL, were significantly reduced by the addition of LL and LH to the diet. This is an established effect of lipid addition to the diets of dairy cows (Grummer, 1991, Palmquist and Jenkins, 1980). The absence of a significant effect on C16:0 in the milk fat from LL an LH fed cows mirrors that which was seen in the plasma of these cows. Fatty acids C6:0 to C10:0 were unaffected by FH, as was the case in the previous experiment and that of Jenkins et al (1996). Lauric acid (C12:0) was not significantly decreased by FH, however, it was lower than the control. Myristic and palmitic acids were significantly decreased by the addition of the FH to the diet. Stearic acid (C18:0) and oleic acid (C18:1) were unaffected by treatments. The proportion of trans-C18:1 was not significantly affected by LL, LH or FH.

An increase in C18:1 may be seen when a protected supplement is fed which is high in oleic acid (Ashes et al, 1992). However, increases in C18:0, C18:1 and in particular, trans-C18:1, in the milk may also serve to indicate that extensive hydrogenation of polyunsaturated fatty acids has occurred in the rumen (Palmquist and Jenkins, 1980). Although this does not appear to be the case in this experiment, it is unlikely that the lignosulfonate treatment provided adequate protection to the fat for the purposes of this study.

Linolenic acid did not show any significant increases in the milk fat of cows fed the lignosulfonate treated flaxseed (0.64, 0.71% for LL and LH respectively versus 0.35% for the control). Diet FH did result in a significant increase in C18:3 (3.51%) as expected (Goodridge and Ingalls, 1997). This represents a 10.33 fold increase in this fatty acid; a large change. Gross transfer efficiencies of C18:3 from the lignosulfonate treated flaxseed were 2.2% (LL) and 1.7% (LH). These results are consistent with that of a poorly protected

supplement (Ashes et al, 1992). In data generated by Atwal et al (1991), feeding canola oil to Holstein cows as Protec, a form of rumen by- pass protein, the transfer efficiency of C18:2 was also 3%. This was interpreted as marginal protection of the fatty acids from hydrogenation in the rumen.

Theoretically, with an intake of 327 g of fat at 54% C18:3 (Table 26), 80% rumen escape (assuming the same protection as in formaldehyde treated products) and a digestibility of 80% with 70% absorbed in the mammary gland, 79 g of C18:3 should have been secreted in the milk fat from diet LH. In vitro degradation of the lignosulfonate treated flaxseed was not determined. Milk fat generally contains only .1% C18:3 (Kennelly, 1994) with a range from trace amounts to 2.06% (Jensen et al, 1991). Therefore, results obtained from this study for the LL and LH fed cows (0.64 and 0.71% respectively) fall within the range of that normally found in milk.

With 354 g intake of fat at 35% C18:3, 80% digestibility, 70% utilization in the mammary gland and 80% rumen escape, the FH would provide 56 g for incorporation into milk fat. In this experiment, 31 grams of C18:3 (Table 27) were secreted into the milk fat, or 55% of the theoretical value. Gross transfer efficiency for this fatty acid was 25%. Adjusting for a digestibility of 80% and rumen escape of 80%, the transfer efficiency is 39%. This is much lower than that found in the previous manuscript (Goodridge and Ingalls, 1997) as well as that reported by Ashes et al (1992). Fatty acid analysis carried out on the fat samples by Palmquist (Ohio State), show C18:3 in the milk fat to be 4.80, 0.87 and 1.00% for FH, LL and LH respectively, with the control at 0.44% (Table 28). Comparing our results with that of Palmquist indicates 73% of C18:3 was found in the milk from our analysis vs that of Palmquist. The results from Palmquist's laboratory show 27% more C18:3. Adjusting for

Table 26. Fatty acid profile and intake of fat from formaldehyde treated flaxseed[†] and lignosulfonate[††] treated flaxseed[†]fed as a top dress.

	Treatments			
Fatty Acid (%):	Flaxseeed-Formaldehyde	SD	Lignosulfonate Flaxseed	SD
C4:0	0.00	n/a	0.00	n/a
C6:0	0.27	0.03	0.00	0.00
C8:0	0.17	0.02	0.01	0.02
C10:0	0.05	0.01	0.04	0.00
C12:0	0.22	0.40	0.27	0.01
C14:0	0.14	0.01	0.06	0.00
C14:1	0.12	0.02	0.03	0.00
C16:0	10.94	0.80	5.60	0.10
C16:1	0.08	0.00	0.03	0.00
C18:0	5.92	0.40	3.73	0.07
C18:1	25.28	1.30	18.47	0.20
C18:2	17.20	0.20	15.69	0.06
C18:3	35.00	2.60	54.35	0.30
C20:0	1.27	0.09	0.94	0.01

[†] formaldehyde protected product courtesy J.R. Ashes, New South Wales, Australia
[††] lignosulfonate treated flaxseed courtesy EXL Milling Ltd., Hassall, Saskatchewan

Table 27. Dietary consumption (C) vs. secretion (S) of fatty acids in milk by cows receiving top dressed formaldehyde treated flaxseed (FH) or lignosulfonate treated flaxseed (LL or LH)

	Treatment			
Fatty Acid (g d^{-1})	Control	FH	LL	LH
C4:0				
C	–	–	–	–
S	14.0	15.0	12.0	12.0
C6:0				
C	2.0	4.0	2.0	2.0
S	12.0	13.0	9.0	10.0
C8:0				
C	–	2.0	–	1.0
S	8.0	9.0	6.0	7.0
C10:0				
C	11.0	11.0	11.0	10.0
S	21.0	21.0	16.0	16.0
C12:0				
C	14.0	15.0	15.0	16.0
S	27.0	24.0	21.0	21.0
C14:0				
C	4.0	5.0	4.0	4.0
S	94.0	78.0	78.0	80.0
C14:1				
C	1.0	2.0	1.0	1.0
S	16.0	11.0	14.0	14.0
C16:0				
C	146.0	218.0	160.0	175.0
S	216.0	177.0	181.0	186.0
C16:1				
C	9.0	9.0	9.0	9.0
S	30.0	16.0	26.0	26.0
C18:0				
C	33.0	74.0	45.0	56.0
S	112.0	136.0	101.0	110.0
C18:1				
C	268.8	438.0	324.0	376.0
S	302.0	285.0	277.0	290.0
C18:2				
C	364.0	473.0	407.0	449.0
S	47.0	56.0	44.0	45.0
C18:3				
C	33.0	280.0	217.0	387.0
S	3.0	34.0	5.0	6.0
C20:0				
C	5.0	14.0	8.0	11.0
S	11.0	11.0	9.0	9.0

Table 28. Effect of formaldehyde treated flaxseed[†] and lignosulfonate treated flaxseed[††] on milk fatty acids as a percentage of milk fat as analysed by Palmquist (least square means and standard errors).

	Treatment				
Fatty Acid (%)	Control	FH	LL	LH	SEM
C4:0	3.49	3.68	3.15	3.36	0.16
C6:0	2.01	2.17	1.78	1.81	0.09
C8:0	1.12^{a,b}	1.21^{a}	0.99^{b}	0.97^{b}	0.04
C10:0	2.48^{a}	2.58^{a}	2.22^{b}	2.12^{b}	0.07
C12:0	3.00^{a}	2.79^{b}	2.78^{b}	2.61^{b}	0.05
C14:0	10.35^{a}	8.81^{c}	10.00^{b}	9.78^{b}	0.09
C14:1	1.36	0.79	1.39	1.33	0.14
C16:0	25.20^{a}	20.76^{b}	24.18^{a}	23.97^{a}	0.46
C16:1	1.91	1.17	1.84	1.70	0.17
C18:0	12.42	15.36	12.66	13.79	0.78
C18:1t*	3.84	3.35	3.83	3.47	0.19
C18:1c*	24.45	23.96	26.12	26.48	0.52
C18:1o*	0.92	0.96	1.12	1.05	0.06
C18:2	3.60	4.68	3.74	3.52	0.28
C18:3	0.44^{b}	4.80^{a}	0.87^{b}	1.00^{b}	0.30
C20:0	0.17	0.27	0.19	0.16	0.03
CLA**	1.47	1.22	1.39	1.32	0.08

a-d Means not followed by the same letter differ significantly at $P< 0.05$ within fatty acid groups.

[†] formaldehyde protected product courtesy J.R. Ashes, New South Wales, Australia.

[††] lignosulfonate treated flaxseed courtesy EXL Milling Ltd., Hassall, Saskatchewan.

* t=trans, c=cis, o=other.

** conjugated linoleic acid.

this factor, 43 g of C18:3 would have been secreted. The gross transfer efficiency would be 31%. Transfer efficiency would be 54% after correcting for digestibility and rumen escape. This is closer to results obtained previously (Goodridge and Ingalls, 1997) with the same supplement. Variability of results for fatty acid analysis between laboratories with respect to formaldehyde protected supplements (Grummer, 1991) is further emphasized by these results. Although a different type of GC column was used by Palmquist (capillary), the results from the first experiment between Palmquist and our laboratory were much closer (Table 17, Appendix 1). The use of a different column may have been a contributory factor but was unlikely the whole explanation.

Linoleic (C18:2) acid and conjugated-linoleic acid (CLA) were not significantly increased as a result of any of the dietary treatments. The supplemental flaxseed did not contribute much more C18:2 than the control diet (364, 473, 407 and 449 grams consumed from the control, FH, LL and LH respectively) and therefore milk from supplemented cows would not be expected to be any higher in the intermediate of linoleic acid, CLA, than that of the control cows.

These results indicate that although blood level of C18:3 was increased, the lignosulfonate treated flaxseed was not adequately protected and did not provide sufficient rumen escape C18:3 to significantly increase the C18:3 content of milk fat. Although milk production parameters and normal rumen metabolism function were unaffected by treatments, the level of fat fed may have been insufficient to observe such changes.

GENERAL DISCUSSION

The use of rumen protected flaxseed significantly alters and improves the fatty acid composition of milk fat, and in particular, linolenic acid (C18:3). The key to success, however, lies in choosing a method which preserves the fatty acids and prevents them from undergoing extensive hydrogenation in the rumen.

Experiment I resulted in an 8.00 fold increase in C18:3 over that of the control while experiment II increased C18:3 10.33 fold when formaldehyde treated flaxseed was fed. The level of linolenic acid in the milk fat was 6.39% (experiment I) and 3.51% (experiment II) of total fatty acids for cows receiving the higher level of formaldehyde treated flax. This represents a significant improvement when compared to the normal C18:3 content of milk fat, which is generally at or less than 1% (Kennelly 1994, Jensen, 1992, Jensen et al, 1991). Fat modified dairy products have resulted in lower plasma cholesterol levels of humans consuming the products (Noakes et al, 1996). In the study conducted by Noakes et al (1996), the fat modified products contained 2.2% C18:3 while the control contained only .7%. Linolenic acid is noted for it's beneficial health effects relating to cardiovascular disease, including lowering plasma cholesterol (Simopoulos, 1996, Leeson and Caston, 1996, Nash et al, 1995).

The feeding of formaldehyde protected flaxseed and Linola resulted in a decrease of medium and long chain saturated fatty acids (C12:0 to C16:0) in the milk from 40% of total fatty acids in the control cows to 33% for both the Linola and flaxseed fed cows (Table 17). All three treatments (FL, FH and Linola) resulted in a significant decrease in medium and long chain saturated fatty acids. Unsaturated fatty acids, C18:1, C18:2 plus C18:3, increased from 32% in the control diet to 38% and 40% of total fatty acids in the milk for Linola and FH

respectively. For experiment II (Table 25) there was a decrease of medium and long chain saturated fatty acids in the milk fat from 35% of the total fatty acids down to 30% and an increase in C18:1, C18:2 and C18:3 from 37% up to 40% of the total fatty acids with the FH treatment. Although this represents a significant improvement in the fatty acid profile of milk from cows fed the formaldehyde protected flaxseed, a further decrease in C12:0, C14:0 and C16:0 may be desirable. The lignosulfonate treated flaxseed was much less successful. Medium and long chain saturated fatty acids, C12:0 to C16:0, were not significantly decreased. Unsaturated fatty acids, C18:1, C18:2 plus C18:3, increased 2 percentage points, from 37% in the control to 39% in both LL and LH. This was a significant increase ($p<.05$). However, linolenic acid comprised only 0.64% and 0.71% of total milk fatty acids in the LL and LH fed cows. The control contained 0.35% C18:3 in milk fat.. Transfer efficiencies of this fatty acid were only 3.26% (LL) and 1.69% (LH), which is consistent with an inadequately protected fat supplement (Ashes et al, 1992, Atwal et al, 1991).

With respect to the formaldehyde treated flaxseed, the first experiment resulted in 56 g of C18:3 in the milk fat of cows fed the FH diet. This was 88% of our theoretical value of 64 g of C18:3. The efficiency of transfer, after adjustment for calculated digestibility and in-vitro protection, was 61%. In the second experiment, only 55% of the theoretical value was achieved with a transfer efficiency of 39%. However, results obtained from a milk fat sample sent to Palmquist (Ohio State Agricultural and Development Research Centre), indicated 77% of the theoretical value was achieved with a transfer efficiency of 54%. Differences and variabilities among laboratories is a noted artifact of analysis of formaldehyde protected fat supplements (Grummer, 1991). These data indicate the improvements in the amount of C18:3 in the milk fat of cows fed formaldehyde treated flaxseed in these studies were

significant regardless of where the analysis was made.

Linoleic acid was significantly increased from 4.83% in the control to 10.34% in the milk from cows fed Linola (experiment I). Linoleic acid has been associated with lowering blood cholesterol levels (Noakes et al, 1996). The proportion of linoleic acid present in the milk fat from experiment II was unaffected by dietary treatments. In experiment I, the level of C18:2 was significantly greater than the control for FH. Comparing the levels of C18:2 found in the milk from cows fed FH for the first experiment resulted in 6.82% C18:2 while the second experiment resulted in a level of 6.00%. Changes in linoleic acid were therefore fairly consistent between the two trials. Conjugated-linoleic acid and trans-C18:1 fatty acids were not affected by the formaldehyde or lignosulfonate treated flaxseed. Milk fat from cows top dressed Linola did show a significant increase ($p<0.05$) in trans C18:1. This may have been due to a lesser degree of rumen protection offered by the Linola supplements. Conjugated-linoleic acid is an intermediate formed in the biohydrogenation of linoleic acid, while trans C18:1 results from hydrogenation of C18:1 in the rumen. In the case of formaldehyde treated flaxseed, rumen protection of the fat was sufficient enough to avoid accumulation of these intermediaries in the milk fat. The amount of linoleic acid present in the lignosulfonate treated flaxseed was probably insufficient to significantly increase the proportion of CLA in the milk. Levels of unsaturated fatty acids (C18:2) present in the diet is an important factor in the levels of C18:2 in the milk (Kelly and Bauman, 1996). Also, some protection of the flaxseed did occur (as indicated by blood plasma results) and thus less CLA or trans-C18:1 would be expected. Conjugated linoleic acid has been identified as a potent anticarcinogen as well as an antioxidant (Kelly and Bauman, 1996) and an increase in the amount of this fatty acid may indeed be beneficial. However, the source of C18:2 fed

would have to be available to the rumen microorganisms for hydrogenation in order for the CLA intermediate to be formed, which then could be absorbed and incorporated into milk triglyceride by the mammary gland. Perhaps a combination of a supplement such as Linola, high in unprotected C18:2 combined with a well protected rumen fat supplement high in C18:3, would result in increased CLA and omega-3-fatty acid C18:3 in milk. Research in this area would prove interesting. The lack of an increase in trans-C18:1 is considered advantageous, as these fatty acids are thought to behave like saturated fatty acids C12:0 to C16:0 in their hypercholesterolemic effects (Grummer, 1991).

Dietary treatments did not result in any detrimental or negative effects on the general status of the animals involved in either study. Feed intake, milk production and milk composition were unaffected. The body condition scores and weight of the cows did not change as a result of either experiment. There were no negative effects associated with the treatments on any rumen metabolism parameters such as VFA or ammonia. Plasma urea nitrogen was not affected in experiment I or II. Feeding high levels of polyunsaturated fatty acids to dairy cows can result in a decrease in the ratio of acetate to propionate resulting in a lower milk fat test (Banks et al, 1982). Also, a consistent result of supplemental fat is a decrease in the protein content of milk (Palmquist and Eastridge, 1991) This was not the case in either study. Fat added to dairy rations, in general, also results in improved reproductive performance and reduced incidence of metabolic disorders (Hutjens, 1993, Grummer and Carroll, 1991). However, if the added dietary fat were to result in the cows gaining too much body fat, health problems associated with over conditioning may occur. This would be particularly true if the weight were to remain through the dry period up until calving (Schmidt et al, 1988). Although this was not indicated in these trials, as supported by a lack of

difference in body condition scores, the experimental periods were too short to measure such changes.

Results of these studies indicate that the addition of protected flaxseed to the diets of dairy cows will improve fatty acid profile as perceived by consumers. Some improvement was also seen even when the supplement was poorly protected, as was the case with the lignosulfonate treated flax. In order to significantly improve the milk fat and especially to increase the C18:3 content, a form of protection comparable to formaldehyde is required. Further research is required to either establish an effective and viable method of protecting fat from hydrogenation in the rumen which would be available for use in Canada or establish the safety or health risks associated when working with formaldehyde protected supplements or consuming products from feeding these supplements to dairy cows. Further work is required in the area of in-vitro protection achieved with formaldehyde protected supplements as well as methods of analysing the fat contained in such supplements. This is required to reduce the variability and inconsistency within and between laboratories. Research into developing a supplement containing high levels of unprotected C18:2 with high levels of protected C18:3 would be very interesting. Flaxseed and Linola could both be utilized in such studies. The CLA level, if increased by such treatments, could provide a natural antioxidant for the higher levels of C18:3 which would result in the milk fat.

SUMMARY

I. Feed intake was not affected by the inclusion of either formaldehyde or lignosulfonate treated flaxseed or Linola containing 400-450 g fat for cows producing approximately 1 kg fat.

II. Milk production parameters including i) yield, ii) fat%, iii) protein % and iv) SNF% were not affected by added flaxseed or Linola to the diets of the cows in either experiment.

III. Normal rumen metabolism and function were unaffected by dietary treatments in either experiment as measured by rumen VFA, pH and ammonia levels.

IV. Plasma urea nitrogen was not affected by the addition of the flaxseed or Linola supplements to the diets.

V. Formaldehyde protected flaxseed and Linola resulted in significant changes in the overall fatty acid composition of milk. Saturated fatty acids, C12:0 to C16:0 were decreased, while total unsaturated fatty acids C18:1, C18:2 and C18:3 were increased.

VI. Formaldehyde protected flaxseed greatly increased the proportion of C18:3 present in the milk fat, while Linola significantly increased the proportion of C18:2.

VII. Lignosulfonate plus heat provided inadequate protection of the fat from biohydrogenation by the rumen microorganisms as measured by lack of change in C18:3 content of milk fat.

VIII. Conjugated-linoleic acid was not significantly increased as a result of any of the dietary treatments in either experiment thus suggesting the Linola was well protected from rumen bacteria.

IX. Trans-C18:1 was not significantly affected by any of the dietary treatments in either

experiment.

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APPENDICES

Appendix 1. Effect of formaldehyde treated flaxseed[†] and Linola[†] on milk fatty acid yield per cow per day (least square means and standard errors).

	Treatment				
Fatty Acid (g):	Control	Linola	FL	FH	SEM
C4:0	11.75^{b}	14.25^{a}	12.50^{a,b}	14.25^{a}	0.43
C6:0	13.25	15.75	14.50	16.00	0.66
C8:0	7.50^{b}	9.00^{a,b}	8.25^{a,b}	9.50^{a}	0.40
C10:0	27.25	29.00	29.00	30.50	1.30
C12:0	33.50	33.00	34.25	34.50	1.40
C14:0	101.00	101.75	96.75	96.50	4.40
C14:1	17.00^{a}	14.25^{a,b}	14.00^{a,b}	13.25^{b}	0.76
C16:0	211.25	206.75	202.25	192.25	9.00
C16:1	27.50	24.25	23.75	22.25	1.40
C18:0	83.00^{b}	124.25^{a}	102.75^{a}	108.50^{a}	5.50
C18:1	224.50	277.75	250.00	252.50	12.60
C18:2	41.75^{b}	106.75^{a}	56.00^{b}	67.50^{b}	6.50
C18:3	7.25^{c}	10.25^{c}	34.50^{b}	63.50^{a}	4.50
C20:0	6.75	9.50	8.25	8.00	1.10

a-d Means not followed by the same letter differ significantly at $P< 0.05$ within rows.
[†] formaldehyde protected product courtesy J.R. Ashes, New South Wales, Australia.

Appendix 2. Effect of formaldehyde treated flaxseed† and Linola† on milk fatty acids as a percentage of milk fat as analyzed by Palmquist (least square means and standard errors).

	Treatment				
Fatty Acid (%)	Control	Linola	FL	FH	SEM
C4:0	3.46	3.51	3.48	3.41	0.10
C6:0	2.31	2.26	2.34	2.38	0.03
C8:0	1.43	1.35	1.44	1.42	0.02
C10:0	3.45^{a}	3.10^{b}	3.43^{a}	3.35^{a}	0.03
C12:0	4.12^{a}	3.42^{d}	3.89^{b}	3.69^{c}	0.04
C14:0	12.29^{a}	10.33^{b}	10.83^{b}	10.21^{b}	0.20
C14:1	1.72^{a}	0.97^{b}	1.08^{b}	0.90^{b}	0.08
C16:0	27.11^{a}	22.14^{c}	23.45^{b}	21.49^{c}	0.30
C16:1	1.71^{a}	$1.18^{b,c}$	1.30^{b}	1.12^{c}	0.04
C18:0	10.76^{b}	13.48^{a}	$12.24^{a,b}$	$12.40^{a,b}$	0.38
C18:1t*	2.046^{b}	2.40^{a}	2.07^{b}	$2.18^{a,b}$	0.06
C18:1c*	21.03	21.18	21.01	20.39	0.24
C18:1o*	0.98	1.04	1.05	0.93	0.04
C18:2	3.57^{d}	9.76^{a}	4.89^{c}	5.88^{b}	0.26
C18:3	1.0^{c}	1.22^{c}	4.70^{b}	7.72^{a}	0.34
C20:0	0.19	0.19	0.20	0.21	0.01
CLA	0.87	0.91	0.84	0.88	0.03

a-d Means not followed by the same letter differ significantly at $P< 0.05$ within fatty acid groups.
† formaldehyde protected product courtesy J.R. Ashes, New South Wales, Australia.
**t=trans, c=cis, o=other.
**CLA=conjugated linoleic acid.

Appendix 3. Effect of formaldehyde treated flaxseed[†] and lignosulfonate treated flaxseed[††] on milk fatty acid yield per cow per day (least square means and standard errors).

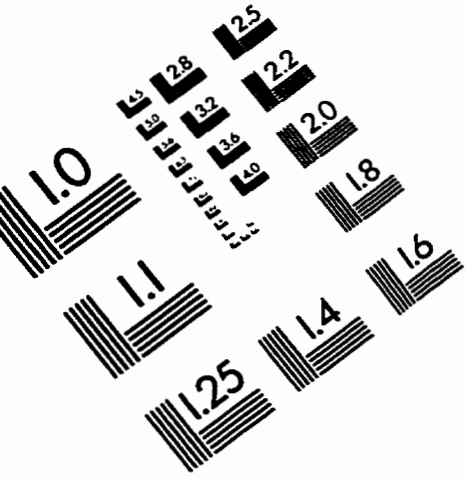
	Treatment				
Fatty Acid (g):	Control	FH	LL	LH	SEM
C4:0	14.00	14.75	12.00	11.50	1.20
C6:0	12.00	13.00	9.50	9.75	0.84
C8:0	8.50	8.50	6.50	6.50	0.56
C10:0	21.25	21.00	16.25	16.25	1.50
C12:0	26.75	23.75	20.75	21.00	1.90
C14:0	93.75	78.00	77.75	79.75	6.90
C14:1	16.25	10.75	14.00	14.25	1.60
C16:0	216.00	176.75	180.75	186.50	17.60
C16:1	30.00	16.00	26.00	26.00	3.60
C18:0	112.50	135.75	100.75	109.75	10.30
C18:1	301.50	285.25	276.75	289.50	26.90
C18:2	47.00	55.75	44.00	45.25	5.40
C18:3	3.75[b]	33.50[a]	5.00[b]	6.25[b]	3.00
C20:0	11.25	11.00	9.25	9.25	0.76

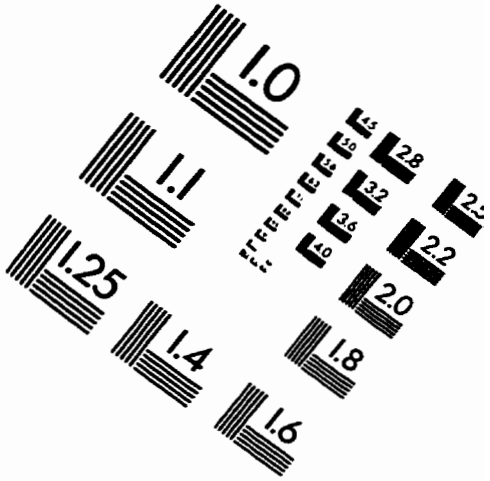
a-d Means not followed by the same letter differ significantly at $P< 0.05$ within rows.

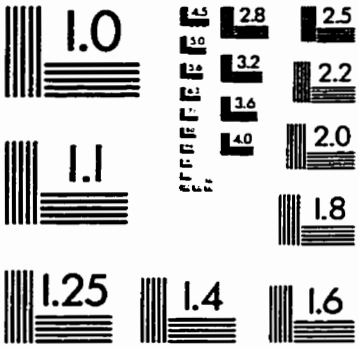
[†] formaldehyde protected product courtesy J.R. Ashes, New South Wales, Australia.

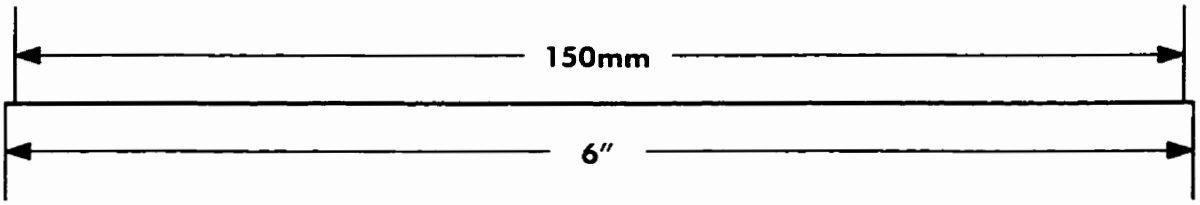
[††] lignosulfonate treated flaxseed courtesy EXL Milling Ltd., Hassall, Saskatchewan.

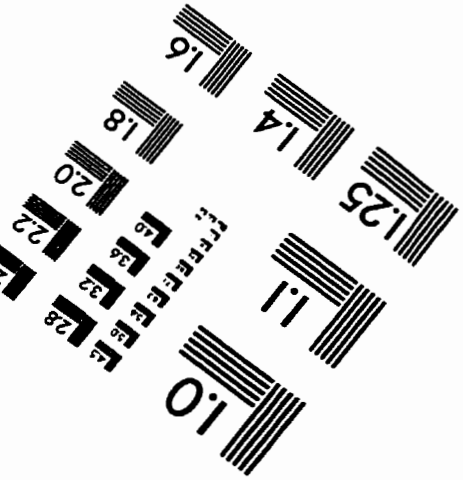
IMAGE EVALUATION
TEST TARGET (QA-3)

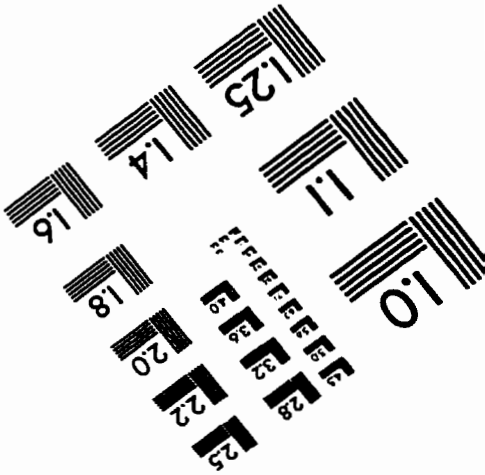












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