MODIFYING FATTY ACID COMPOSITION OF BOVINE MILK BY ABOMASAL INFUSION OR DIETARY SUPPLEMENTATION OF SEED OILS OR FISH OIL

By

Aloka B. P. A. Bandara

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Animal Science (Dairy)

Joseph H. Herbein, Jr., Chair

Susan E. Duncan

Thomas W. Keenan

Michael L. McGilliard

William E. Vinson

Kenneth E. Webb, Jr.

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The United States of America.

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(ABSTRACT)

The potential for enhancing oleic acid (*cis*-18:1) and linoleic acid (18:2) content and lowering medium chain fatty acid (MCFA) content of bovine milk was investigated by abomasal infusion or dietary supplementation of oils. In experiment 1, olive oil, sesame oil, sunflower oil, or fish oil was abomasally infused (155 to 219 g/d) into Jersey cows during the last 6 d of each of four 14-d periods. In experiment 2, canola oil, olive oil, high-oleic sunflower oil, or distilled water (control) was abomasally infused (342 to 371 g/d) into three Holsteins and three Jerseys during the last 5 d of each of four 10-d periods. The intestinal digestibility and concentration of *cis*-18:1 and 18:2 in milk were proportional to flow of these fatty acids to the duodenum. Also, greater concentration of *cis*-18:1 in milk was associated with lowered yield of MCFA. During olive oil or sesame oil infusion in experiment 1, for each 100 g of *cis*-18:1 infused into the abomasum, milk *cis*-18:1 yield was increased by an average of 47 g, and MCFA yield was reduced by 42 g. The yield of 18:2 in milk was increased by approximately 46 g for each 100 g of infused 18:2 during olive oil or sesame oil infusion. Milk produced during sesame oil infusion, however, had an off-flavor when evaluated by a taste panel. In experiment 2, each 100 g of *cis*-18:1 infused daily increased milk *cis*-18:1 yield in Holsteins and Jerseys by 41 and 39 g/d, respectively, whereas recovery of infused 18:2 was 34 g/d for Jerseys and 42 g/d for Holsteins. In experiment 3, 22 Jersey cows were fed a basal diet, or the basal diet supplemented with 3.5% high-oleic canola oil, 3.5% soybean oil, or 1.75% high-oleic canola oil plus 1.75% soybean oil for 5 wk. Dietary canola oil supplementation increased conjugated linoleic acid (CLA) percentage in milk to a moderate level without raising *trans*-18:1 percentage, whereas feeding either supplement containing soybean oil

raised both CLA and *trans*-18:1 percentages. Concentrations of *trans*-18:1 and CLA in milk apparently reflected the extent of unsaturated fatty acid biohydrogenation in the rumen. Dietary supplementation with canola oil increased yield of *cis*-18:1 in milk by 21 g for each 100 g of supplemental *cis*-18:1 intake. Yield of 18:2 in milk was raised by 3 g for each 100 g of supplemental 18:2 intake by cows fed soybean oil. Using abomasal infusion as an indicator of the maximum potential for apparent recovery of *cis*-18:1 in milk (39 to 49%), *cis*-18:1 recovery in response to supplemental *cis*-18:1 in the diet was approximately half of the potential response due to partial biohydrogenation in the rumen. The apparent recovery of dietary 18:2 in milk was reduced to only one-tenth of the potential yield (31 to 47%) indicated by abomasal infusion of seed oils. Results indicated that the fatty acid profile of bovine milk was altered in a manner that would be beneficial to human health when cows were fed supplemental oleic acid, but further research should focus on safe and economical methods to protect dietary unsaturated fatty acids from biohydrogenation.

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CHAPTER 1

INTRODUCTION

Saturated, medium-chain fatty acids (MCFA), namely lauric acid (12:0), myristic acid (14:0) and palmitic acid (16:0), are among major dietary factors responsible for raising plasma low-density-lipoprotein (LDL)-cholesterol (Denky and Grundy, 1992; Keys et al., 1965; Nestel et al., 1994). *Trans* fats (mainly elaidic acid [*trans*-18:1]) also have been shown to increase serum LDL-cholesterol (Judd et al., 1994 and Keys et al., 1986). In contrast, unsaturated fatty acids (UFA), mainly oleic acid (*cis*-18:1), linoleic acid (18:2) and linolenic acid (18:3), have been shown to lower plasma LDL-cholesterol (Garg et al., 1994; Grundy and Denky, 1990; Keys et al., 1970; Mattson and Grundy, 1985). Increased blood cholesterol concentration (hypercholesterolemia) leads to gradual deposition of cholesterol on the inner wall of arteries, a process known as atherosclerosis, which is considered a principal cause for coronary heart disease (CHD). Bovine milk contains significantly higher concentrations of MCFA (approximately 41% of total fatty acids) and relatively lower concentrations of UFA (approximately 31% of total fatty acids) compared with other dietary sources of vegetable and animal fat (Berner, 1993; Kennelley, 1996).

The trend towards more widespread supplementation of dairy cattle diets with fat provides an opportunity to favorably alter milk fatty acid composition. For this purpose, UFA-rich seeds or seed oils could be supplemented in dairy diets (Kennelley, 1996). However, dietary UFA are saturated by rumen microorganisms, a process known as biohydrogenation (Harfoot, 1978; Harfoot and Hazlewood, 1988; Palmquist and Jenkins, 1980). Additionally, dietary UFA have the potential to disrupt the digestion of non-lipid energy sources in the rumen (Ikwuegbu and Sutton, 1982; Jenkins and Palmquist, 1984).

Alternative methods for feeding UFA have been suggested. Fat encapsulated in a matrix of formaldehyde-treated protein was found to bypass ruminal biohydrogenation (Faichney et al., 1972; Scott et al., 1971). When diets were supplemented with formaldehyde treated canola seeds at 6.5% of the diet dry matter (DM), milk UFA yield was increased by 54% and MCFA yield was reduced by 10% (Ashes et al., 1992). Fotouhi and Jenkins (1992 a, 1992b) reported that treating

UFA with primary amines produced fatty acyl amides that resisted rumen biohydrogenation and caused no disruption of ruminal fermentation. When sheep were fed diets supplemented with 5% butylsoyamide, a product made by treating soybean oil with butylamine, the linoleic acid content of plasma neutral lipids was increased by 65%, compared with only a 16% increase when the diet was supplemented with 5% soybean oil (Jenkins, 1990). When fed to dairy cows at 3.5% of diet DM, the butylsoyamide supplement had no effect on ruminal volatile fatty acid (VFA) production; thus, indicating the inertness of the protected fat in the rumen (Jenkins et al., 1995).

The impact of fat-modified dairy products on plasma cholesterol of humans was examined by Noakes et al. (1995). The subjects who consumed fat-modified products had significantly lower total and LDL-cholesterol in plasma, representing a 9% reduction in the risk of developing CHD.

Further understanding of the flow of dietary UFA from the intestine to the blood and to the mammary gland in the bovine is needed. In the present study, several aspects of fatty acid transfer from the intestine to the mammary gland were evaluated in an effort to estimate the maximum potential of dietary UFA to alter milk fat composition. In two experiments, UFA-rich seed oils or fish oil were abomasally infused into Holstein and Jersey cows to investigate the relationships between flow of individual fatty acids to the duodenum and their digestibility in the intestine. Flow of oleic and linoleic acid to the duodenum was compared with apparent recovery of these fatty acids in milk. In a third experiment, UFA-rich seed oils were supplemented in diets of Jersey cows to evaluate the apparent recovery of the supplemented fatty acids in milk. Also, the influence of biohydrogenation in the rumen on apparent production and transfer of *trans* fatty acids to milk in response to dietary UFA was evaluated.

CHAPTER 2

REVIEW OF LITERATURE

Nutritional value of bovine milk

Hippocrates, often referred to as the father of medicine, described milk as "the most nearly perfect food" (Ensminger, 1993). Newborn mammals grow rapidly with a digestive tract that is poorly developed, so they must obtain all nutrient requirements from milk. Therefore, milk must be nutritionally complete, and easily digested and absorbed. If milk were anything less than complete, survival of young would be difficult (Schmidt, et al., 1988).

The protein in milk has a biological value of 85 as compared to 50 to 65 in cereal grains. This high value indicates that the milk protein is highly digestible and contains a well-balanced amino acid profile. In addition, The protein to calorie ratio of milk is very favorable and therefore consumers do not ingest calories in unnecessary amounts (Ensminger, 1993). Milk contains 120 mg of calcium per 100 g whereas most meats contain only 5 to 15 mg. Additionally, the calcium to phosphorus ratio of milk is very balanced (1.4:1; Krause and Mahan, 1984). Milk also provides an excellent source of vitamin A and riboflavin (Bath et al., 1985). Milk fat consists primarily of short- and medium-chain fatty acids. These fatty acids are more readily digested and absorbed than the long-chain fatty acids found in vegetable fats. Milk fat is the fraction of milk that carries vitamin A (Ensminger, 1993).

Health Problems associated with consumption of dairy milk

Even though milk is nutritious it is not favorable for the health of every human. Certain individuals show metabolic disorders when milk is introduced into their diet, and as such these people find difficulties in exploiting the nutritional benefits of this foodstuff.

Cow's Milk Protein Allergy (CMPA): This is thought to affect from 0.3 to 12 per cent of the pediatric population. The average age of onset is approximately three months (Hutchins and Walker-Smith, 1982). CMPA commonly occurs in infants with a family history of atopy or

CMPA. However, milk allergies are not severe. The symptoms in most cases include vomiting, abdominal pain, rash and diarrhea (Krause and Mahan, 1984).

Lactose Intolerance: This is the condition of maldigestion of lactose due to an insufficiency of the enzyme lactase. Lactase is responsible for splitting lactose into the absorbable sugars, galactose and glucose. Most populations except for Northern European Caucasians and white American ethnic groups have high incidences of adult lactose intolerance (Anderson et al., 1982). Low amounts of lactase are found in the digestive tract of 70% of the blacks and 6 to 12% of the whites in the United States (Schmidt et al., 1988).

The retention of disaccharides in the lumen provides an osmotic force for water and sodium to move into the lumen. Thus, the volume of chyme increases (Christoper and Bayless, 1971). Studies of Bond and Levitt (1975) and Debongnie et al. (1979) point to more rapid transit through the small bowel when lactose absorption is incomplete. Bacterial hydrolysis of sugars that enter the colon produces organic acids, which further increase the osmolarity of lumen contents (Christoper and Bayless, 1971). These factors ultimately lead to cramps and diarrhea (Ensminger, 1993).

Coronary Heart Diseases (CHD): Nearly one million people in the US die each year due to the numerous types of heart diseases including, hypertension, cerebrovascular disease (stroke), congestive heart failure, and CHD. Diet has been implicated in a number of these diseases, and much attention has been given to the role of animal fats in CHD. A positive correlation has been found between the consumption of saturated fat and the occurrence of CHD (Keys, 1970; Keys et al., 1957; Keys et al., 1986).

Intake of milk and butter has been clearly associated with higher CHD rates in different countries (Solonen and Vohlonen, 1982). The correlation with CHD mortality is likely to be mediated by the effect of dairy fats on plasma LDL-cholesterol concentration, which is a risk factor for CHD (American Heart Association/Heart, Lung and Blood Institute, 1990). The cholesterol-enhancing (hypercholesterolemic) effect of dairy products was demonstrated conclusively in numerous

controlled studies. In 1957, Ahrens et al. revealed that butter was hypercholesterolemic compared with polyunsaturated vegetable oil. These findings were confirmed by Keys et al. (1957, 1965), Hegsted et al. (1965), Mensink and Katan (1989), and Denke and Grundy (1991). In two well-controlled trials in which whole milk and skim milk within isoenergetic diets were compared, whole milk consumption caused an elevation of total cholesterol by 7 to 13% (Roberts et al., 1982; Kristi et al., 1994). According to Spady et al. (1993), saturated fatty acids were responsible for the increase in plasma cholesterol level.

Trend in milk and milk product consumption in U. S. A.

Americans have become health conscious in recent years, leading to decreased per capita consumption of fluid whole milk and cream and an increased per capita consumption of low-fat milk (Schmidt et al., 1988). From 1950 to 1988, per capita whole milk consumption declined 64%, low-fat milk consumption increased 746%, butter consumption declined 59%, evaporated and condensed milk consumption declined 62%, and total cheese consumption increased 206% (Ensminger, 1993). Much of the drop in consumption of milk equivalents was caused by the decrease in consumption of butter, fluid milk and cream. The decreased consumption of these components could be partly attributed to the consumers' alertness about heart diseases. An overall trend towards reduced consumption of foods rich in fat, especially saturated fat, has been shown during past few decades (Schmidt et al., 1988).

Saturated fatty acids (SFA) in human foodstuffs

By definition, an SFA is an aliphatic carboxylic acid with no double bonds present between any of its carbon atoms. Table 2.1 provides systemic names, shorthand notations, trivial names and dietary sources of most common and nutritionally important SFA.

Unsaturated fatty acids (UFA) in human foodstuffs

The UFA are aliphatic carboxylic acids with one or more double bonds in the carbon chain. A fatty acid with a single double bond is known as a monounsaturated fatty acid and a fatty acid with two or more bonds in its structure is known as a polyunsaturated fatty acid. The presence of

Table 2.1. Typical saturated fatty acids in foods

Systematic name	Shorthand notation	Trivial name	Major sources
Tetranoic	4:0	Butyric	Butter
Hexanoic	6:0	Caproic	Butter
Octanoic	8:0	Caprylic	Coconut
Decanoic	10:0	Capric	
Dodecanoic	12:0	Lauric	Palm kernel, Coconut
Tetradecanoic	14:0	Myristic	Palm kernel, Coconut
Hexadecanoic	16:0	Palmitic	Palm
Octadecanoic	18:0	Stearic	Most animal fats, cocoa
Eicosanoic	20:0	Arachidic	Peanut
Docosanoic	22:0	Behenic	Seeds
Tetracosanoic	24:0	Lignoceric	Peanut

Source: Perkins (1991).

the double bond allows for configurational isomerism in *cis* or *trans* positions. Naturally occurring UFA are mostly in the *cis* configuration. Table 2.2 provides information for common UFA available in food sources. Oleic acid and linoleic acid are examples of a monounsaturated and a polyunsaturated fatty acid, respectively.

Dietary fatty acids and blood lipoprotein and cholesterol levels

Absorption of fat after a meal is associated with a large increase in lipid concentration of the blood, referred to as lipidemia (Helfant and Banka, 1978). Blood lipids consist of dietary lipids absorbed from the intestine, as well as lipids mobilized from depot stores and from synthesis in body tissues, especially the liver and adipose tissues. The diet is the primary source of cholesterol to the body. Cholesterol is also synthesized in the liver (Helfant and Banka, 1978).

Lipids, together with cholesterol, are transported in blood as lipoproteins ranging from very low density to high density. The density is increased as the proportion of protein in the complex increases and the lipid decreases. Density, composition, and electrophoretic mobility have been used to divide lipoproteins into four major classes: chylomicrons, very- low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL; Church and Pond, 1988). The compositions of these lipoprotein types are summarized in Table 2.3.

Diets rich in SFA are believed to increase plasma cholesterol levels (Spady et al., 1993) by increasing the blood concentration of LDL which contain about 51 to 58% cholesterol (Church and Pond, 1988). This condition is known as hypercholesterolemia. Very little is known about the mechanism by which saturated dietary fat influences blood LDL-cholesterol. Substitution of UFA for SFA in the diet tends to decrease the levels of LDL (Bagio et al., 1994; Mattson and Grundy, 1985; Spady et al., 1993), and increase the levels of HDL, which contains only 18 to 25% cholesterol (Church and Pond, 1988). Though high levels of HDL are associated statistically with decreased blood cholesterol levels, the mechanism involved is not known. It is known that one function of HDL is to remove cholesterol from peripheral tissues and return it to the liver. A major metabolic fate of cholesterol in the liver is the synthesis of bile acids, which are secreted into the intestine (Starr, 1994).

Table 2.2. Unsaturated fatty acids in foods

Systematic name	Shorthand Notation	Trivial name	Major sources
Monounsaturated			
Cis			
9-Tetradecanoic	14:1n5	Myristoleic	Butter
9-Hexadecanoic	16:1n7	Palmitoleic	Seafood, beef
9-Octadecanoic	18:1n9	Oleic	Olive, Canola
11-Octadecanoic	18:1n7	Vaccenic	Seafood
13-Docosenoic	22:1n9	Eruic	Rapeseed
Trans			
9-Octadecanoic	*trans*-18:1n9	Elaidic	Hydrogenated fats
11-Octadecanoic	*trans*-18:1n7	Transvaccenic	Hydrogenated fats, butter
Polyunsaturated			
All cis			
9,12-Octadienoic	18:2n6	Linoleic	Sunflower, safflower
6,9,12-Octadecatrienoic	18:3n6	γ-Linolenic	Primrose
8,11,14-Eicosatrienoic	20:3n6	dihomo-γ-linoleic	Shark liver
5,8,11,14-Eicosatetraenoic	20:4n6	Arachidonic	Eggs, most animal fats
9,12,15-Octatrienoic	18:3n3	Linolenic	Soybean, canola
5,8,11,14,17-Eicosapentaenoic	20:5n3	Timnodonic	Seafood
7,10,13,16,19-Docosapentaenoic	22:5n3	Clupadonic	Seafood
4,7,10,13,16,19-Docosahexaenoic	22:6n3	Cervonic	Seafood

Source: Perkins (1991).

Table 2.3. Composition of blood lipoproteins

Lipoprotein Class[a]	Density (g/mL)	Composition (weight)			
		Protein	Triglyceride	Phospholipid	Cholesterol
Chylomicrons	<0.94	1-2	85-95	3-6	3-7
VLDL (β-lipoprotein)	0.94-1.006	6-10	50-65	15-20	20-30
LDL (β-lipoprotein)	1.006-1.063	18-22	4-8	18-24	51-58
HDL (α-lipoprotein)	1.063-1.21	45-55	2-7	26-32	18-25

[a]VLDL denotes very-low-density lipoprotein, LDL low-density lipoprotein, and HDL high-density lipoprotein.
(Source: Church and Pond, 1988).

Effects of individual fatty acids on blood cholesterol level: There is agreement that certain, but not all, SFA raise plasma levels of LDL-cholesterol. Short chain fatty acids (butyric acid [4:0], caproic acid [6:0], caprylic acid [8:0], and capric acid [10:0]), although present in a small number of food sources, do not appear to raise LDL-cholesterol levels (Berner, 1993). Medium-chain saturated fatty acids (MCFA), (lauric acid [12:0], myristic acid [14:0], and palmitic acid [16:0]) are generally considered to be the serum total and LDL-cholesterol raising SFA (Keys et al., 1965; Hegsted et al., 1965). These three fatty acids account for approximately 41% of milk fat (Berner, 1993). It has been suggested that 14:0 is more hypercholesterolemic than 16:0 (Zock et. al., 1994). The other 59% of milk fatty acids are not hypercholesterolemic compared with MCFA. These fatty acids are short chain fatty acids, stearic acid (18:0), monounsaturated fatty acids, polyunsaturated fatty acids, and traces of others (Berner, 1993).

Trans fatty acids and hypercholesterolemia: *Trans* fatty acids are formed when oils are hydrogenated so as to harden them and reduce the rate of spoilage by oxidation. According to Judd et al. (1994) and Keys et al. (1986), *trans* fats have been shown to increase serum total and LDL-cholesterol. *Trans* fatty acids may also lower serum HDL (Temple, 1996). Several studies have revealed that persons with an increased intake of *trans* fats or an increased adipose tissue level are at a raised risk of CHD (Willett and Ascherio, 1994). Intake of margarine, the chief source of *trans* fats, has been associated with risk of CHD in a case-control study in Greece (Tzonou et al., 1993).

Blood cholesterol level and atherosclerosis

Atherosclerosis is the condition of thickening and losing elasticity of the arterial wall, and narrowing of the arterial lumen due to building up of cholesterol and other lipids on the arterial wall. Cholesterol released into blood in the form of LDL can infiltrate arterial walls. At the sites where LDL-cholesterol infiltrated into the arterial wall, abnormal smooth muscle cells are multiplied and connective tissue components are increased. Cholesterol accumulates in cells and in extra-cellular spaces of the wall endothelial lining. Calcium salts are deposited on top of the lipids,

and a fibrous net is formed over the mass. This atherosclerotic plaque projects into the arterial lumen (Shillingford, 1981; Starr, 1994).

Atherosclerosis and CHD

When blood platelets get caught on an atherosclerotic plaque's rough edges, they secrete chemicals that initiate clot formation. Growth of the plaque and clot can narrow or block the artery. Blood flow to the tissues serviced by the artery may decrease to a trickle or stop entirely (Shillingford, 1981; Starr, 1994).

Coronary arteries and their branches have narrow diameters. They are extremely vulnerable to clogging by a plaque or clot. Atherosclerosis causes further narrowing of the interior of coronary arteries. This leads to lack of oxygen and blood to the heart muscles, a condition known as myocardial ischemia. Mild shortage of blood supply to the heart muscles gives rise to chest pain (angina pectoris; Helfant and Banka, 1978; Vlodaver et al, 1976). The severe shortage of oxygen and nutrients to the heart muscles causes the death of heart muscle tissues leading to heart attack (myocardial infarction; Helfant and Banka, 1978; Shillingford, 1981).

There is convincing evidence that an elevated blood cholesterol level causes clinical CHD and that to a large extent, this occurs by way of atherosclerosis. Law and Wald (1994 a and b) reported a much stronger relationship than that generally reported elsewhere. Based on the results obtained from international studies, these workers indicated that in middle age a 0.6 mmol/L lower serum cholesterol (about 10% of western values) corresponds to a reduced risk of CHD death (38% in men, 31% in women, 54% at age 40, and 20% at age 70; Law and Wald, 1994 b). In recent years, angiography has been used to quantify the extent of atherosclerosis. Angiograms of 723 men under the age of 40 admitted to the Cleaveland Clinic (Cleaveland, OH, USA) revealed that the extent of arterial closure steadily increased with serum cholesterol level (Welch et al., 1970).

The dividing line between normal and high serum cholesterol levels is usually placed around 6.2 to 7.0 mmol/L. Strong evidence shows that atherosclerosois does not develop when serum cholesterol is below 4.0 mmol/L, but steadily develops at higher levels (Diehl, 1994; Gillman et

al., 1995). According to Keys et al. (1957 and 1958) a level of 5.0 mmol/L is atherogenic, however less so than 6.0 mmol/L.

Other factors responsible for CHD

The habit of cigarette smoking and a raised blood pressure are additional risk factors in the development of CHD. There would also appear to be a familial tendency; the disease often runs in families. It is difficult to separate out whether this is due to an inherited characteristic or whether these families share a similar diet together with the other risk factors, such as smoking (Shillingford, 1981).

Preventing and reversing CHD

According to the above evidence, it is clear that lowering blood cholesterol both prevents and treats CHD. It is believed that greater decreases in serum cholesterol would bring about reversal of atherosclerosis.

Dietary manipulation: For the prevention and treatment of CHD, vigorous dietary intervention is needed to lower the serum cholesterol level by at least 6% (Temple, 1996). A diet reduced from 20 to 30% of energy as fat with SFA to 5 to 7% fat can be expected to lower cholesterol level by 6% to over 20%. For this purpose, the foods of animal origin should be eaten sparingly, and more prominence should be given to the foods rich in carbohydrate and fiber, especially fruit and vegetables. If acceptability of the diet becomes a problem and people desire more fat in their diet, then the UFA rich oils such as olive or canola could be incorporated in the diet (Temple, 1996).

The role of UFA: Oleic acid (*cis*-18:1) is the dominant monounsaturated fatty acid in both animal and vegetable fats. Linoleic acid (18:2) is the most common n6 polyunsaturated fatty acid found in many vegetable oils such as safflower, corn, sunflower, and soybean. Linolenic acid (18:3), eicosapentaenoic (20:5) and docosahexaenoic (22:6) acids are the major n3 polyunsaturated fatty acids and they are present in elevated concentrations in green leafy vegetables, marine foods and fish oils, respectively (Goodnight et al., 1982). In many diet intervention trials, SFA have been replaced by polyunsaturated fat. Studies have revealed that tissue levels of linoleic acid, the most

common polyunsaturated fatty acid, are lower in CHD cases than in controls (Wood and Oliver, 1992). Changes in national intake of UFA appear to partly explain trends in CHD mortality rates: intake of UFA has increased considerably in Australia and the US where CHD mortality has fallen most (Kesteloot and Joossens, 1992).

In recent years researchers have focused on the usefulness of monounsaturated fats. Keys et al. (1970) demonstrated that the Mediterranean countries have CHD mortality rates two- to three-fold lower than that found in northern Europe or the US. Traditional Mediterranean diets are typically rich in olive oil, a very good source of oleic acid. Further analysis revealed a correlation of 0.66 between the ratio of monounsaturated fat to saturated fats and the 15-year mortality rate for CHD (Keys et al., 1986). These data indicate that CHD may be greatly reduced by minimizing the intake of animal fats (and therefore saturated fat) and replacing them with unsaturated fatty acid sources.

Rivellese et al. (1994) fed diets rich in monounsaturated fats to hyperlipidemic subjects in Italy. One experimental diet contained 27% fat with 17% monounsaturated and 4% polyunsaturated fatty acids while the other diet contained 36% fat with 19% monounsaturated fatty acids and 10% polyunsaturated fatty acids. Both diets contained only 6 to 7% SFA. Each diet reduced plasma cholesterol by about 9%. This indicates that, provided the intake of SFA is reduced, cholesterol control can be achieved with diets rich in monounsaturated fat.

Garg et al. (1994) compared two diets using patients with type II diabetes. The first (low fat) contained 30% of energy as fat and the second contained 45% of energy as fat, much of it being from monounsaturated fat. No differences were seen in plasma cholesterol, LDL-cholesterol or HDL-cholesterol. However, the low fat diet caused elevated plasma glucose, insulin, and triglyceride, and these conditions persisted 14 weeks. This indicates that an increased intake of monounsaturated fat can be beneficial for type II diabetes.

The role of omega3 (n3) fatty acids: Studies on Greenland Inuit (Eskimos) living on their traditional diets revealed a high intake of n3 fats, a prolonged bleeding time and a low risk of

CHD (Bang et al., 1980). There is evidence that fish oil may prevent CHD at relatively low intakes. A study in the Netherlands revealed that small amounts of fish are protective against CHD mortality (Kromhout et al., 1985). In the Multiple Risk Factor Intervention Trial, a nested case-control study, the serum level of n3 fatty acids was found negatively related to risk of CHD (Simon et al., 1995).

Drug therapy: An alternative approach to the management of elevated cholesterol levels has been the use of hypolipidemic drugs. Recently, there has been controversy concerning an excess of non-CHD deaths associated with these drugs. Smith et al. (1993) reexamined the results from drug trials and concluded that drugs lead to an excess of non-CHD deaths, which may exceed the numbers of CHD deaths prevented. Clearly, if this is the case, then there is little point in using such drugs. However, more evidence is needed before proper identification of patients for whom the benefits of hypolipidaemic drugs will exceed the risks. Certainly, a patient at exceptionally high risk of CHD and who has failed to control his blood cholesterol by diet should be given these drugs. A typical person in this class is a man with serum cholesterol of over 7 mmol/L plus clinically evident CHD (Temple, 1996).

Milk fatty acid profile

Milk fat contains significantly higher concentrations of short-chain fatty acids and MCFA and relatively lower concentrations of UFA as compared to other dietary sources of vegetable and animal fat (Berner, 1993; Kennelley, 1996). Milk fat has been criticized because it contains a less desirable balance of fatty acids than vegetable fat or fish oil. Two principal MCFA in butterfat, myristic acid and palmitic acid, have been identified as major dietary factors responsible for raising plasma LDL cholesterol (Keys et al., 1965; Hegsted et al., 1965; Grande, et al., 1970; Denky and Grundy, 1992; Nestel et al., 1994). In particular, myristic acid, of which dairy products are a major source, is reputedly more potent than palmitic acid in cholesterol-raising effects (Zock et al., 1994).

Altering milk fatty acid intake

There have been a number of options put forward to modify the cholesterol raising properties of milk (Berner, 1993; Jensen et al., 1991; Ney, 1991) including cholesterol removal, milk fat fractionation, and changes in the feeding of cows.

Whole milk versus skim milk: The studies of Roberts et al. (1982) and Kristi et al. (1994) suggest that the effects of changing from whole to skim milk consumption will result in a reduction in total cholesterol. However, it is argued that skim milk is less palatable than full-fat milk because of the mouth-feel characteristics of fat. Furthermore, it is technically more difficult to reduce fat in some high-fat dairy products, notably cheese, ice cream, and cream. These products heavily rely on their fat content for texture and palatability. Lower-fat versions of these products, despite the use of fat substitutes in some cases, have yet to achieve a large market share.

Fatty acid modified milk: Milk fatty acids are partly derived from dietary long-chain fatty acids, microbial synthesis of fatty acids and body fat stores. The remainder is synthesized in the mammary system from short-chain fatty acids, primarily acetate and β-hydroxy butyrate arising from microbial digestion of carbohydrate in the rumen (Chilliard, 1993; Jenkins, 1993; Kemp et al., 1984; Kennelley, 1996). Milk fatty acid profile could be altered substantially by manipulating the diet of the animal (Gaynor et al., 1994; Kennelley and Fenton, 1982; Kennally and Khorasani, 1992; Khorasani et al., 1991).

Increased levels of oleic acid and linoleic acid at the expense of lauric, myristic and palmitic acids is considered desirable from a human health perspective and also offers the additional benefit of resulting in softer butter. This alteration in the fatty acid profile of dairy products, if applied to populations typical of developed Western countries, represents a potential strategy to lower the risk of CHD without any appreciable change in customary eating patterns.

Modifying dairy milk fatty acid profile using feed technology

The trend towards more widespread supplementation of dairy cattle diets with fat provides an opportunity to alter the milk fatty acid composition (Kennelley, 1996). For this purpose, UFA rich seeds or seed oils should be selected (Table 2.4) and included in the dairy diet in appropriate levels.

UFA flow to the duodenum and recovery in milk: Researchers have attempted to investigate the relationship between fatty acid flow to the abomasum and milk composition by infusing fatty acid mixtures or UFA-rich oils into the abomasum of dairy cows. Gaynor et al. (1994) reported that when *cis*-18:1-rich fat (65% high-oleic sunflower oil plus 35% cocoa butter) was abomasally infused in Holstein cows at 750 g/d milk content of *cis*-18:1 and 18:2 were increased up to 34.7 and 4.7%, respectively, from 23.3 and 3.8% in uninfused cows. They further revealed that *cis*-18:1infusion lowered 10:0, 12:0, 14:0 and 16:0 content of milk. Drackley et al. (1992) abomasally infused 168 g of meat solubles (carrier for fatty acids) [control], control plus 450 g of mostly unsaturated fatty acids, control plus 450 g of mostly saturated fatty acids or control plus 450 g of a mixture of saturated and unsaturated fatty acids daily in Holstein cows. In their experiment, the lowest content of MCFA was 34.9% during infusion of mostly unsaturated fatty acids compared with 46% for the control. In addition, infusion of mostly unsaturated fatty acids resulted in 17.7% 18:1 (16.6% in control) and 13.3% 18:2 (2.3% in control) in milk fat.

Chilliard et al. (1991) reported that duodenal rapeseed oil infusion (1.0 to 1.1 kg/d) in early- and mid-lactation Holstein cows resulted in lowered 16:0 in milk fat. Additionally, milk 18:1 content did not differ due to infusion during the first week of lactation, but increased during the second week (33% compared with 29% for control) and mid-lactation (wk 19 to 26) (27% compared with 19% for control). They also found that milk 18:2 content increased due to infusion during early-lactation (4.5 to 5.2% compared with 2.1 to 2.5% for control) and mid-lactation (6.7% compared with 2.6% for control).

UFA flow to the duodenum and digestibility in the intestine: Klusmeyer and Clark (1991) reported that increased flow of *cis*-18:1 and 18:2 to the duodenum enhances the digestibility of these fatty acids in the intestine. They reported 90.3, 89.6, 82.6, and 79.6% digestibilities of *cis*-18:1 in intestine when flows to the duodenum were 290, 235, 102, and 94 g/d, respectively. They further reported 90, 82.6, and 79.6% intestinal digestibilities of 18:2, respectively, when flows of 18:2 to the duodenum were 63, 43, and 41 g/d. Palmquist (1991) reported a linear decline in true digestibility of total fatty acids from 100% (at 1% fat in the diet) to 78% (at 8% fat in the diet), but the total fatty acid digestibility did not differ due to source of fat (animal-vegetable blend, Ca-soap, hydrogenated animal fat, saturated fatty acids, tallow, or basal diet). Elliott et al. (1996) reported a decline in total fatty acid digestibility from 81.3% (control diet) to average of 71% when diets supplemented with 5 to 6.1% of fat (calcium salts of long chain fatty acid distillate) were fed.

Supplementation of UFA-rich seeds or seed oils in dairy rations

Supplementation of UFA-rich seeds or seed oils in raw forms in dairy cow diets may not result in the modifications in milk fatty acid profile observed during abomasal infusion of fatty acids or oils. This is due to the interaction between dietary fat and the microorganisms in the rumen.

Fate of unsaturated fatty acids in the rumen: Two important microbial fermentation processes have been found to occur with respect to metabolism of fatty acids: lipolysis and biohydrogenation (Harfoot, 1978; Harfoot and Hazlewood, 1988; Palmquist and Jenkins, 1980). Lipolysis results in release of free fatty acids from esterified plant lipids. This is followed by biohydrogenation, which reduces the number of double bonds.

Biohydrogenation of 18:2 and 18:3 involves an isomerization reaction which converts the *cis*-12 double bond to a *trans*-11 isomer, followed by reduction to *trans*-11 18:1 and ultimately to 18:0 which is the principal end product of microbial hydrogenation of *cis*-18:1, 18:2 and 18:3 fatty acids (Jenkins, 1993). Biohydrogenation leads to minimal flow of UFA to the duodenum (Jenkins, 1993). Accordingly, addition of plant oils to ruminant diets increases unsaturation of body tissues

Table 2.4. Fatty acid composition of potential unsaturated fat supplements

Supplement	g/100g fatty acids		
	Polyunsaturated	Monounsaturated	Saturated
Corn	59	35	16
Linseed	68	21	11
Olive	7	83	10
Rapeseed	23	71	6
Safflower	78	13	9
Sesame	43	42	15
Soybean	59	25	16

Source: Hudson (1996).

only slightly. This obstructs the efforts intended to alter the fatty acid composition of tissue or milk fat in cattle.

A portion of the *trans* isomers produced in the rumen escape further biohydrogenation and ultimately are absorbed from the intestine and incorporated into storage lipids and milk fat (Wu et al., 1991). Loss of fatty acids from the rumen either by absorption across the ruminal epithilium or by catabolism to volatile fatty acids (VFA) or CO_2 was minimal according to most reports. In addition, microbes synthesize fatty acids de novo from carbohydrate precursors. Therefore, lipid reaching the duodenum consists of fatty acids from both dietary and microbial origins (Jenkins, 1993).

Microbial synthesis of branched and odd-numbered chain fatty acids (e.g. 15:0) occurs in the rumen, and these fatty acids are present in carcass and milk lipids (Timmen and Patton, 1988). SFA such as 18:0 reaching the duodenum are subject, in part, to desaturation by both intestinal and mammary desaturase enzymes. As a result, the ratio of 18:0 to 18:1 is lower in milk than in intestinal digesta. This is a mechanism used by the ruminants to preserve the fluidity of milk fat.

Supplemental fat and ruminal fermentation: Lipid supplements in the ruminant diet have the potential to disrupt the digestion of non-lipid energy sources in the rumen. Ruminal digestion of structural carbohydrates was lowered by 50% or more by diets with less than 10% added fat (Ikwuegbu and Sutton, 1982; Jenkins and Palmquist, 1984). Two main mechanisms have been suggested to explain the relationship between the dietary fat and the disruption of ruminal digestion (Jenkins, 1992). According to one theory, fat covers the feed particles, thus preventing contact with microbial enzymes. The other theory suggests that a high level of dietary fat is toxic to the ruminal microbes.

Feeding the UFA supplements in protected forms

Fat supplements must be fed in a protected form to avoid interactions between the dietary fat and ruminal microbes. This facilitates greater passage UFA to the duodenum without affecting the ruminal digestion of non-fat nutrients.

Formaldehyde treatment: Fat encapsulated in a matrix of formaldehyde treated protein has been found to bypass ruminal biohydogenation (Faichney et al., 1972; Scott et al., 1971). Ashes et al., (1992) tested the usefulness of this technique to modify the milk fatty acid profile. When diets supplemented with formaldehyde treated canola seeds at 6.5% of the diet DM, the milk UFA yield was increased by 54% (143 g/d) and MCFA yield was reduced by 10% (38 g/d). No significant differences were reported in feed intake or milk yield. This indicates that the protected fat did not cause any major disruption in ruminal fermentation.

Feeding UFA in amide form: Fotouhi and Jenkins (1992 a, 1992b) reported that the reaction of UFA with primary amines produced fatty acyl amides that resist ruminal biohydrogenation and cause less disruption of ruminal fermentation. Similar results were reported by Jenkins (1995) when sheep diets were supplemented with 5% butylsoymide, a product made by treating soybean oil with butylamine. In this study, the butylsoymide supplement increased linoleic acid content in plasma neutral lipids by 65%, compared with only 16% increase in response to a diet supplemented with 5% soybean oil.

As indicated above, Jenkins et al. (1995) fed cows with diets supplemented with 3.5% soybean oil or butylsoymide. Soybean oil supplementation reduced total VFA and acetate concentration in the rumen and reduced the fat percentage of milk. These results indicate that fat fed in the raw form disrupts ruminal fermentation. However, supplementation of butylsoymide showed no influence on ruminal VFA concentration or milk fat percent, indicating the rumen inertness of the protected fat. The proportion of linoleic acid in plasma fatty acids was higher in cows fed butylsoymide (59%), compared to soybean oil (54%) and control (52%). Butylsoymide supplementation also resulted in milk fat containing 6.3% linoleic acid compared with 3.6% for the control. MCFA content of milk fat was reduced from 56.9% for control to 53.6% for the butylsoymide treatment.

Other methods of protecting dietary UFA: Feeding calcium salts of long-chain fatty acids (4.0 to 5.0% of diet DM) appeared to overcome the adverse effects of feeding unprotected fat on nutrient digestibility (Grummer, 1988; Jenkins and Palmquist, 1984). Depending upon the

chemical composition of the fat used in making such salts, significant alterations in the milk fatty acid profile could be obtained. Intact oil seeds also provide a degree of protection from biohydrogenation. The seed coat may provide complete or partial resistance to microbial enzyme activity (Cadden and Kennelly, 1984; Handy and Kennelly, 1983; Kennelly and Khorasani, 1992).

Other uses of feeding protected fat: Recent use of fat supplements in the diet of dairy cattle has helped to provide a dietary strategy to offset the negative energy balance that occurs in early lactation (Grummer et al., 1990). The fat supplements must be relatively inert in the rumen to reduce the detrimental effect of fat on ruminal fermentation (Grummer et al., 1990). In this context, feeding protected fat supplements to lactating cows will increase energy concentration in the diet and improve metabolic efficiency (Kronfeld et al., 1980). Moreover, depending upon the chemical composition of the fat used, there will be significant changes in the fatty acid profile of milk fat (Macdonald and Scott, 1977).

Fat-modified bovine milk and CHD

The impact of fat-modified dairy fats on plasma cholesterol of humans was examined by Noakes et al. (1995). In order to obtain the fat modified milk for this purpose, a group of cows were fed a diet containing a low amount of roughage and a lipid supplement derived from canola and soybean meal protected by formaldehyde treatment. The reduced level of dietary roughage was to lower acetate production in the rumen, thus minimizing de novo synthesis of short and medium chain fatty acids in the mammary system. Consequently, the milk produced by cows had reduced levels of palmitic and myristic acids and increased levels of oleic and linoleic acids. When such milk was included in diets of humans the dietary intakes of palmitic acid and myristic acids fell relative to the control diet by 37% and 35%, respectively, and intakes of oleic, linoleic and linoleinic acids increased by 31, 59, and 133%. During the test periods, the fat-modified dairy products resulted in a significant (0.28 mmol/L or 4.3%) lowering of total cholesterol. Most of this decrease was in LDL cholesterol, which decreased by 0.24 mmol/L, whereas HDL cholesterol and triacylglycerols remained essentially unchanged. The 4.3% reduction in cholesterol, if applied to the population, represents a 9% reduction in the risk of developing CHD (LRCP, 1984).

Appropriate UFA in bovine milk - oleic acid versus linoleic acid

Most early studies aimed at modifying milk fatty acid profile used diets with protected sunflower seeds. The milk produced in these experiments contained substantially increased levels of linoleic acid and reduced levels of MCFA (Cook et al., 1970; Nestel et al., 1973; Nestel et al., 1974). However, these linoleic acid-enriched products had a decreased shelf life because they were more susceptible to autoxidation (McDonald and Scott, 1977), making them less viable commercially. It became necessary to add butylated hydroxytoluene as an antioxidant to the milk.

Oleic acid, a monounsaturated fatty acid, has been found to be more stable to oxidation than linoleic acid. Cadden and Kennelly (1984) reported that butter made from the milk of cows fed canola seed or protected canola seeds was softer and had similar organoleptic properties to control butter.

Processing quality and consumer acceptance of fat-modified milk

The influence of dietary supplemental fat on the processing quality of milk and milk products will depend on the extent to which the fatty acid composition is altered. Elevating the level of polyunsaturated fatty acids in milk results in softer butter and a lighter color but it tends to be more susceptible to oiling off at 10 °C or higher (Kennelly, 1996). The major concern associated with increased concentrations of polyunsaturated fatty acids is that milk is more susceptible to autoxidation. Approaches taken to control autoxidation include supplementing the cow diet with α-tocopherol, direct addition of antioxidants to milk and modifications to the processing system involved in the production of butter, cheese and other dairy products. Increasing the level of α-tocopherol in milk by dietary supplementation or intramuscular injection has recently been successfully used to control oxidized flavor in milk (Charmley and Nicholson, 1993; Charmley et al., 1993). Hagemeister et al. (1991) observed a marked reduction in peroxide value of milk containing in excess of 10% n3 fatty acids when α-tocopherol was infused into the abomasum with linseed oil at the rate of 4 g DL-α-tocopherol per kg oil. Cadden and Kennelly (1984) highlighted the suitability of increasing oleic acid rather than linoleic acid in milk in order to have better texture and organoleptic properties of milk products.

Alternative ways to modify milk fatty acid profile - biotechnology

Seeds and seed oils with higher contents of fatty acids favorable for human health have been developed via breeding and genetic engineering. Canola, corn, soybean and sunflower varities with higher contents of oleic acid in seeds have been introduced to the market (Cline and Re, 1997). The possibility to use such fat sources to modify the bovine milk fatty acid profile should be investigated.

Scientists have been studying the possibility of using recombinant DNA tecnology to alter the de novo fatty acid synthesis pattern in mammary tissues. More attention is being focused on regulating the transcription rate of fatty acid desaturase and fatty acyl-CoA carboxylase enzymes. Clark and Jump (1994) reported that, by introducing different fatty acid isomers to mammary cells, the transcription of specific genes involved in milk fat synthesis could be regulated. It is evident that further studies are needed to establish the usefulness of biotechnology to produce fatty acid modified milk from dairy cows.

CHAPTER 3

MODIFYING MILK FATTY ACID PROFILE OF JERSEY COWS BY ABOMASAL INFUSION OF OLIVE OIL, SESAME OIL, SUNFLOWER OIL OR FISH OIL

ABSTRACT

The potential for enhancing oleic acid (*cis*-18:1) and linoleic acid (18:2) content and lowering medium chain fatty acid (MCFA) content of bovine milk was investigated by abomasal infusion of olive oil (82% *cis*-18:1, 3% 18:2), sesame oil (71% *cis*-18:1, 11% 18:2), sunflower oil (52% *cis*-18:1, 26% 18:2) or fish oil (27% *cis*-18:1, 52% 18:2) in Jersey cows. After an initial 14-d preliminary period, 155 to 219 g/d of oil was infused continuously during the last 6 d of each of four 14-d periods in a 4x4 Latin square design. Digestibility of *cis*-18:1 in the intestine was greater during olive oil infusion (94%) than during sunflower oil infusion (84%). Olive oil or sesame oil infusion provided the highest *cis*-18:1 content in milk (35.6 and 33.6%, respectively), and sunflower oil infusion resulted in the highest 18:2 content (2.6%). During olive oil or sesame oil infusion, for each 100 g of *cis*-18:1 infused into the abomasum, milk *cis*-18:1 yield increased by 45 and 49 g, respectively, and milk MCFA yield was lowered by 42 to 43 g. In addition, during olive oil or sesame oil infusion, 18:2 yield in milk increased by 47 and 46 g, respectively, for each 100 g of infused 18:2. The digestibility of *cis*-18:1 and 18:2 in the intestine and recovery in milk were proportional to the flow of these fatty acids to the duodenum. Milk produced during sesame oil infusion had an off-flavor when evaluated by a taste panel, but milk produced during other oil infusions was within sensory analysis specifications. Overall, results indicated olive oil was the most appropriate of the four oils to modify milk fatty acid composition in a manner that would be beneficial for human health.

Key words: oleic acid, linoleic acid, medium chain fatty acids, digestibility, milk flavor.

INTRODUCTION

Elevated concentration of low-density-lipoprotein (LDL)-cholesterol in plasma is a risk factor for coronary heart disease (American Heart Association/Heart, Lung and Blood Institute, 1990). Three of the principal medium-chain, saturated fatty acids (MCFA) in bovine milk fat, lauric acid (12:0), myristic acid (14:0), and palmitic acid (16:0), are among the dietary factors that raise plasma LDL-cholesterol (Denky and Grundy, 1992; Nestel et al., 1994). However, long-chain unsaturated fatty acids (UFA), primarily oleic acid (*cis*-18:1), linoleic acid (18:2) and linolenic acid (18:3), apparently lower plasma cholesterol (Grundy and Denky, 1990; Mattson and Grundy, 1985; Rivellese et al., 1994). Bovine milk fat contains relatively higher concentrations of MCFA and lower concentrations of UFA as compared with other dietary sources of animal or vegetable fat (Berner, 1993; Kennelly, 1996).

Biohydrogenation by ruminal microbes minimizes the flow of dietary UFA to the duodenum; thus, dietary UFA supplements increase the ratio of UFA to MCFA only slightly. Moreover, excess dietary UFA interfere with microbial function and fiber digestion in the rumen (Ikwuegbu and Sutton, 1982; Jenkins, 1993; Jenkins and Palmquist, 1984). Feeding dietary UFA sources encapsulated in a matrix of formaldehyde-treated protein (Ashes et al., 1992; Faichney et al., 1972; Scott et al., 1971) or as fatty acyl amides (Fotouhi and Jenkins, 1992 a, b; Jenkins, 1995) increased the quantity of UFA available for absorption by ruminants and consequently increased milk UFA content. The beneficial effect of consuming milk and dairy products with elevated UFA content on plasma LDL-cholesterol was clearly demonstrated by Noakes et al. (1996), who obtained fat-modified milk from cows fed protected canola seeds.

Potential sources of UFA-rich supplements that could be protected and fed to dairy cattle to improve the fatty acid profile of milk need to be evaluated. The extent of digestion and absorption of dietary fatty acids in the intestine and the efficiency with which the mammary gland incorporates the absorbed fatty acids may vary with source.

OBJECTIVES

Olive oil, sesame oil, sunflower oil or fish oil, which differed in their *cis*-18:1 and 18:2 contents, were infused into the abomasum of Jersey cows to evaluate the potential of these oils to modify milk fatty acid profile. Enhanced flow of *cis*-18:1 and 18:2 to the duodenum was used to increase the yields of *cis*-18:1 and 18:2 and decrease the yield of MCFA in milk. In addition, sensory quality of processed whole milk was determined to evaluate the consumer acceptance of milk with a modified fatty acid profile.

MATERIALS AND METHODS

Cows and diet

Four Jersey cows in mid-lactation, each with a duodenal and a rumen cannula were housed in a tie-stall barn and fed a basal diet (Table 3. 1) at 0730 and 1930 h daily. Sufficient diet was fed for ad libitum intake, and orts (feed refusals) were weighed at 0700 h. Cows were milked at 0700 and 1900 h daily, and allowed to adjust to the diet and the barn environment during a 14-d preliminary period. With the exception of oil infusion, cows were managed and sampled during the preliminary period as described below for the 14-d treatment periods.

Treatments and infusion procedure

The treatments, which differed primarily in their content of oleic acid (*cis*-18:1) and linoleic acid (18:2) (Table 3.2), were fish oil (crude menhaden oil, Zapta Protein (USA), Inc., Mandeville, LA), olive oil (Hunt Wesson, Inc., Fullerton, LA), sesame oil (Loriva®, Supreme Foods, Inc., Hauppauge, NY), and sunflower oil (Richfood, Inc., Richmond, VA). Oil was continuously infused to the abomasum of each cow at a rate of 155 to 219 g/d from d 9 through 14 of four treatment periods in a 4x4 Latin square design. The assignment of cows to treatments is shown in Appendix Table 1.

A peristaltic pump (Multistatic®, Haake Buchler Instruments, Inc., Saddle Brook, NJ) and Tygon® tubing (0.16 cm i.d., 0.31 cm o.d.; Fisher Scientific Company, Pittsburgh, PA) were used for infusion. Tubing was routed through an opening in the rumen cannula, then through the omasal orifice into the abomasum. The end of the tubing was passed through a 60 mL Nalgene® bottle (4 cm diameter) to prevent it from being pulled out of the abomasum during rumen contractions. The presence of tubing in the abomasum was confirmed on d 9, 12, and 14 of each period. The reservoir for each oil infusion was a 250 mL plastic bag (Baxter®, Baxter Healthcare Corporation, Deerfield, IL 60015), which was weighed at the start and the end of each 24 h. Oil from each reservoir passed through one pump set to deliver approximately 0.14 mL/min. However, actual oil flow rates varied due to differing viscosities.

Digesta marker

Chromium (Cr)-mordanted fiber was used as the digesta marker. The mordanting procedure of Uden et al. (1980) was used to attach Cr to washed fecal fibers (6 g Cr/100 g fecal fibers) collected from non-lactating cows fed orchard-grass hay. Cr-mordanted fecal fibers (15 g/d) were placed in the rumen throughout the experiment, with 50% of each dose given at 0700 h and 50% at 1900 h.

Sample collection

All sampling was done between 2000 h on d 12 and 2000 h on d 14 of each period, including the preliminary period. One sample of corn silage, alfalfa haylage, and concentrate mixture was collected in each period and dried to a constant weight at 60 ^{0}C. Six duodenal digesta and six fecal samples were collected from each cow in each period (2000 h on d 12, 0400 and 1200 h on d 13, and 0000, 0800 and 1600 h on d 14). Fecal and duodenal samples were stored at -20 ^{0}C. Fecal samples were dried to constant weight at 60 ^{0}C. Duodenal samples were freeze-dried. All dry, composited samples were then ground through a 1 mm screen in a Cyclone mill (UD Corporation, Boulder, Colorado).

Blood (10 mL) was collected from the coccygeal artery at 2000 h on d 12. Plasma was separated by centrifugation at 3,000 x *g* for 15 min then stored at -20 ^{0}C. Milk samples were collected from

each milking during the last 2 d of each period for fat, lactose, protein, and SNF determinations by the Dairy Herd Improvement Association laboratory at Virginia Tech. Additional samples from each milking during d 14 of each period were centrifuged at 11,000 x *g* for 60 min to extract the fat layer for fatty acid analysis. In addition, 10 L of milk were obtained from each cow on d 14 of each period for sensory quality testing conducted by the Department of Food Science and Technology at Virginia Tech.

Chemical analysis

Forages, concentrates, duodenal digesta, and feces were analyzed for ether extract (EE) (AOAC, 1990), crude protein (CP) (AOAC, 1990) and organic matter (OM) (AOAC, 1990). The Cr concentration in duodenal and fecal samples was determined using atomic absorption spectrophotometry following acid digestion as described by Scandell (1950). The dry matter flow at the duodenum was calculated using the method described by Armentano and Russell (1985).

Fatty acid concentrations in forages, concentrates, duodenal digesta, feces, milk fat and plasma were determined following transesterification (Outen et al., 1976). Undecenoic acid (Nu-Check Prep, Elysian, MN) was used as the internal standard. Samples were injected by auto-sampler into a Hewlett Packard, 5890A gas chromatograph equipped with a flame ionization detector (Hewlett Packard, Co., Sunnyvale, CA) using procedures described by Wonsil (1994). The split ratios were 15:1 for feed, duodenal digesta, fecal and plasma samples and 80:1 for oils and milk fat samples.

Milk quality

Raw milk from each cow was processed within 24 h of collection. Milk was pre-warmed to 60 ^{0}C and homogenized (first stage = 13.6 Mpa, second stage = 3.4 Mpa) using a laboratory homogenizer (APV Gaulin, Inc., Model 15MR, Everett, MA). This was immediately followed by pasteurization at 74 ^{0}C for 15 sec in a laboratory-scale high-temperature short-time pasteurizing system (UHT/HTST Lab-25, Microthermics, Inc., Raleigh, NC). Processed product was cooled to 10 ^{0}C and stored at 3.3 ^{0}C in stainless steel cans with lids.

Homogenized, pasteurized samples were evaluated for sensory acceptance using the "In/Out" method (Bodyfelt et al., 1988), which is used to accept or reject samples that fall beyond a preset specification or standard. Specifications for milk quality determinations are listed in Table 3.3. The product was considered acceptable if 50% or more of the panelists identified the product as "in" specification. Panelists (n=10) had been trained in terminology and sensory characteristics of milk through a dairy products sensory course or collegiate dairy products evaluation team. Panelists were seated in separated sensory booths. Samples (30 mL) were presented simultaneously, at 9 ^{0}C, in coded 57 g plastic cups with lids. Sample order was randomized. Panelists were instructed to smell and taste each sample and expectorate. The product was rated as "in" or "out" of specification, and reasons for "out" of specification were requested. Panelists rinsed with water between samples.

Statistical analysis

Effects of treatments, cows and periods were analyzed using General Linear Model (SAS, 1985). The model was:

$$Y_{ijk} = \mu + C_i + P_j + T_k + E_{(ijk)}$$

Where Y_{ijk} = dependent variable

μ = overall mean

C_i = effect of cow (i = 1, 2, 3, and 4)

P_j = effect of period (j = 1, 2, 3, and 4)

T_k = effect of treatment (k = 1, 2, 3, and 4)

$E_{(ijk)}$ = residual error

Treatment means were compared using Turkey's pair-wise comparison procedure (SAS, 1985), and were considered significantly different at $P < 0.05$. An ANOVA example is shown in Appendix Table 1. Paired-t-tests were used to compare effects of oil infusion versus no infusion (preliminary period) on intake, flow to the duodenum, and digestibility of OM, CP, and EE. For this purpose, observations during the preliminary period (no infusion and no catheter placed in the omasal orifice and abomasum) were compared with those during the first infusion period (regardless of type of oil infused).

RESULTS AND DISCUSSION

Feed intake and milk production

Daily dry matter intake (DMI) did not differ due to the type of oil infused into the abomasum. Compared with DMI (19.2 kg/d) during the preliminary period, however, all cows had numerically lower average DMI (18.3 kg/d) during the oil infusion periods. Daily milk yields were similar among treatments, and averaged 19.7 kg/d (Table 3.4). Infusion of oil apparently reduced milk yield by 1 to 1.3 kg/d, and this may have been associated with lower DMI or advancing stage of lactation compared with the preliminary period. Milk component percentages and yields also did not differ due to treatment.

Intake, flow and digestibility of organic matter, crude pretein and ether extract

Intake, flow to the duodenum and digestibility of organic matter (OM) did not vary with type of oil infused (Table 3.5). Compared with that of the preliminary period, however, OM and crude pretein (CP) intake were numerically lower, but flow of OM and CP to the duodenum were numerically higher during infusion periods. Infused oil accounted for only 0.16 to 0.22 kg (Table 3.5) of the estimated 1.8 kg increase in average OM flow during infusion periods compared with the preliminary period. The remainder of the difference apparently was due to oil in the abomasum and (or) the presence of the infusion catheter in the omasal orifice and abomasum. Paired-t-test results, however, indicated no significant difference between the preliminary period and the first infusion period for intake, flow to the duodenum, or intestinal digestibility of OM and CP. The inability to detect a significant difference probably was due to variation associated with rate of flow and detection of the digesta marker (chromium) in the duodenal samples. Cows apparently compensated for the greater flow of digesta to the duodenum during the infusion periods, compared with the preliminary period, by increasing the apparent digestibility of OM and CP between the duodenal sampling site and the feces.

The amount of additional ether extract (EE) flow to the duodenum detected during the oil infusion periods (approximately 200 g/d), compared with the preliminary period, was similar to the average amount of oil infused daily. Average apparent digestibility of EE in the intestine

during the oil infusion periods was 75%, compared with 72% during the preliminary period. Paired t-tests detected ($P < 0.05$) the nearly doubled rate of EE flow to the duodenum, but did not indicate a significant change in EE digestibility between the preliminary period and the first infusion period.

Flow and digestibility of fatty acids

Compared with those of the preliminary period, average amounts of all fatty acids, except *trans*-18:1, that flowed to the duodenum were numerically greater during the oil infusion periods (Table 3.6). However, flow of fatty acids to the duodenum and fatty acids absorbed from the intestine (Appendix Table 3) did not vary significantly among treatments. All fatty acids, except 18:3, were digested in the intestine at rates above 75%. More than 83% of *cis*-18:1 and 18:2 flowing to the intestine were absorbed. However, only *cis*-18:1 digestibility differed among treatments. The *cis*-18:1 digestibility was greater during olive oil infusion than sunflower oil infusion. When ranked numerically, olive oil infusion provided the greatest flow and amount of *cis*-18:1 absorbed, followed by sesame oil, sunflower oil and fish oil. Thus, amounts of *cis*-18:1 flow to and absorption from the intestine were proportional to the *cis*-18:1 content of the oils (Table 3.2).

Klusmeyer and Clark (1991) reported that, in Holstein cows, increased flow of *cis*-18:1 and 18:2 to the duodenum enhanced their digestibility in the intestine. When flows of *cis*-18:1 to the duodenum were 290, 235, 102, and 94 g/d, respectively, digestibilities of *cis*-18:1 in the intestine were 90.3, 89.6, 82.6 and 79.6%. They also reported 90, 82.6, and 79.6% intestinal digestibilities of 18:2 when flows of 18:2 to the duodenum were 63, 43, and 41 g/d. The above digestibility values are slightly lower than *cis*-18:1 and 18:2 digestibilities reported in the present experiment, possibly due to differences in efficiency of nutrient utilization between Holstein and Jersey cows. Palmquist (1991) demonstrated that true digestibility of total fatty acids declined from 100% (at 1% fat in the diet) to 78% (at 8% fat in the diet), but the total fatty acid digestibility did not differ due to source of fat (animal-vegetable blend, Ca-soap, hydrogenated animal fat, saturated fatty acids, tallow, or basal diet). Elliott et al. (1996) reported a decline in total fatty acid digestibility from 81.3% for a control diet to an average of 71% for diets supplemented with 5 to 6.1% fat

(calcium salts of long chain fatty acids, prilled fatty acids, or prilled or flaked hydrogenated palm fatty acid distillate).

Fatty acid concentration in blood plasma

Olive oil infusion elevated *cis*-18:1 content of blood plasma compared with sesame oil and sunflower oil infusions (Table 3.7). Plasma *cis*-18:1 (75 μg/mL) was 7.9% of total fatty acids during the preliminary period compared with 9.6% during olive oil infusion. Gaynor et al. (1993) reported that *cis*-18:1 accounted for 6.3% of total plasma fatty acids in control cows and 11.4 % in cows abomasally infused daily with 750 g of *cis*-18:1. Wonsil (1990) reported plasma 18:1 concentrations of 6 versus 8% when cows were fed a control diet or diets with 3% tallow. Average plasma 18:2 content during the infusion periods was numerically greater than during the preliminary period, but concentrations did not differ due to type of oil infused.

Milk fatty acid composition

Neutral fatty acids

The fatty acids believed to have neither cholesterol raising nor cholesterol lowering effects were classified as neutral (Berner, 1993). The major neutral fatty acids in milk fat are short chain fatty acids (SCFA) [butyric acid (4:0), caproic acid (6:0), caprylic acid (8:0), and capric acid (10:0)] and stearic acid (18:0). Oil infusion treatments did not affect 4:0, 6:0, or 8:0 content of milk (Table 3.8), but 10:0 was greater during sunflower oil infusion than olive oil infusion. Additionally, infusion of olive oil, which supplied the numerically lowest amount of 18:0 (Table 3.2), resulted in a lower 18:0 content in milk, compared with infusion of sunflower oil, which had the numerically highest 18:0 content, or fish oil. Drackley et al. (1992) reported no difference in milk SCFA or 18:0 content when Holstein cows were abomasally infused daily with 450 g of mostly unsaturated fatty acids. According to Wonsil et al. (1993), cows fed diets with 3% soybean oil did not exhibit changes in milk 4:0, 6:0, or 8:0, but had lower 10:0 (1.9%) compared with the control (2.8%). Ashes et al. (1992) reported no change in SCFA content of milk when cows were fed protected canola seeds at 6.5% of the diet DM, but 18:0 content was 9.2% compared with 7.1% when cows were fed the control diet.

Cholesterol-raising fatty acids

According to Berner (1993), MCFA [lauric acid (12:0), myristic acid (14:0) and palmitic acid (16:0)] are considered to be cholesterol-raising (hypocholesterolemic) fatty acids. The content of each of these fatty acids in milk was significantly altered by the treatments, and infusion of oil numerically reduced their content relative to the preliminary period (Table 3.8). Infusion of olive oil, the relatively richest source of *cis*-18:1 (Table 3.2), resulted in lower content of 12:0 in milk, compared with infusion of sunflower oil. Olive oil infusion also resulted in lower 14:0 content compared with sunflower oil or fish oil infusion. Olive oil and sesame oil reduced 16:0 content of milk to a greater extent than fish oil.

Gaynor et al. (1994) observed that when *cis*-18:1 was infused abomasally at 750 g/d, the concentration of MCFA in milk dropped from 52.5% to 39.6%. According to Drackley et al. (1992), the MCFA content of milk produced by cows infused abomasally with 450 g of unsaturated fatty acids daily was 34.9% versus 46% for the control. When cows were fed with protected canola seeds at 6.5% of the diet DM, Ashes et al. (1992) observed a total of 33% MCFA compared with 42.8% for the control. Palmquist et al. (1993) hypothesized that supplemental dietary fat depresses the de novo synthesis of MCFA in cow mammary tissue.

In addition to MCFA, *trans*-18:1 also is considered hypercholesterolemic (Judd et al., 1994 and Keys et al., 1986). Milk *trans*-18:1 content did not vary due to type of oil infused because abomasal infusion of oil had no significant effect on *trans*-18:1 flow to or absorption from the intestine (Table 3.6).

Cholesterol-lowering fatty acids

Cis-18:1 is a cholesterol-lowering (hypercholesterolemic) fatty acid in human foodstuffs (Berner, 1993). The average *cis*-18:1 content of milk during oil-infusion periods (32%) was numerically greater compared with the preliminary period (28%) (Table 3.8). Also, *cis*-18:1 in milk reflected *cis*-18:1 content of the oils (Table 3.1) and amount absorbed in the intestine (Appendix Table 2). Olive or sesame oil infusions resulted in greater amounts of *cis*-18:1 in milk (35.6 and 33.6% respectively) compared with sunflower oil (29.6%) or fish oil (29.4%) infusions. These

observations agree with the findings of Gaynor et al. (1994), who reported 34.7% *cis*-18:1 in milk when *cis*-18:1 was infused abomasally at 750 g/d. Klusmeyer and Clark (1991) reported 27.1% *cis*-18:1 in milk when lactating cows were fed with calcium salts of long chain fatty acids at 4% of diet DM, compared with 21.3% when cows received a control diet. The long chain fatty acid mixture used in their experiment had a moderate *cis*-18:1 content, and the milk *cis*-18:1 content reported by these workers was lower than that of the present study or Gaynor et al. (1994). Ashes et al. (1992) reported 29.2% *cis*-18:1 in milk when cows were fed 6.5% protected canola seed in the diet versus 23.8% for their control group.

Content of 18:2 in milk was improved due to oil infusion compared with the preliminary period. Sunflower oil infusion elevated milk 18:2 content to the highest level (2.56%) compared with the other oil infusions (0.9 to 1.5%) or the preliminary period (0.6%). Jenkins et al. (1995) reported that milk 18:2 content could be increased from 3.6 to 4.8 or 6.3% when soybean oil or butylsoyamide was fed to dairy cows at 3.5% of diet DM. Drackley et al. (1992) reported that milk 18:2 could be increased from 2.3 to 13.3% when an unsaturated fatty acid mixture was abomasally infused at 450 g/d. However, the amount of fatty acids fed (Jenkins et al., 1995) or infused (Drackley et al., 1992) was much higher than the amount infused in the present study.

Other fatty acids

Concentrations of odd chain fatty acids (15:0 and 17:0), medium chain unsaturated fatty acids (14:1, 16:1 and 17:1), and 20:0 in milk were numerically lower during infusion compared with the preliminary period (Table 3.8). These fatty acids accounted for 5% or less of milk fatty acids. However, increased content of unsaturated fatty acids in milk due to oil infusion apparently reduced the percentages of these fatty acids in milk. Reasons for the small, but significant differences in response to type of oil infused are not apparent.

Summary of milk fatty acid composition

Olive oil infusion resulted in a greater content of hypocholesterolemic fatty acids in milk (36.4%) compared with infusion of sunflower oil (32.2%) or fish oil (30.9%) (Table 3.8). In addition, lower concentrations of hypercholesterolemic fatty acids resulted from olive oil (37.8%) or

sesame oil (38.2) infusions, relative to the infusion of fish oil (41.1%). As a result, olive oil infusion increased the ratio of unsaturated to saturated fatty acids (0.66) in milk to a greater extent than fish oil (0.51). The results, therefore, indicated that oils containing 80% or more *cis*-18:1 may have the greatest potential for improving the fatty acid profile of bovine milk in a manner that makes fluid milk or manufactured milk products more desirable with respect to prevention of hypercholesterolemia in humans.

The most consistant results, with respect to improved fatty acid profile of milk, were obtained from olive oil and sesame oil infusion. During olive oil or sesame oil infusion, yield of *cis*-18:1 was increased by 45 and 49 g, respectively, for each 100 g of *cis*-18:1 infused into the abomasum (Table 3.9). In addition, infusion of these oils reduced MCFA yield by 43 and 42 g, respectively, for each 100 g of infused *cis*-18:1. In contrast, 18:2 yield was increased by approximately 46 g/d for each 100 g of infused 18:2 from either oil. Responses to sunflower oil and fish oil were less consistant, most likely due to their high 18:2 content.

Consumer acceptance of fat-modified milk

Sensory analyses indicated that milk produced during sesame oil infusion was considered “out” of specification, because the milk of three out of four cows failed to receive 50% or more "in" responses (Table 3.10). Panelist comments were variable but many panelists agreed that there was an aroma and aftertaste. Rancid or microbial off-flavors also were suggested. Some panelists criticized the product for being too cooked but this would be a processing variable unrelated to the oil-infusion treatments. Shahidi et al. (1997) reported that the content of sesamin, the major antioxidant found in sesame seeds was reduced to 20% or less of its original level during oil processing. They also reported that reduction of sesamolin and γ-tocopherol, the other antioxidants found in sesame seeds, were more drastic than that of sesamin during processing. Perhaps products of antioxidant degradation or products of fatty acid oxidation in milk produced during infusion of sesame oil were partially responsible for the off-flavor of the milk.

Other than milk from cow 3 when infused with sunflower oil, all other milk samples obtained during oil infusions were acceptable. However, the percentage of “in” responses were highest for

all cows during the preliminary period. As suggested by Shahidi et al. (1997) regarding sesame oil, inadequate content of antioxidants might cause milk with a high ratio of unsaturated to saturated fatty acids to be more susceptible to oxidation during pasteurization. This might be a reason for relatively lower “in” responses for milk produced during oil infusions compared with milk from the preliminary period.

IMPLICATIONS

The study revealed that nearly 45 to 49% of supplemental *cis*-18:1 and 18:2 flow to the duodenum was recovered in milk fat when olive oil or sesame oil was infused. Use of oils rich in *cis*-18:1 may be appropriate to modify the fatty acid profile of bovine milk in a manner that would be beneficial for human health. Olive oil infusion did not alter milk flavor. Olive oil also provided a relatively greater content of hypocholesterolemic fatty acids, a higher ratio of unsaturated to saturated fatty acids, and a lower content of hypercholesterolemic fatty acids when compared with other oils. The use of other oils with high *cis*-18:1 content for this purpose should be investigated. The seed oil from canola, soybean, and sunflower that have been bred or genetically engineered to contain high *cis*-18:1 content may be good choices.

Table 3.1. Dietary ingredients, chemical composition, and fatty acid content of the basal diet

Ingredient, % of dry matter	
Corn silage	25.3
Alfalfa haylage	28.5
Orchard grass hay, chopped	2.7
Corn	28.4
Dried distiller's grain	14.0
Mineral/vitamin premix[1]	1.1
Chemical composition, % of dry matter	
Organic matter	94.5
Crude protein	14.0
Ether extract	3.1
Fatty acids, µg/g feed	
4:0	5.2
14:0	45.8
15:0	24.0
15:1	3.6
16:0	6408.4
16:1	146.6
17:0	30.8
18:0	1257.0
cis -18:1	5199.4
18:2	15833.2
18:3	115.0
20:0	1704.4
Total	30782.8

[1] Contained 6.5% P, 16.0% Ca, 4.3% NaCl, 2.2% Mg, 3.5% K, 3.2% S, 0.11% Mn, 0.13% Zn, 0.03% Fe, 0.13% Cu, 0.002% I, 0.0003% Co, 0.0005% Se, 110,000 IU vitamin A/kg, 44,000 IU vitamin D_3/kg, and 1,350 IU vitamin E/kg.

Table 3.2. Average fatty acid composition (g/100 g fatty acids) of oils used in the study

Fatty acid	Olive oil	Sesame oil	Sunflower oil	Fish oil
16:0	10.5	9.1	10.1	10.1
16:1	2.0	0.1	-	0.1
17:0	-	-	-	0.1
18:0	2.5	8.3	10.8	4.4
cis-18:1	81.6	71.1	51.7	26.9
18:2	2.8	10.6	26.0	51.8
18:3	0.2	0.5	0.4	0.4
20:0	0.4	0.4	0.9	5.4
20:3	-	-	-	0.5
22:1	-	-	-	0.3

Table 3.3. Specifications for Milk Quality

Critical Attributes	"In/Out" Limits
Cooked	May have slightly cooked flavor and aroma, sweet but no indication of caramel-like flavors. If product is slightly (or more) reminiscent of canned milk, then product is "out" of specification. A scorched flavor is "out" of specification.
Feed	May have slight aroma, but no aftertaste. Strong aroma or slight aftertaste reminiscent of hay, silage, onion, garlic, etc. is "out" of specification. Astringent feeling on tongue after expectoration is "out" of specification.
Microbiological off-flavor	No aroma or flavor indicative of microbiological problems is acceptable. This includes any fermented, fruity, malty, unclean aromas, flavor or aftertaste. If present, the product is "out" of specification.
Rancid	No aroma or flavor indicative or rancidity is acceptable. If evident, the product is "out" of specification.
Oxidized	May have a slight aroma, at level easily confused with cooked aroma, but no oxidized flavor. If strong oxidized aroma or any oxidized flavor is evident, the product is "out" of specification.
Bitter	No bitter flavor is acceptable. If evident, the product is "out" of specification.

Source: Bodyfelt et al. (1988).

Table 3.4. Daily milk production, milk composition, and milk component yields of Jersey cows infused abomasally with olive oil, sesame oil, sunflower oil, or fish oil

		Oil infusion				
	Preliminary	Olive	Sesame	Sunflower	Fish	SE
Milk, kg/d	20.8 ± 1.9[a]	19.5	19.6	19.7	19.8	0.4
Composition:						
Fat, %	5.1 ± 0.1	5.3	5.2	5.3	5.3	0.1
Lactose, %	4.8 ± 0.1	4.8	4.7	4.8	4.8	0.1
Protein, %	3.8 ± 0.3	3.7	3.7	3.7	3.7	0.1
Solids-not-fat, %	9.3 ± 0.1	9.2	9.3	9.3	9.3	0.1
Yield:						
Fat, kg/d	1.0 ± 0.1	1.0	1.1	1.0	1.1	0.1
Lactose, kg/d	1.0 ± 0.1	0.9	0.9	1.0	1.0	0.1
Protein, kg/d	0.8 ± 0.1	0.7	0.7	0.7	0.8	0.1
Solids-not-fat, kg/d	1.9 ± 0.1	1.8	1.8	1.8	1.8	0.1

[a] Mean ± SE (n = 4).

Table 3.5. Intake, flow to the duodenum, and intestinal digestibility of organic matter (OM), crude protein (CP), and ether extract (EE) of Jersey cows during abomasal infusion of olive oil, sesame oil, sunflower oil, or fish oil

		Oil infusion					
	Preliminary	Olive	Sesame	Sunflower	Fish	SE	t-value[b]
Intake, kg/d							
OM	18.2 ± 0.5 [a]	17.1	17.5	17.3	17.3	0.5	1.30
CP	2.7 ± 0.1	2.5	2.6	2.6	2.6	0.1	1.14
EE	0.40 ± 0.02	0.38	0.39	0.38	0.38	0.01	1.29
Oil infused, kg/d	-	0.19	0.17	0.22	0.16	0.03	
Flow to the duodenum, kg/d							
OM	8.4 ± 0.1	9.9	10.3	10.5	9.9	0.6	0.65
CP	1.8 ± 0.1	2.2	2.2	2.2	2.2	0.1	0.12
EE	0.33 ± 0.07	0.55	0.54	0.49	0.56	0.01	2.70
Apparent digestibility in intestine, %							
OM	33.5 ± 4.8	42.8	36.3	38.3	44.4	5.4	0.85
CP	38.7 ± 6.1	49.1	40.5	43.2	48.9	4.6	1.34
EE	72.0 ± 3.0	76.8	73.2	74.4	74.9	1.3	0.74

[a] Mean ± SE (n = 4).

[b] Calculated t-value from paired-t-test, comparing means of the preliminary period and the first infusion period. A significant t-value ($P < 0.05$), using three degrees of freedom, is equal to or greater than 2.35.

Table 3.6. Fatty acid (FA) flow and digestibility in the intestine of Jersey cows infused abomasally with olive oil, sesame oil, sunflower oil, or fish oil

		Oil infusion				
	Preliminary	Olive	Sesame	Sunflower	Fish	SE
FA flow to duodenum, g/d						
16:0	77.0 ± 28.7[1]	98.3	93.3	99.0	103.3	16.5
18:0	338.0 ±158.4	394.0	394.3	420.3	431.5	74.6
cis-18:1	75.3 ± 19.6	219.0	137.8	127.5	116.3	23.3
trans-18:1	28.5 ± 14.4	17.7	42.5	16.8	29.8	10.4
18:2	13.0 ± 2.7	17.5	20.5	31.8	22.5	5.1
18:3	2.4 ± 1.0	3.1	3.2	3.4	3.5	0.6
Apparent FA digestibility in the intestine, %						
16:0	78.5 ± 2.1	83.0	75.5	76.8	82.3	3.5
18:0	75.5 ± 3.9	83.5	76.3	77.0	79.4	4.2
cis-18:1	80.0 ± 4.8	93.5[a]	85.5[ab]	83.5[b]	89.5[ab]	2.0
trans-18:1	91.5 ± 4.3	85.5	89.0	81.0	91.3	3.5
18:2	79.8 ± 6.4	86.3	83.0	88.0	88.5	2.1
18:3	66.3 ± 8.3	75.8	65.0	66.3	73.0	5.2

[1]Mean ± SE (n = 4).

[a, b] Means for oil infusion periods with same subscripts do not differ ($P < 0.05$).

Table 3.7. Fatty acid content (μg/mL) of arterial blood plasma of Jersey cows infused abomasally with olive oil, sesame oil, sunflower oil, or fish oil.

		Oil infusion				
	Preliminary	Olive	Sesame	Sunflower	Fish	SE
16:0	134.3 ± 6.4[1]	149.5	140.8	126.5	152.5	20.8
18:0	201.5 ± 4.6	205.5	214.3	206.5	244.8	15.1
trans-18:1	28.3 ± 2.5	27.3	28.0	25.0	33.3	2.8
cis-18:1	75.3 ± 7.9	106.3[a]	71.3[b]	53.8[b]	75.5[ab]	7.1
18:2	515.3 ± 59.1	613.8	569.0	628.5	809.0	54.2

[1]Mean ± SE (n = 4).

[a, b] Means for oil infusion periods with same subscripts do not differ (P < 0.05).

Table 3.8. Milk fatty acids (g/100g fatty acids) of Jersey cows in response to abomasal infusion of olive oil, sesame oil, sunflower oil, or fish oil, listed according to their influence on plasma cholesterol when included in the diet of humans[1]

		Oil infusion				
	Preliminary	Olive	Sesame	Sunflower	Fish	SE
Neutral						
4:0	3.3 ± 0.1[2]	3.0	3.0	3.1	3.0	0.1
6:0	1.6 ± 0.1	1.5	1.6	1.6	1.6	0.1
8:0	1.3 ± 0.1	1.2	1.3	1.4	1.3	0.1
10:0	1.51 ± 1.20	1.35[b]	1.40[ab]	1.57[a]	1.44[ab]	
0.04						
18:0	15.7 ± 1.0	15.1[b]	16.2[ab]	16.9[a]	16.8[a]	0.3
Total	23.4 ± 0.7	22.2[b]	23.4[ab]	24.6[a]	24.2[ab]	0.3
Hypercholesterolemic						
12:0	5.6 ± 0.4	4.8[b]	4.9[ab]	5.5[a]	5.3[ab]	0.1
14:0	8.7 ± 0.3	7.3[b]	7.5[ab]	8.0[a]	8.0[a]	0.2
16:0	28.9 ± 0.7	24.7[b]	24.7[b]	25.3[ab]	26.9[a]	0.4
trans-18:1	1.0 ± 0.1	1.1	1.1	0.9	1.0	0.1
Total	44.2 ± 1.3	37.8[b]	38.2[b]	39.7[ab]	41.1[a]	0.4
Hypocholesterolemic						
cis-18:1	27.6 ± 1.3	35.6[a]	33.6[a]	29.6[b]	29.4[b]	0.6
18:2	0.6 ± 0.1	0.9[b]	1.5[b]	2.6[a]	1.5[b]	0.1
Total	28.2 ± 1.4	36.4[a]	35.1[ab]	32.2[bc]	30.9[c]	0.4
Others						
14:1	0.9 ± 0.1	0.7	0.6	0.7	0.6	0.1
15:0	0.72 ± 0.06	0.56[b]	0.61[ab]	0.62[ab]	0.63[a]	0.01
16:1	1.4 ± 0.2	1.3[a]	1.1[b]	1.0[b]	1.2[ab]	0.1
17:0	0.56 ± 0.08	0.49[b]	0.54[a]	0.53[a]	0.55[a]	0.01
20:0	0.4 ± 0.1	0.4[b]	0.5[b]	0.6[ab]	0.7[a]	0.1
Total	5.1 ± 0.7	3.6[b]	3.3[c]	3.5[bc]	3.8[a]	0.1
Unsat / saturated[3]	0.46 ± 0.03	0.66[a]	0.61[ab]	0.53[bc]	0.51[c]	0.01

[1] Berner (1993).
[2] Mean ± SE (n = 4).
[3]Ratio of unsaturated fatty acids (total of 14:1, 16:1, *cis*-18:1, *trans*-18:1, and 18:2) to saturated fatty acids (total of 4:0, 6:0, 8:0, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0).
[a, b, c]Means for oil infusion periods with same subscripts do not differ ($P < 0.05$).

Table 3.9. Amount of fatty acid infused and change in yield of fatty acids in milk of Jersey cows in response to abomasal infusion of olive oil, sesame oil, sunflower oil, or fish oil, compared with yield of fatty acids during the preliminary period.

	Oil infusion				
	Olive	Sesame	Sunflower	Fish	SE
Average amounts infused (g/d)					
Cis-18:1	155.0	122.5	113.1	40.4	
18:2	5.3	18.2	56.9	80.2	
Apparent change in yield (g/d)					
MCFA[1]	-43.2	-41.8	-33.8	-72.6	37.7
Cis-18:1[2]	45.1	49.0	18.5	48.2	18.5
18:2[3]	46.7[a]	46.3[a]	31.0[ab]	9.8[b]	5.6

[a, b, c]Means for oil infusion periods with same subscripts do not differ ($P < 0.05$).

[1]Change in MCFA (total of 12:0, 14:0, and 16:0) yield (g) for each 100 g of infused *cis*-18:1 compared with MCFA yield during the control period.

[2]Change in *cis*-18:1 yield (g) for each 100 g of infused *cis*-18:1 compared with *cis*-18:1 yield during the control period.

[3]Change in 18:2 yield (g) for each 100 g of infused 18:2 compared with 18:2 yield during the control period.

Table 3.10. Percentage of “in” responses for milk from Jersey cows infused abomasally with olive oil, sesame oil, sunflower oil, or fish oil.

Treatment	Cow 1	Cow 2	Cow 3	Cow 4
Preliminary	89	100	89	89
Olive	80	50	70	89
Sesame	63	30*	40*	20*
Sunflower	60	90	40*	63
Fish	70	90	75	50

* Milk with "in" responses below 50% are considered unacceptable to the consumers.

CHAPTER 4

MODIFYING MILK FATTY ACID PROFILE OF HOLSTEIN AND JERSEY COWS BY ABOMASAL INFUSION OF CANOLA OIL, OLIVE OIL, OR HIGH-OLEIC SUNFLOWER OIL.

ABSTRACT

The potential for enhancing oleic acid (*cis*-18:1) and linoleic acid (18:2) content of bovine milk was investigated by abomasal infusion of canola oil (61% *cis*-18:1, 21% 18:2), olive oil (72% *cis*-18:1, 11% 18:2), high-oleic sunflower oil (82% *cis*-18:1, 9% 18:2), or distilled water (control). Oils (342 to 371 g/d) or distilled water (400 to 429 g/d) were infused into three Holstein cows and three Jersey cows continuously during the last 5 d of each of four 10-d periods in an Incomplete Block design. All oil infusions increased *cis*-18:1 flow to the duodenum (198 to 296 g/d versus 71 g/d for control), *cis*-18:1 digestibility in intestine (91 to 94% versus 85% in control), and *cis*-18:1 content in milk fat (21 to 25% versus 15% in control). Infusion of canola oil provided a numerically greater flow of 18:2 to the duodenum (76 g/d versus 47 g/d in control), higher intestinal digestibility of 18:2 (92% versus 83% in control), and higher milk content of 18:2 (4.5% versus 2.0% in control). All oil infusions lowered the 14:0 and 16:0 content of milk. Holstein milk contained more *trans*-18:1 and conjugated-linoleic-acid (2.3 and 0.6%, respectively) than Jersey milk (1.6 and 0.4%, respectively). Out of each 100 g of *cis*-18:1 infused abomasally, 39 (Jerseys) to 41 g (Holsteins) were apparently recovered in milk. Milk apparent recovery of 18:2 was 34 (Jerseys) to 42 g (Holsteins) for each 100 g infused. Results indicated that flow of supplemental *cis*-18:1 and 18:2 to the duodenum increased their digestibility in the intestine and apparent recovery in milk. The three oils were equally effective in both breeds for modifying milk fatty acid profile in a manner that would be beneficial for human health.

Key words: oleic acid, linoleic acid, *trans*-18:1, conjugated-linoleic-acid, medium chain fatty acids.

INTRODUCTION

Human health concerns regarding saturated fatty acids in animal food products have indicated a need to alter the fatty acid composition of ruminant tissues (Jenkins et al., 1996). In many countries the incidence of coronary heart disease has been elevated due to milk and butter consumption (Salonen and Vohlonen, 1982). A challenge for milk producers and processors is to partially replace saturated, medium-chain fatty acids (MCFA) and *trans*-18:1 with unsaturated fatty acids. Noakes et al. (1996) reported that the plasma cholesterol levels of individuals who consumed fat-modified milk products were lower compared with those who consumed conventional milk products.

Diets containing protected sunflower seeds were used in most early studies aimed at modifying milk fatty acid profile. The milk produced in these experiments contained increased levels of linoleic acid (18:2) and reduced levels of MCFA (Cook et al, 1970; Nestel et al., 1973; Nestel et al., 1974). However, milk products enriched with 18:2 had decreased shelf life, making them nonviable commercially. It was necessary to add butylated hydroxytoluene as an antioxidant to 18:2-enriched milk. Oleic acid (*cis*-18:1) is more resistant to oxidation than 18:2 (Cadden and Kennelley, 1984).

In our previous experiment (Chapter 3), when olive oil (82% *cis*-18:1) was infused into the abomasum of Jersey cows, 45% of infused *cis*-18:1 and 47% of infused 18:2 were recovered in milk fat. The potential to use dietary *cis*-18:1 to modify the milk fatty acid profile of dairy cows needs further evaluation. Holsteins and Jerseys are the prominent dairy cattle breeds in most counties in the temperate region of the world, but the breeds greatly differ in milk and milk fat production (Ensminger, 1993). The effect of breed on the extent of digestion and absorption of dietary *cis*-18:1 and 18:2 in the intestine and the ability of the mammary system to incorporate the absorbed fatty acids into milk fat also needs to be evaluated.

OBJECTIVES

Canola oil, olive oil, high-oleic sunflower oil, or distilled water were infused into the abomasum of Holstein and Jersey cows, to study the extent of alterations in fatty acid profile of milk fat. The relationships between *cis*-18:1 and 18:2 flow to the duodenum, digestibility in the intestine, and the rate of recovery in milk fat were evaluated to determine the effect of breed on response to treatments.

MATERIALS AND METHODS

Cows and diet

Three Holsteins and three Jerseys in early lactation, each with a duodenal and a rumen cannula, were housed in a tie-stall barn and fed a basal diet (Table 4.1) for ad libitum intake. Feed was provided at 0730 and 1930 h, and orts were weighed at 0700 h daily. The cows were milked at 0700 and 1900 h daily. The cows were allowed to adjust to the diet and the barn environment during an initial 30-d preliminary period. This was followed by four 10-d treatment periods using an Incomplete Block design. Assignment of cows to treatments is shown in Appendix Table 4.

Treatments and infusion procedure

Treatments with varying concentrations of oleic acid (*cis*-18:1) and linoleic acid (18:2; Table 4.2), were canola oil (Canola salad oil, Cargill Inc., Minneapolis, MN), olive oil (Bunicci olive oil, Bunge Foods, Bradley, IL), and high-oleic sunflower oil (TRISUN® 80, SVO Speciality products, Inc., Eastlake, OH). Distilled water was used as the control. Oil (342 to 371 g/d) or distilled water (400 to 429 g/d) was continuously infused to the abomasum of each cow from d 6 through d 10 of each period. A greater amount of oil (compared with 155 to 219 g/d in experiment 1) was infused in the present experiment, anticipating a relatively greater milk production from early lactation Holstein and Jerseys.

Oil was infused using a peristaltic pump (Harvard Apparatus®, South Natick, MA) and Tygon® tubing (0.16 cm i.d. x 0.31 cm o.d., Fisher Scientific Company, Pittsburgh, PA). The tubing was

inserted into the rumen through an opening in the ruminal canula then through the omasal orifice to the abomasum. A 60 mL Nalgene® bottle (4 cm diameter) was connected to the end of the tubing to prevent it from being pulled out of the abomasum during ruminal contractions. The presence of tubing in the abomasum was confirmed on d 6, 8, and 10 of each period by touching the bottle at the end of tubing. Oil or water was placed into a 500 mL plastic bag (Baxter Healthcare Corporation, Deerfield, IL). Bags were weighed then connected to the infusion system at 1900 h. Oil or water from each bag passed through one pump set to deliver approximately 0.25 mL/min. However, actual flow rates varied due to differing viscocities. After each 24 h of infusion, the bag was removed, weighed, and replaced with a newly-filled bag.

Digesta marker

Chromium (Cr)-mordanted fibers were used as the digesta marker in the experiment. The mordanting procedure described by Uden et al. (1980) was used to attach Cr to washed fecal fibers (6 g Cr/100 g fecal fibers) that were collected from non-lactating cows fed orchard-grass hay. Thirty grams of Cr-mordanted fecal fibers were administered daily via the rumen cannula, with 50% of each dose given at 0700 h and 50% at 1900 h through out the experiment.

Sample collection

All sampling was done between 2000 h of d 8 and 2000 h of d 10 of each period. Two forage samples and two samples of each concentrate mixture were collected in each period. Six duodenal digesta samples and six fecal samples were collected from each cow in each period (at 2000 h of d 8, 0400 and 1200 h of d 9, and 0000, 0800 and 1600 h of d 10). Duodenal and fecal samples were stored at -20 ^{0}C. Fecal samples were composited and dried to a constant weight at 60 ^{0}C. Duodenal samples were freeze dried, then composited. All dried samples were ground through a 1 mm screen in a Cyclone mill (UD Corporation, Boulder, Colorado).

A10 mL blood sample was collected from the coccygeal artery at 2000 h on d 12. Plasma was separated from blood by centrifuging at 3,000 x *g* for 15 min, then stored at -20 ^{0}C. Milk samples were collected from each milking on the last 2 d of each period for fat, lactose, protein, and SNF determinations by the Dairy Herd Improvement Association laboratory of Virginia Tech.

Additional samples from each cow on d 10 of each period were centrifuged at 11,000 x *g* for 60 min to extract the milk fat layer for fatty acid analysis.

Chemical analysis

Feed, duodenal digesta and feces were analyzed for ether extract (AOAC, 1990), crude protein (AOAC, 1990) and organic matter (AOAC, 1990). The Cr concentration of duodenal and fecal samples was determined using atomic absorption spectrophotometry following acid digestion described by Scandell (1950). Dry matter flow through the digestive tract was calculated using the method described by Armentano and Russell (1985).

Fatty acid concentrations in feed, duodenal digesta, feces, milk fat and plasma were determined following transesterification (Outen et al., 1976). Undecenoic acid (Nu-Check Prep, Elysian, MN) was used as the internal standard. Samples were injected by auto-sampler into a Hewlett Packard 5890A gas chromatograph equipped with a flame ionization detector (Hewlett Packard, Co., Sunnyvale, CA) using procedures described by Wonsil (1994). Samples were split onto a glass capillary column (Supelco SP 2380, Supelco, Inc., Bellefonte, PA). The split ratios were 15:1 for feed, duodenal digesta, fecal and plasma samples and 80:1 for oils and milk fat samples.
A temperature program initiated runs at 60 ^{o}C, held for 3 min, warmed to 205 ^{o}C at 5 ^{o}C /min, held for 12 min, warmed to 215 ^{o}C at 5 ^{o}C /min, held for 5 min, warmed to 220 ^{o}C at 5 ^{o}C /min, then held for 2 min.

Statistical analysis

Effects of treatments, breeds, treatment x breed interactions, and period were analyzed using General Linear Model (SAS, 1985). The model was:

$$Y_{ijk} = \mu + B_i + P_j + T_k + (BT)_{ijk} + E_{(ijk)}$$

Where Y_{ijk} = dependent variable

μ = overall mean

B_i = effect of breed (i = 1, and 2 [three cows nested under each breed])

P_j = effect of period (j = 1, 2, 3, and 4)

T_k = effect of treatment (k = 1, 2, 3, and 4)

$(\beta T)_{ij}$ = effect of breed x treatment interaction

$E_{(ijk)}$ = residual error

Treatment means were compared using Turkey's pair-wise comparison procedure (SAS, 1985), and were considered significantly different at $P < 0.05$. An ANOVA example is shown in Appendix Table 4.

RESULTS AND DISCUSSION

Feed intake

Daily dry matter intake (DMI) by Holsteins was significantly higher (22.3 kg/d) than that by Jerseys (16.0 kg/d). However, DMI did not vary due to treatment or treatment x breed interaction. This result is contrary to the results of Gaynor et al., (1994) who reported a decline in DMI from 24.2 to 22.8 kg/d when *cis* or *trans* fatty acids were abomasally infused into Holstein cows at 750 g/d. Drackley et al. (1992) also reported a decline in DMI from 24.5 to 22.7 kg/d for Holstein cows when 450 g of unsaturated fatty acids were infused abomasally. Palmquist (1990) pointed out that when fed with supplemental fat, cows reduce the feed intake to regulate their blood fatty acid concentration. The amount of oil infused in the present study (340 to 370 g/d) apparently was not large enough to significantly affect feed intake.

Milk yield, milk composition and milk component yields

Average daily milk yield (Table 4.3) of Holsteins was significantly higher (41.8 kg/d) than that of Jerseys (23.3 kg/d). However, the daily milk yield of each breed did not differ due to treatment or treatment x breed interaction. This might be a reflection of unchanged DMI of cows in response to treatments. Holstein cows produced milk with lower fat, greater lactose, lower protein, and lower solids-not-fat (SNF) percentages compared with Jerseys. However, daily yields of fat, lactose, protein and SNF were greater for Holsteins due to greater daily milk yields. Milk composition and component yields did not differ due to treatment or breed x treatment interactions.

Intake, flow and digestibility of organic matter, crude protein and ether extract

Due to greater daily DMI, Holstein cows had higher organic matter (OM) intakes (20.9 kg/d) and tended ($P < 0.09$) to have higher flows to the duodenum (15.7 kg/d) compared with those of Jerseys (15.3 and 11.1 kg/d, respectively) (Table 4.4). However, Jersey cows tended ($P < 0.06$) to digest OM more efficiently (58.8%) than did Holsteins (52.8%). Compared with control, oil infusions did not alter the intake, flow or digestibility of OM. Patterns of intake, digestibility and flow of crude protein (CP) and ether extract (EE) for each breed were similar to those of OM. However, the difference between duodenal EE flow for the control (0.66 kg EE flow per day) and the average for all oil infusions (0.93 kg EE) was only 0.27 kg EE, which was less than the average amount of oil infused (0.36 kg).

Flow and digestibility of fatty acids

The flows of medium-chain fatty acids (MCFA) [lauric acid (12:0), myristic acid (14:0) and palmitic acid (16:0)] to the duodenum were greater in Holstein cows (total of 179 g/d) than in Jersey cows (136 g/d; Table 4.5) due to higher DMI. In the intestine, 12:0, 14:0 and 16:0 were digested at average rates of 68, 65, and 77%, respectively, and did not vary due to treatment or breed x treatment interactions. Jersey cows apparently digested 14:0 more efficiently than Holsteins.

The flows to the abomasum and digestibility in intestine of stearic acid (18:0), oleic acid (*cis*-18:1) and linoleic acid (18:2) did not differ due to breed or breed x treatment interactions. The flow of *trans* vaccenic acid (*trans*-18:1) to the duodenum was higher in Holstein cows (54.4 g/d) than in Jersey cows (37.3 g/d). Again, this may be due to higher DMI providing more UFA for microbial biohydrogenation in the rumen; thus, providing more *trans*-18:1 flow out of the rumen. The treatments did not impact flow to the duodenum or intestinal digestibility of 18:0 and *trans*-18:1, because these fatty acids were derived primarily from biohydrogenation of dietary UFA, and not from abomasal infusion.

Flow and digestion of *cis*-18:1 were influenced by treatment. Infusion of high-oleic sunflower oil (82% *cis*-18:1) or olive oil (72% *cis*-18:1; Table 4.2) provided the greater flows of *cis*-18:1 to the

duodenum (296 and 212 g/d, respectively versus 71 g/d in control). Oil infusions enhanced *cis*-18:1 digestibility from 85% (control) to an average of 93%. All oil infusions tended ($P < 0.08$) to enhance the flow of 18:2 to the duodenum. The digestibility of 18:2 was greater under infusion of canola oil, which contained 21% 18:2 (Table 4.2), compared with control.

In the previous experiment, (Chapter 3), fish oil, olive oil, sesame oil or sunflower oil infusion also improved *cis*-18:1 and 18:2 digestibility in the intestine when compared with the preliminary period. Klusmeyer and Clark (1991) reported that increased flow of *cis*-18:1 and 18:2 to the duodenum enhances the digestibility of these fatty acids in intestine. They reported 90.3, 89.6, 82.6 and 79.6% digestibility of *cis*-18:1 in intestine when flows to the duodenum were 290, 235, 102, and 94 g/d, respectively. They further reported 90, 82.6, and 79.6% intestinal digestibility of 18:2 when flows of 18:2 to the duodenum were 63, 43, and 41 g/d, respectively. Palmquist (1991) demonstrated a linear decline in true digestibility of total fatty acids from 100% (at 1% fat in the diet) to 78% (at 8% fat in the diet), but the total fatty acid digestibility did not differ due to source of fat (animal-vegetable blend, Ca-soap, hydrogenated animal fat, saturated fatty acids, tallow, or basal diet). Elliott et al. (1996) reported a decline in total fatty acid digestibility from 81.3% for the control diet to an average of 71% for diets supplemented with 5 to 6.1% of fat (calcium salts of long chain fatty acids, prilled fatty acids, or prilled or flaked hydrogenated palm fatty acid distillate). Thus, digestibility of UFA increase as UFA flow increases; whereas, digestibility of saturated fatty acids decreases as their flow to the intestine increases.

Fatty acid concentration in blood plasma

Concentrations of all fatty acids in blood plasma (Table 4.6) of Holstein cows were higher than those of Jersey cows. However, fatty acid concentrations did not differ due to treatments or treatment x breed interactions.

In the previous experiment (Chapter 3), infusion of olive oil caused an increase in plasma *cis*-18:1 concentration (106 μg/mL, 15% of total fatty acids) compared with the preliminary period (75 μg/mL, 8% of total fatty acids). Gaynor et al. (1993) reported plasma *cis*-18:1 content increased from 6.3% of total fatty acids to 11.4% when cows were abomasally infused daily with 750 g of a

mixture of *cis*-18:1-rich seed oils. Wonsil (1990) reported plasma 18:1 concentrations increased from 5.8% (control) to 8.3, and 8.2% when the cows were fed diets supplemented with *cis*-18:1 in the form of tallow or tallow coated with casein and corn syrup solids at 3% of DM. Plasma concentrations of 18:2 in Holstein cows fed a control diet, or diets supplemented with soybean oil or butylsoyamide (rumen protected form of soybean oil) at 3.5% of the diet DM were 54.3, 52 and 59%, respectively (Jenkins et al., 1995).

Milk fatty acid composition

Neutral fatty acids

The fatty acids which have neither cholesterol-raising nor cholesterol-lowering effects are classified as neutral fatty acids (Berner, 1993). The major neutral fatty acids in milk fat are short-chain fatty acids (SCFA) (butyric acid [4:0], caproic acid [6:0], caprylic acid [8:0], and capric acid [10:0]) and stearic acid (18:0). Milk from Holsteins had higher 4:0 content and lower 10:0 content than that from Jerseys (Table 4.7). Milk 6:0, 8:0, and 18:0 content, however, did not vary due to breed.

The total neutral fatty acid content of milk during oil infusions (23.2 to 23.5%) did not deviate significantly from that of the control (23.5%). These results agree with those of the previous study in which neutral fatty acids were 23.6% during infusion and 23.4% during the preliminary period (Chapter 3). Drackley et al. (1992) reported no difference in milk SCFA or 18:0 content when Holstein cows were abomasally infused daily with 450 g of mostly unsaturated fatty acids. According to Wonsil et al. (1993), feeding cows diets with 3% soybean oil did not show changes in milk 4:0, 6:0, and 8:0, but 10:0 content was lower (1.9% compared with 2.8% in control). Ashes et al. (1992) found SCFA were not affected when cows were fed protected canola seeds at 6.5% of the diet DM, but 18:0 increased from 7.1% (control) to 9.2%.

Cholesterol raising fatty acids

According to Berner, (1993) MCFA are considered cholesterol-raising (hypercholesterolemic) fatty acids. Milk 12:0 content was higher in Holsteins than in Jerseys, whereas 14:0 or 16:0 content did not vary due to breed or breed x treatment interaction (Table 4.7). MCFA content of

milk was lowered by all oil infusions. In addition to MCFA, *trans*-18:1 is also considered hypercholesterolemic (Judd et al., 1994 and Keys et al., 1986). Treatment and breed x treatment interaction did not influence *trans*-18:1 content of milk, but *trans*-18:1 content was greater for Holstein cows than Jersey cows. This might have been due to higher DMI providing more UFA substrate for biohydrogenation in the rumen. Thus, total hypercholesterolemic fatty acid content of milk was lowered primarily due to the decrease in MCFA content. The MCFA content of milk in the present experiment (43.3 to 53.3%) was greater than the content observed (37.8 to 44.2%) in the previous experiment.

In the previous experiment (Chapter 3), infusion of olive oil lowered milk MCFA content to 37.8% from 44.2% in the preliminary period. Gaynor et al. (1994) observed that abomasal infusion of *cis*-18:1 at 750 g/d decreased MCFA to 39.6% from 52.5% (control). According to Drackley et al. (1992), the MCFA content of milk from cows infused abomasally with 450 g of unsaturated fatty acids daily was 34.9% compared with 46% for the control. When cows were fed protected canola seeds at 6.5% of the diet DM, Ashes et al. (1992) observed 33% MCFA compared with 42.8% (control). Palmquist et al. (1993) hypothesized that supplemental dietary fat depresses the rate of de novo synthesis of MCFA in the mammary gland.

Cholesterol lowering fatty acids

All oil infusions increased the *cis*-18:1 content of milk (Table 4.7). No difference recorded in milk *cis*-18:1 content among treatments. The range in *cis*-18:1 content of milk recorded during oil infusions in the present experiment (21.4% to 25.3%) was relatively smaller than the range reported in the previous experiment (29.4 to 35.6%). This might be due to the higher MCFA content of milk in the present experiment compared with that observed in the previous experiment. The greater milk yield of cows in early lactation in this experiment, compared with cows in late lactation in the previous experiment, apparently was associated with greater rates of de novo MCFA synthesis in the mammary gland.

The increase in *cis*-18:1 content of milk recorded during oil infusion in this study and the previous study (35.6% *cis*-18:1 during olive oil infusion versus 27.6% in the preliminary period) agree with

the findings of other workers. Gaynor et al. (1994) reported 34.7% *cis*-18:1 in milk when *cis*-18:1 was infused abomasally at 750 g/d. Klusmeyer and Clark (1991) reported 27.1% *cis*-18:1 in milk when lactating cows were fed with calcium salts of long chain fatty acids (4% of diet DM), compared with 21.3% for cows fed a control diet. The long chain fatty acid mixture used in their experiment had a moderate *cis*-18:1 content, and accordingly the incremental increase in *cis*-18:1 content of milk was lower than that indicated in Table 4.7 or by Gaynor et al. (1994). Ashes et al. (1992) reported 29.2% *cis*-18:1 in milk when cows were fed 6.5% protected canola seed diet compared with 23.8% (control).

Infusion of canola oil, which was the richest 18:2 source (Table 4.2) and digested in the intestine at a higher efficiency (Table 4.5), resulted in a greater 18:2 content in milk (4.5%) compared with control (2.0%). In our previous experiment, infusion of sunflower oil (with a lower *cis*-18:1 content and higher 18:2 content than the oil used in this experiment) elevated milk 18:2 content to the highest level (2.6%) compared with the other oil infusions and preliminary period (0.6 to 1.5%). Jenkins et al. (1995) reported that milk 18:2 content could be increased from 3.6% to 4.8 and 6.3% when soybean oil or butylsoyamide was fed to dairy cows at 3.5 of diet DM. Drackley et al. (1992) reported that milk 18:2 increased from 2.3 to 13.3% when an unsaturated fatty acid mixture was abomasally infused at 450 g/d. The amount of 18:2 fed (Jenkins et al., 1995) or infused (Drackley et al., 1992) was higher than the amount infused in the present experiment. Additionally, the above reports did not account for several fatty acids, including saturated or unsaturated odd chain fatty acids, in their calculations. The present study accounted for these fatty acids (as shown in Table 4.7) in the calculation of total fatty acids. Thus, the higher value for total fatty acids caused the percentage of 18:2 to be lower than those reported previously.

Other fatty acids

The concentrations of 14:1, 15:0, 16:1, 17:0, and 17:1, in general, were lowered due to oil infusions; whereas, 20:0 concentration was elevated due to oil infusions (Table 4.7). Other long chain fatty acids (20:3, 20:4, 22:1 and 22:5) did not differ due to treatment, but Jersey cows had higher concentrations of 22:1, and 22:5 compared with Holsteins.

Conjugated linoleic acid (CLA) is an anti-carcinogenic fatty acid found in ruminant milk and adipose tissue (Ha et al., 1987 and 1989). CLA is synthesized in the rumen due to isomerization and biohydroganation of dietary long-chain fatty acids (Ha et al., 1987). In the present study, the milk CLA content did not differ due to treatment or breed x treatment interactions. Holstein milk however, contained more CLA (0.6%) than Jersey milk (0.4%). This might be due to the higher DMI of Holsteins providing more dietary UFA as substrate for biohydrogenation in the rumen.

Milk fatty acid composition - summary

According to the results shown in Table 4.7, all oil infusions resulted in a greater cholesterol-lowering fatty acid content (average of 27.8% versus 17.6% for control) and lower cholesterol-raising fatty acid content (average of 44.2% versus 53.3% for control). Canola oil and high-oleic sunflower oil infusions significantly improved the ratio of unsaturated to saturated fatty acids in milk (0.50 versus 0.29 for control).

Out of each 100 g of *cis*-18:1 infused into the abomasum, 41 g in Holsteins and 39 g in Jerseys were recovered in milk fat (Table 4.8). In addition, for each 100 g of infused *cis*-18:1, the daily yield of MCFA in milk was reduced by 8 g in Holstein cows and 34 g in Jersey cows. In the previous experiment (Chapter 3), abomasal infusion of fish oil, olive oil, sesame oil or sunflower oil in Jersey cows reduced MCFA yield by approximately 33 g for each 100 g of infused *cis*-18:1 when compared with the preliminary period. In our previous experiment, milk *cis*-18:1 yield was increased by 45 to 49 g, and MCFA yield was lowered by 42 to 43 g for each 100 g of *cis*-18:1 infused as olive oil or sesame oil.

In the present study, for every 100 g of 18:2 infused, the yield of 18:2 in milk of Holstein cows increased by 42 g; whereas the increase in 18:2 yield was 34 g for Jerseys (Table 4.8). In the previous experiment (Chapter 3), 18:2 yield in milk was increased by 46 to 47 g per 100 g of 18:2 infused as olive oil or sesame oil.

IMPLICATIONS

The study revealed that nearly 39 to 41% of *cis*-18:1 and 34 to 42% of 18:2 flow to the duodenum is apparently recovered in milk fat. All three oil infusions similarly altered milk fatty acid profile in a manner that would be more beneficial for human health. The potential of other oils for enhancing hypocholesterolemic properties of bovine milk also should be evaluated. The seed oil from canola and soybean that have been bred or genetically engineered to contain a high *cis*-18:1 content (Cline and Re, 1997) may be good choices. A practical method to achieve greater flow of *cis*-18:1 to the duodenum may benefit the dairy industry in terms of more desirable milk and milk products for health-conscious consumers.

Table 4.1. Dietary ingredients, chemical composition, and fatty acid content of the basal diet

Ingredient, % of dry matter	
Corn silage	23.4
Alfalfa haylage	27.6
Orchard grass hay, chopped	3.2
Corn grains	28.1
Dried distiller's grains	16.2
Mineral/vitamin premix[1]	1.4
Chemical composition, % of dry matter	
Organic matter	94.5
Crude protein	15.1
Ether extract	2.9
Fatty acids, μg/g dry matter	
12:0	61.4
14:0	246.9
16:0	5685.3
18:0	948.8
cis-18:1	5510.7
trans-18:1	114.6
18:2	12849.7
18:3	2979.5
20:0	279.8
20:3	256.6
20:5	123.8

[1] Contained 6.5% P, 16.0% Ca, 4.3% NaCl, 2.2% Mg, 3.5% K, 3.2% S, 0.11% Mn, 0.13% Zn, 0.03% Fe, 0.13% Cu, 0.002% I, 0.0003% Co, 0.0005% Se, 110,000 IU vitamin A/kg, 44,000 IU vitamin D_3/kg, and 1,350 IU vitamin E/kg.

Table 4.2. Fatty acid composition of oils (g/100 g total fatty acids) infused into the abomasum

Fatty acid	canola oil	olive oil	high-oleic sunflower oil
16:0	4.4	11.9	3.1
16:1	0.3	0.8	-
18:0	2.2	3.0	4.4
cis-18:1	60.8	72.0	81.5
trans-18:1	0.4	-	-
18:2	21.0	10.8	8.8
18:3	0.6	0.6	0.4
20:0	9.0	0.4	0.3
20:3	0.4	0.2	1.2
20:4	0.5	-	-
22:1	0.3	0.1	0.4
22:4	0.2	-	-

Table 4.3. Milk yield, milk composition and milk component yields of Holstein and Jersey cows in response to abomasal infusion of distilled water (control), canola oil, olive oil, or high-oleic sunflower oil (HO-Sun).

	Treatment averages				Breed averages			P < [1]		
	Control	Canola	Olive	HO-Sun	Holstein	Jersey	SE[2]	Treatment	Breed	T*Breed
Milk, kg/d	32.9	31.9	33.3	32.2	41.8	23.3	2.2	0.97	0.01	0.95
Fat, %	4.0	4.6	4.3	4.3	3.5	5.1	0.2	0.23	0.01	0.99
Lactose, %	4.8	4.7	4.8	4.8	4.9	4.7	0.1	0.81	0.01	0.91
Protein, %	3.2	3.2	3.2	3.2	2.8	3.5	0.1	0.98	0.01	0.34
SNF, %	8.7	8.6	8.7	8.7	8.4	8.9	0.1	0.92	0.01	0.85
Fat, kg/d	1.3	1.4	1.4	1.3	1.5	1.2	0.1	0.84	0.02	0.96
Lactose, kg/d	1.6	1.5	1.6	1.6	2.0	1.1	0.1	0.96	0.01	0.95
Protein, kg/d	1.0	1.0	1.0	1.0	1.2	0.8	0.1	0.96	0.01	0.81
SNF, kg/d	2.8	2.7	2.9	2.8	3.5	2.1	0.2	0.97	0.01	0.94

[1] Effects of treatment (T), breed, or T x breed interactions are considered significant when P < 0.05.

[2] Treatment Standard Error.

Table 4.4. Intake, flow to the duodenum, and apparent digestibility in intestine of organic matter (OM), crude protein (CP), and ether extract (EE) in Holstein and Jersey cows in response to abomasal infusion of distilled water (control), canola oil, olive oil, or high-oleic sunflower oil (HO-Sun).

	Treatment averages				Breed averages			P <[1]		
	Control	Canola	Olive	HO-Sun	Holstein	Jersey	SE[2]	Treatment	Breed	T*Breed
Intake, kg/d										
OM	18.1	17.2	18.4	18.6	20.9	15.3	1.3	0.91	0.01	0.46
CP	2.9	2.8	3.0	3.0	3.4	2.5	0.2	0.89	0.01	0.45
EE	0.57	0.54	0.57	0.58	0.65	0.48	0.04	0.91	0.01	0.45
Oil infused, kg/d	-	0.37	0.34	0.36						
Flow to the duodenum, kg/d										
OM	12.4	12.9	14.4	13.9	15.7	11.1	1.6	0.79	0.03	0.49
CP	2.2	2.4	2.6	2.4	2.8	2.0	0.2	0.90	0.01	0.46
EE	0.66	0.95	0.92	0.93	0.92	0.81	0.08	0.65	0.01	0.44
Apparent digestibility in intestine, (%)										
OM	50.1	57.9	58.0	57.4	52.8	58.8	3.0	0.25	0.08	0.73
CP	39.8	48.7	52.7	49.1	44.3	50.8	5.3	0.43	0.33	0.89
EE	79.6	85.0	85.0	82.6	81.5	84.6	2.6	0.38	0.18	0.86

[1] Effects of treatment (T), breed, or T x breed interactions are considered significant when $P < 0.05$.

[2] Treatment Standard Error.

Table 4.5. Flow to the duodenum and intestinal digestibility of fatty acids in Holstein and Jersey cows in response to abomasal infusion of distilled water (control), canola oil, olive oil, or high-oleic sunflower oil (HO-Sun).

	Treatment averages				Breed averages			P < [1]		
	Control	Canola	Olive	HO-Sun	Holstein	Jersey	SE[2]	Treatment	Breed	T*Breed
12:0										
Flow to the duodenum, g/d	2.5	3.0	2.9	2.6	3.2	2.2	0.3	0.64	0.01	0.56
Intestinal digestibility, (%)	64.6	73.7	65.6	67.4	66.3	69.4	2.6	0.10	0.25	0.36
14:0										
Flow to the duodenum, g/d	9.7	11.4	10.0	9.6	11.9	8.5	1.1	0.58	0.02	0.34
Intestinal digestibility, (%)	62.4	71.2	64.8	61.9	62.1	68.0	2.8	0.11	0.05	0.15
16:0										
Flow to the duodenum, g/d	126.7	152.9	163.8	135.1	163.9	125.3	14.3	0.26	0.02	0.39
Intestinal digestibility, (%)	75.6	80.0	77.2	77.1	76.2	78.7	2.0	0.50	0.23	0.22
18:0										
Flow to the duodenum, g/d	324.3	427.2	361.8	343.3	403.4	324.9	42.2	0.36	0.08	0.82
Intestinal digestibility, (%)	76.2	77.6	74.2	78.1	79.1	73.9	3.7	0.86	0.18	0.41
cis-18:1										
Flow to the duodenum, g/d	70.7[b]	198.0[ab]	212.3[a]	296.3[a]	193.8	194.9	33.7	0.01	0.97	0.12
Intestinal digestibility, (%)	84.7[b]	92.4[a]	91.1[a]	94.4[a]	90.0	91.4	1.6	0.01	0.39	0.15
trans-18:1										
Flow to the duodenum, g/d	46.8	50.1	43.8	42.7	54.4	37.3	7.8	0.91	0.04	0.94
Intestinal digestibility, (%)	83.2	78.8	82.5	80.4	82.6	79.8	3.7	0.83	0.46	0.56
18:2										
Flow to the duodenum, g/d	46.7	75.8	69.6	76.9	74.2	60.3	8.6	0.08	0.13	0.12
Intestinal digestibility, (%)	82.6[b]	91.5[a]	88.6[ab]	90.3[ab]	87.4	89.2	2.1	0.04	0.41	0.68

[1] Effects of treatment (T), breed, or T x breed interactions are considered significant when P < 0.05.
[2] Treatment Standard Error.

Table 4.6. Fatty acid composition (μg/mL) of arterial (coccegyal) blood plasma of Holstein and Jersey cows in response to abomasal infusion of distilled water (control), canola oil, olive oil, or high-oleic sunflower oil (HO-Sun).

	Treatment averages				Breed averages			P <[1]		
	Control	Canola	Olive	HO-Sun	Holstein	Jersey	SE[2]	Treatment	Breed	T*Breed
12:0	0.4	0.4	0.4	0.6	0.6	0.3	0.3	0.86	0.01	0.87
14:0	1.8	2.0	2.0	1.6	2.1	1.6	0.2	0.37	0.04	0.52
16:0	98.8	101.8	91.7	82.5	107.0	80.5	8.3	0.38	0.01	0.93
18:0	126.3	137.3	121.6	115.4	148.4	101.9	12.3	0.64	0.01	0.81
cis-18:1	83.6	101.2	101.4	117.5	111.6	90.3	10.5	0.21	0.05	0.78
trans-8:1	8.6	11.0	9.0	6.2	11.2	6.2	1.6	0.29	0.01	0.89
18:2	780.2	902.6	788.6	694.0	956.7	626.0	86.1	0.40	0.01	0.57

[1] Effects of treatment (T), breed, or T x breed interactions are considered significant when $P < 0.05$.

[2] Treatment Standard Error.

Table 4.7. Fatty acid composition (g/100 g fatty acids) of milk from Holstein and Jersey cows in response to abomasal infusion of distilled water (control), canola oil, olive oil, or high-oleic sunflower oil (HO-Sun) listed according to their influence on plasma cholesterol when included in the diet of humans[1].

	Treatment averages				Breed averages			P <[2]		
	Control	Canola	Olive	HO-Sun	Holstein	Jersey	SE[3]	Treatment	Breed	T*Breed
Neutral[1]										
4:0	3.5	4.0	3.8	3.8	4.3	2.4	0.3	0.62	0.01	0.46
6:0	3.7	3.6	3.6	3.5	3.6	3.6	0.1	0.55	0.82	0.83
8:0	2.0	1.9	1.8	1.8	1.8	1.9	0.1	0.44	0.31	0.63
10:0	5.7	5.0	4.8	5.0	4.8	5.5	0.3	0.14	0.03	0.51
18:0	8.5	9.1	9.2	9.3	9.0	9.1	0.4	0.61	0.92	0.18
Total	23.5	23.5	23.2	23.5	23.5	23.3	0.6	0.95	0.78	0.59
Hypercholesterolemic[1]										
12:0	4.9[a]	3.9[b]	3.9[b]	4.0[ab]	3.9	4.4	0.2	0.03	0.03	0.34
14:0	14.6[a]	12.3[b]	12.4[b]	12.3[b]	12.7	13.1	0.4	0.01	0.25	0.57
16:0	32.0[a]	25.8[b]	27.1[b]	25.1[b]	26.5	28.5	1.2	0.01	0.12	0.86
trans-18:1	1.9	2.1	2.0	1.9	2.3	1.6	0.2	0.95	0.01	0.26
Total	53.3[a]	44.1[b]	45.3[b]	43.3[b]	45.3	47.6	1.5	0.01	0.13	0.69
Hypocholesterolemic[1]										
cis-18:1	15.1[b]	21.4[a]	23.0[a]	25.3[a]	21.6	20.8	1.3	0.01	0.54	0.73
18:2	2.0[b]	4.5[a]	3.1[ab]	3.0[ab]	3.4	2.9	0.4	0.01	0.29	0.45
18:3	0.6[b]	2.0[a]	0.7[b]	0.6[b]	1.1	0.8	0.2	0.01	0.19	0.63
Total	17.6[b]	27.9[a]	26.7[a]	28.8[a]	26.1	24.5	1.8	0.01	0.23	0.81

(Table 4.7 continues on the next page)

Table 4.7 (Continued).

	Treatment averages				Breed averages			P <[2]		
	Control	Canola	Olive	HO-Sun	Holstein	Jersey	SE[3]	Treatment	Breed	T*Breed
Others										
14:1	1.7[a]	1.1[b]	1.2[b]	1.1[b]	1.4	1.6	0.1	0.01	0.13	0.47
15:0	1.6[a]	1.3[b]	1.3[b]	1.3[b]	1.4	1.3	0.1	0.01	0.06	0.81
16:1	0.40[a]	0.31[b]	0.35[ab]	0.35[ab]	0.40	0.31	0.02	0.02	0.01	0.84
17:0	0.60[a]	0.51[b]	0.57[ab]	0.53[b]	0.58	0.52	0.01	0.01	0.01	0.49
17:1	0.3	0.2	0.2	0.2	0.2	0.2	0.1	0.03	0.30	0.60
CLA[4]	0.5	0.5	0.5	0.4	0.6	0.4	0.1	0.97	0.04	0.58
20:0	0.20[b]	0.26[a]	0.25[a]	0.23[ab]	0.23	0.24	0.01	0.01	0.36	0.23
20:3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.18	0.01	0.84
20:4	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.79	0.15	0.97
22:1	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.10	0.01	0.03
22:5	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.54	0.03	0.89
Total	7.5	6.6	6.8	6.3	7.4	6.2	0.4	0.24	0.01	0.28
Unsat / saturated[5]	0.29[b]	0.49[a]	0.46[ab]	0.50[a]	0.46	0.41	0.1	0.01	0.23	0.91

[1] Berner (1993).

[2] Effects of treatment (T), breed, or T x breed interactions are considered significant when $P < 0.05$.

[3] Treatment Standard Error.

[4] Conjugated linoleic acid (*cis*-9-*trans*-11- linoleic acid).

[5] Ratio of unsaturated fatty acids (total of 14:1, 16:1, 17:1, *cis*-18:1, *trans*-18:1, 18:2, 18:3, CLA, 20:3, 20:4, 22:1, and 22:5) to saturated fatty acids (total of 4:0, 6:0, 8:0, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0).

Table 4.8. Amount of fatty acids infused and apparent change in yield of fatty acids in milk of Holstein and Jersey cows in response to abomasal infusion of canola oil, olive oil, or high-oleic sunflower oil (HO-Sun) compared with yield of fatty acids during the control period.

	Treatment averages				Breed averages			P <[1]		
	Control	Canola	Olive	HO-Sun	Holstein	Jersey	SE[2]	Treatment	Breed	T*Breed
Average amounts of fatty acid infused (g/d)										
Cis-18:1	-	222.9	247.6	294.0						
18:2	-	76.8	37.1	31.8						
Apparent change in milk yield (g/d)										
MCFA[3]	-	-22.1	-15.9	-24.7	-7.5	-34.2	6.0	0.58	0.01	0.54
Cis-18:1 yield[4]	-	38.7	40.7	40.1	41.2	38.5	8.3	0.98	0.78	0.85
18:2 yield[5]	-	40.0	37.2	38.0	42.3	34.4	7.8	0.97	0.40	0.20

[1] Effects of treatment (T), breed, or T x breed interactions are considered significant when $P < 0.05$.

[2] Treatment Standard Error.

[3] Change in MCFA (total of 12:0, 14:0, and 16:0) yield (g) for each 100 g of infused *cis*-18:1 compared with MCFA yield during the control period.

[4] Change in *cis*-18:1 yield (g) for each 100 g of infused *cis*-18:1 compared with *cis*-18:1 yield during the control period.

[5] Change in 18:2 yield (g) for each 100 g of infused 18:2 compared with 18:2 yield during the control period.

CHAPTER 5

MODIFYING MILK FATTY ACID PROFILE BY FEEDING DIETS SUPPLEMENTED WITH CANOLA OIL AND (OR) SOYBEAN OIL TO JERSEY COWS

ABSTRACT

In previous experiments, when unsaturated fatty acid (UFA)-rich seed oils were abomasally infused into Jersey cows, 39 to 49% of infused oleic acid (*cis*-18:1) and 31 to 47% of infused linoleic acid (18:2) were apparently recovered in milk fat. The present study was conducted to investigate the changes in *cis*-18:1, 18:2, and MCFA content of milk when UFA-rich oils were supplemented in the diet. Twenty-four Jersey cows in mid lactation were fed a basal diet [Control] or the basal diet with 3.5% high-oleic canola oil (74% *cis*-18:1, 20% 18:2), 3.5% soybean oil (14% *cis*-18:1, 56% 18:2), or 1.75% high-oleic canola oil plus 1.75% soybean oil for 5 wk using a Complete Randomized Design with repeated measurements. Milk MCFA content was reduced, and the concentrations of 18:0, *cis*-18:1, 18:2, and 18:3 were elevated due to dietary oil supplementation. Additionally, canola oil supplementation elevated CLA content of milk whereas the other two supplementations raised both CLA and *trans*-18:1 contents. For each 100 g of *cis*-18:1 added to the diet by canola oil supplementation, the yield of *cis*-18:1 in milk increased by approximately 21 g/d (approximately half of the potential recovery). For each 100 g of 18:2 provided to the diet by soybean oil supplementation, 18:2 yield in milk increased by approximately 3 g/d (approximately one-tenth of the potential recovery). Thus, to further enhance *cis*-18:1 and 18:2 content of milk by feeding UFA-rich oils, future research should focus on methods to protect UFA from biohydrogenation in the rumen.

Key words: Unsaturated fatty acids, medium chain fatty acids, *trans*-fatty acids, conjugated linoleic acid.

INTRODUCTION

The concentration of low-density-lipoprotein (LDL)-cholesterol in plasma, which is a risk factor for coronary heart disease, is likely to be mediated by the fatty acid composition of dietary ingredients (Ahrens et al., 1957; American Heart Association, 1990). Medium-chain fatty acids (MCFA), (lauric acid [12:0], myristic acid [14:0] and palmitic acid [16:0]), and elaidic acid (*trans*-18:1) are generally considered to be among the factors that elevate serum total and LDL cholesterol (Keys et al., 1965; Hegsted et al., 1965). Unsaturated fatty acids (UFA), mainly oleic acid (*cis*-18:1), linoleic acid (18:2) and linolenic acid (18:3), are considered cholesterol-lowering fatty acids. Milk fat has been criticized due to its relatively high MCFA content and low UFA content, and recommendations to modify milk fatty acid profile have been discussed (Berner, 1993; Jensen et al., 1991).

Microbial biohydrogenation in the rumen minimizes flow of dietary UFA to the intestine. A number of studies revealed that fat sources could be fed in protected forms to reduce the microbial influence. Feeding fat coated with formaldehyde-treated-casein to dairy and beef cattle substantially reduced the 16:0 content and increased the 18:2 content of milk and beef fats (Cook et al., 1970). Ashes et al. (1992) enhanced the oleic acid content of milk by including formaldehyde-protected canola seeds in the diet. Feeding soybean oil in the form of acyl amides enhanced milk linoleic acid content (Jenkins, 1995). When humans consumed milk and milk products with higher UFA and lower saturated fatty acids, they had lower plasma total cholesterol compared with those who consumed conventional milk (Noakes et al., 1996).

In our previous studies where UFA-rich seed oils were infused into the abomasum, it was shown that extra flows of *cis*-18:1 and 18:2 to the abomasum favorably alters the milk fatty acid profile. Holstein and Jersey cows could apparently incorporate nearly 39 to 49% of infused *cis*-18:1 into milk fat. The incorporation rate for 18:2 was approximately 42% in Holsteins and 31 to 34% in Jerseys. In addition, for each 100 g of infused *cis*-18:1, milk MCFA yield was lowered by 8 g in Holsteins and by 34 to 43 g in Jerseys (Chapters 3 and 4).

OBJECTIVES

Diets supplemented with high-oleic canola oil and (or) soybean oil were fed to Jersey cows to investigate the extent of recovery of supplementatal *cis*-18:1 and 18:2 in milk fat. The findings were compared with the previous experiments to evaluate the extent of biohydrogenation of supplemental, unprotected oils in dairy cattle diets.

MATERIALS AND METHODS

Diets and cows

Twenty-four Jersey cows in mid lactation were housed in a free-stall facility equipped with Calan® doors for determination of individual feed intake. The cows were fed for ad libitum intake at 1700 h daily, and orts were removed and weighed at 1630 h the following day. The cows were milked at 0100 and 1300 h daily.

Treatments and experimental design

High-oleic canola oil (Intermountain Canola®, Clear Valley 75, Idaho Falls, ID) and Soybean oil (Monarch Regency, PYA/Monarch Inc., Greensville, SC) were used as *cis*-18:1 and 18:2 supplements (Table 5.1) in diets. Diets were formulated by supplementing a control diet with 3.5% high-oleic canola oil (Canola), 3.5% soybean oil (Soybean), or 1.75% high-oleic canola oil plus 1.75% soybean oil (Can-Soy). All diets contained 57% forage and 43% concentrate (Table 5.2). Fatty acid content of the total-mixed diets is given in Table 5.3.

Six cows were assigned to each treatment in a Complete Randomized design. All cows were fed the basal diet during an initial 14-d preliminary period, followed by feeding of assigned treatment diets for 5 wk. The second wk of the preliminary period was used as a covariate for statistical analysis. During the third wk of the treatment period, a cow fed soybean oil gained access to the ration fed to one of the cows in the control group. Both cows were omitted from the experimental

design, thus reducing total cows to 22 with only five in the Control and Soybean and six in the Canola and Can-Soy groups.

Sample collection

Samples of the forage mixture (corn silage plus alfalfa haylage) and each concentrate mixture (Control, Canola, Soybean and Can-Soy) were collected each week, composited, dried to a constant weight at 60 ^{0}C, and ground through 1 mm screen in a cyclone-mill (UD Corporation, Boulder, Colorado). A10 mL blood sample was collected from the jagular vein of each cow on d 14 of the preliminary period and during wk 4. Plasma was separated by centrifuging at 3,000 x *g* for 15 min.

Immediately after obtaining blood samples, samples of ruminal fluid were collected from each cow using a stomach tube with a screen on the rumen-end of the tube. If a cow reacted adversely to attempts to pass the sampling tube into the esophagus, the sampling procedure was terminated. For this reason, the number of cows sampled per treatment was four for Canola and five for Control, Can-Soy, and Soybean. A 5 mL aliquot of rumen liquid was preserved with 1 mL 25% H_3PO_4 and 1 mL of 30 μM isocaproic acid. Aliquots were stored at 4 ºC until analyses for volatile fatty acid (VFA). Another 1 mL of aliquot was preserved with 1 mL 25% H_3PO_4, and stored at 4 ºC until analyses for ammonia-nitrogen (N). Another aliquot of rumen fluid was collected to a 60 mL cup and stored at -20 ºC, until they were feeze-dried and analysed for medium- and long-chain fatty acids.

Milk samples were collected from each milking on the last 2 d of the preliminary period and d 6 and 7 of each wk in the treatment period for estimating milk fat, lactose, protein, and solids-not-fat contents, by the Dairy Herd Improvement Association laboratory at Virginia Tech. Milk samples also were collected from the 1300 h milking on d 5 of each week for centrifugation at 11,000 x *g* for 60 minutes to obtain milk fat for fatty acid analysis. Additionally, a 10 L milk sample from each cow was collected for sensory evaluation by the Department of Food Science and Technology of Virginia Tech (data for sensory and physical evaluation of creams are not included in this report).

Chemical analysis

Forages and concentrates were analyzed for ether extract (AOAC, 1984), crude protein (AOAC, 1980) and organic matter (AOAC, 1980). Fatty acid concentrations in feed, rumen fluid, plasma and milk fat were determined following transesterification (Outen et al., 1976). Undecenoic acid (Nu-Check Prep, Elysian, MN) was used as the internal standard. Samples were injected by auto-sampler into a Hewlett Packard 5890A gas chromatograph equipped with a flame ionization detector (Hewlett Packard, Co., Sunnyvale, CA) using procedures described by Wonsil et al. (1994). Samples were split onto a 30 mm glass capillary column (Supelco SP 2380, Supelco, Inc., Bellefonte, PA). The split ratios were 15:1 for feed, ruminal fluid and plasma samples and 83:1 for oils and milk fat samples. The column temperature program began at 60 ^{o}C, held for 3 min, warmed to 205 ^{o}C at 5 ^{o}C /min, held for 12 min, warmed to 215 ^{o}C at 5 ^{o}C /min, held for 5 min, warmed to 220 ^{o}C at 5 ^{o}C /min, and held for 2 min.

Ruminal fluid was analyzed for ammonia-N without the use of urease (Weatherburn, 1967). Ruminal fluid VFA content was determined by flame ionization detection on a Varian Vista 6000 chromatograph equipped with a Varian 4270 integrator (Varian Instruments, Palo Alto, CA). Five μL of sample were injected into a 18.3 m x 0.64 cm o.d. and 2 mm i.d. glass column (Supelco, Inc., Belfonte, PA) packed with GP 10% SP-1200/1% H3PO4 on 80/100 Chromosorb WAW. Column, injector, and detector temperatures were 115, 170, and 180 ºC, respectively. The carrier gas (N) flow was set at 80 mL/min with detector gases (hydrogen and air) set at 40 and 60 mL/min, respectively.

Statistical analysis

The measurements recorded during the week prior to introducing test diets were used as covariates in statistical analysis. Data for dry matter intake, milk yield, percentages and daily yields of fat, protein, lactose and solid-not-fat, and milk fatty acid composition were analyzed by repeated measures analysis of covariance with the General Linear Model procedure of SAS (SAS/STAT Version 6, SAS Institute, Cary, NC). The following model was used for statistical analysis:

$$Y_{ijk} = \mu + T_i + \beta_1(X_{(i)j} - \overline{X}..) + C_{(i)j} + W_k + (TW)_{ik} + E_{ijk}$$

Data for plasma fatty acids, ruminal volatile fatty acids, ruminal ammonia and medium- and long-chain fatty acids in ruminal fluid were analyzed using the General Linear Model procedure of SAS. The following model was used for statistical analysis on these variables:

$$Y_{ij} = \mu + T_i + \beta_1(X_{(i)j} - \bar{X}..) + E_{ij}$$

where Y_{ijk} (or Y_{ij})= dependent variable

μ = overall mean

T_i = effect of treatment (i = 1, 2, 3, or 4)

$\beta_1(X_{(i)j} - \bar{X}..)$ = effect of covariate ($X_{(i)j}$ = average effect of cows allocated for treatment i before introducing treatment; $\bar{X}..$ = average effect of cows allocated for all treatments before introducing treatments)

$C_{(i)j}$ = effect of cow within treatment (j = 1, 2, 3, 4, 5, or 6) (number of cows differed, but number / treatment is given in each table)

W_k = effect of week (k = 1, 2, 3, 4, or 5)

$(TW)_{ik}$ = effect of treatment x week interaction

E_{ijk} (or E_{ij}) = residual error.

Individual treatments were compared using the Orthogonal Contrast procedure (SAS, 1985). The contrasts were: Control versus other treatments, Canola versus Soybean, and Can-Soy versus Canola + Soybean. Means or interactions were considered significantly different at $P < 0.05$. ANOVA examples are shown in Appendix Table 7.

RESULTS AND DISCUSSION

Feed intake

The dry matter intake (DMI) of cows did not differ due to oil supplementation or type of oil supplemented (overall average = 16.6 kg/d). Moreover, DMI did not vary with time (wk 1 through wk 5) or time x treatment interaction. In the two previous experiments when cows were abomasally infused with various oils at 155 to 219 or 330 to 370 g/d, DMI also did not differ due

to treatment (Chapters 3 and 4). Jenkins et al. (1995) observed no difference in DMI when soybean oil was fed at 3.5% of the diet to Holstein cows. Wonsil (1990) reported no difference in intake when Holstein cows were fed a diet containing 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil. Feed intake also did not differ when cows were fed whole canola seeds, ground canola seeds or protected canola seeds at 6% of diet DM (Handy and Kennelly, 1983).

Ruminal ammonia and volatile fatty acids

The rumen samples collected by stomach tube contained rumen fluid with a mixture of small feed particles and associated bacteria, fungi and protozoa. Because the fluid was collected through a screen on the rumen-end of the tube, larger feed particles were not included in samples.

Rumen ammonia concentration (Table 5.4) was lowered due to feeding canola oil compared with feeding soybean oil. Canola oil might disrupt the proteolytic activity of bacteria and protozoa in the rumen, thus reducing microbial protein synthesis. Infusion of linseed oil into the rumen of sheep decreased ruminal protein digestion, which lead to decreased ruminal ammonia concentrations (Ikwuegbu and Sutton, 1992). Similar changes occurred when sheep were fed additional lipid as either corn oil or lecithin (Jenkins and Fotouhi, 1990).

Concentrations of volatile fatty acids (VFA) in the rumen were measured to evaluate the effects of oil supplementation on carbohydrate fermentation. Total VFA concentration in the rumen (Table 5.4) did not differ due to treatment. Acetic acid percentage of total VFA also was not influenced by treatment. However, propionic acid concentration was increased in cows fed oil-supplemented diets compared with Control. As a result, the ratio of acetate to propionate in the rumen was reduced due to oil supplementation.

Wonsil et al. (1994) observed no changes in the concentration of individual VFA or the ratio of acetate to propionate when diets with soybean oil plus partially hydrogenated soybean oil were fed to Holstein cows. Jenkins et al. (1995) reported reduced total VFA concentration (107 versus

113 mM) and acetate percentage (55.8 versus 57.6%), when diets containing 3.5% soybean oil were fed to Holstein cows. However, when soybean oil was fed as fatty acyl amide (butylsoymide), total VFA and acetate were not affected.

Long-chain fatty acids in ruminal fluid

Concentrations of 12:0, 14:0, 16:1, 18:2, 20:0, 20:3 and 20:5 in ruminal fluid did not differ among treatments (Table 5.5). Contents of 16:0 or 18:0 did not differ from Control due to oil supplementation, but cows fed canola oil had higher concentrations of these fatty acids than cows fed soybean oil. Concentrations of 14:1, 15:0, 17:0, and 18:3 were increased and *cis*-18:1 and *trans*-18:1 were reduced due to oil supplementation. Conjugated linoleic acid (CLA) content of ruminal fluid tended ($P < 0.06$) to increase due to oil supplementation, and canola oil supplementation tended ($P < 0.07$) to cause higher CLA than did soybean oil. It is rather unexpected that concentrations of 18:0, *cis*-18:1, *trans*-18:1, and 18:2 did not increase when cows were fed oil-supplemented diets. The oils were expected to adhere to feed particles. The ruminal fluid sample collected through a screen contained mostly ruminal liquid with associated bacteria and protozoa, so oil coated particles may have been excluded. This might be a reason for our unexpected results. Wonsil (1990) reported no difference in the concentration of 18:0, 18:1, and 18:2 in mixed ruminal bacteria from Holstein cows fed control diet or diets with tallow, partially hydrogenated tallow, or tallow coated with casein and corn syrup at 3% of diet DM. Bauchart et al. (1990) reported 26.9% 18:0 in liquid-associated rumen bacteria when cows were fed soybean oil (87 g/kg diet DM) compared with 22.2% when fed a control diet. Bauchart et al. (1990) also reported 23% 18:1 in liquid-associated ruminal bacteria and 35% in solid-adherent bacteria when cows were fed rapeseed oil (94 g/kg diet DM) compared with 11 and 16%, respectively, for the control.

Fatty acid concentration in blood plasma

Dietary oil supplementation, compared with Control increased 17:0, *cis*-18:1, *trans*-18:1, 18:2, and CLA concentrations in blood plasma (Table 5.6). Concentrations of 14:1, 16:1, 17:1, and *cis* -18:1 in plasma of cows fed Canola were greater than in those fed Soybean. Concentration of *cis* -18:1 in plasma appeared to be proportional to *cis* -18:1 intake. A similar trend was not apparent

for 18:2, for which a higher concentration in cows fed Soybean compared with those fed Canola could be expected if the amount of 18:2 escaping ruminal fluid biohydrogenation was proportional to the amount in the diet.

Wonsil (1990) reported increased plasma *cis*-18:1 when *cis*-18:1-rich tallow and tallow coated with casein and corn syrup solids were supplemented in diets of lactating cows at 3% of diet DM. Jenkins et al. (1996) reported similar proportions of 18:2 in plasma when cows were fed a control diet and a diet supplemented with soybean oil at 3.5% diet DM, but plasma 18:2 was elevated only when soybean oil was fed as fatty acyl amides (butylsoymide). Thus, unprotected *cis*-18:1 apparently escapes ruminal biohydrogenation in amounts capable of elevating plasma *cis*-18:1, but unprotected dietary 18:2 must be more susceptible to biohydrogenation. The concentration of *trans*-18:1 in blood plasma was raised in response to feeding all oil-supplemented diets. There were only tendencies for *trans*-18:1 ($P < 0.12$) and CLA ($P < 0.19$) in plasma to be higher when Soybean was fed compared with Canola. However, as indicated in the following section, concentrations of *trans*-18:1 and CLA in milk fat reflect the extent of biohydrogenation of 18:2 in the rumen.

Milk and milk component yields

Milk production, composition and component yield data are shown in Table 5.7. Daily average milk yield of cows did not differ significantly due to treatment, time, or time x treatment interaction. Milk yield also did not differ in the previous experiments (Chapters 3 and 4) when fish oil or seed oils were abomasally infused. Jenkins et al. (1995) reported no difference in milk yield when Holstein cows were fed soybean oil (3.5% of diet DM). Daily milk yield did not change when cows were fed diets containing: protected canola seeds at 6.5% of diet DM (Ashes et al., 1992); whole canola seeds, ground canola seeds, or protected canola seeds at 6% of diet DM (Handy and Kennelly, 1983); or 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil (Wonsil, 1990).

As compared with Control, oil supplementation reduced milk protein percentage, but did not affect fat, lactose, or SNF percentages. This is in agreement with Mohamed et al. (1987), who

observed decreased milk protein percentage when soybean oil was added at 4% of diet DM. However, Handy and Kennelly (1983), Ashes et al. (1991), Jenkins et al. (1995) and Wonsil (1990) reported no difference in milk protein percentage when diets were supplemented with seeds or seed oils rich in unsaturated fatty acid.

Although milk protein percentage differed due to treatments, milk protein yield did not. Yield of milk protein also did not differ due to time or time x treatment interaction. In contrast, milk fat yield was greater for cows fed oil supplements compared with Control. Among oil supplements, cows fed Soybean had greater milk fat yield than cows fed Canola.

Handy and Kennelly (1983) and Wonsil (1990) reported no change in milk fat percentage when UFA-rich oil supplements were fed. However, Gaynor et al. (1994) reported a reduction from 3.5 to 2.6% when the cows were abomasally infused with 450 g of *cis*-18:1. Astrup et al. (1976), and Selber and Shultz (1980) hypothesized that supplemental dietary fatty acids inhibit de-novo synthesis of saturated fatty acids in the mammary gland, eventually reducing milk fat percentage. However, Palmquist (1976) explained that uptake of long chain fatty acids by the mammary gland can be greater than the compensatory reduction in de novo fatty acid synthesis, resulting higher milk fat yield. His theory is supported by the findings of Ashes et al. (1991) which indicated increased milk fat percentage and daily milk fat yield due to feeding protected canola seeds.

Milk fatty acids

Neutral fatty acids

The fatty acids which are believed to have neither cholesterol-raising nor cholesterol-lowering effects fall into this category (Berner, 1993). The major neutral fatty acids in milk fat are short chain fatty acids (SCFA) (butyric acid [4:0], caproic acid [6:0], caprylic acid [8:0], and capric acid [10:0]), and stearic acid (18:0).

The 4:0 content of milk was increased, and 6:0 and 10:0 were reduced due to oil supplementation (Table 5.8). In addition, 18:0 content of milk increased due to oil supplementation. Primarily due to the increase in 18:0 content, the total amount of neutral fatty acids in milk was elevated in

response to dietary oil supplementation. Handy and Kennelly (1983) reported increased milk 18:0 content when canola seeds were supplemented at 6% of diet DM as whole, ground or protected (Protec®) forms. Ashes et al. (1992) also reported an increase in 18:0 content of milk, with no changes in SCFA, when protected canola seeds were fed to Holstein cows at 6.5% of diet DM. The increase in 18:0 could be due to biohydrogenation of a portion of the supplemented UFA to 18:0 in the rumen. Jenkins et al. (1995) reported increased 18:0 content of milk when Holstein cows were fed 3.5% soybean oil, but no difference was observed when soybean oil was fed as butylsoymide.

Cholesterol raising fatty acids

According to Berner (1993), Judd et al. (1994), and Keys et al. (1986), MCFA and *trans*-18:1 are considered cholesterol-raising (hyprecholesterolemic) fatty acids. Oil-supplemented diets lowered the content of MCFA in milk (Table 5.8), but increased *trans*-18:1 content. The elevated *trans*-18:1 content of milk in response to oil supplements was due primarily to Soybean and Can-Soy.

Gaynor et al. (1994) observed a reduction in MCFA in milk from 53% to 40% when *cis*-18:1 was infused abomasally (750 g/d). According to Drackley et al. (1992), MCFA content of milk from cows infused abomasally with unsaturated fatty acids (450 g/d) was 35% compared with 46% for their control. When cows were fed protected canola seeds at 6.5% of diet DM, Ashes et al. (1992) observed a total of 33% MCFA compared with 43% for their control. Palmquist et al. (1993) hypothesized that supplemental dietary fat depresses the de novo synthesis of MCFA in cow mammary tissue.

Cholesterol lowering fatty acids

Dietary oil supplementation increased *cis*-18:1 content of milk (Table 5.8). Concentration of *cis*-18:1 was similar in response to the three oil supplements, despite differences in *cis*-18:1 content of the oils and blood plasma (Table 6). In contrast, 18:2 content of milk was proportional to 18:2 content of the supplemental oils, but not plasma 18:2 content. All oil supplements increased the content of 18:3 in milk. As far as the enhancement of total unsaturated fatty acid content of milk

is concerned, three oil supplements enhanced total hypocholesterolemic fatty acids to the same extent.

Other fatty acids

Conjugated linoleic acid, found in animal fat sources, is considered an anticarcinogenic fatty acid (Ha et al., 1987 and 1989). CLA originates in the rumen as a product of isomerization and biohydrogenation of dietary unsaturated fatty acids (Ha et al., 1987). The three oil supplements increased CLA content of milk, with concentration in response to Soybean being greater than in response to Canola. The CLA level reported in the present experiment was greater than the level reported for both Holsteins (0.6%) and Jerseys (0.4%) in the second experiment (Chapter 4). The biohydrogenation of dietary 18:2 might be responsible for the greater CLA content of milk in this experiment.

Milk 16:1, 20:3, 20:4 and 20:5 content did not vary due to oil-supplemented diets. Cows fed the oil-supplemented diets had reduced 14:1, 15:0, and 17:0 in milk, and increased 20:0 and 22:5. Cows fed Canola had a greater concentration of 20:0 and lower 20:3 than cows fed Soybean. The total content of these fatty acids in milk did not differ due to feeding oil-supplemented diets.

Milk fatty acid composition - summary

Cows fed the oil-supplemented diets had a reduced content of hypercholesterolemic fatty acids and increased content of hypocholesterolemic fatty acids. The unsaturated to saturated fatty acid ratio of milk (Table 5.8) was raised equally by feeding all three supplements. Cows fed Can-Soy or Soybean had elevated percentages of CLA and *trans*-18:1, whereas cows fed Canola had elevated CLA percentage with no change in *trans*-18:1 percentage compared with Control.

Out of each 100 g/d of *cis*-18:1 supplemented by canola oil, 21 g/d was apparently recovered in milk fat (Table 5.9). In addition, milk MCFA yield was reduced by 48 g/d, for each 100 g/d of supplemental *cis*-18:1. Out of 100 g/d 18:2 supplemented by soybean oil, 3 g/d was apparently recovered in milk fat.

IMPLICATIONS

Feeding oil supplemented diets apparently raised hypocholesterolemic fatty acid content and CLA content, and lowered hypercholeterolemic fatty acid content of milk. *Trans*-18:1 content of milk was raised in response to Soybean and Can-Soy, but not Canola. Accordingly, in future, the potential of other *cis*-18:1-rich supplements in diets should be investigated. The oils of corn, soybean, and sunflower that are genetically improved to contain high *cis*-18:1 (Cline and Re, 1977) may be good choices.

In our previous experiments in which oils were abomasally infused in Jersey cows, for each 100 g of infused *cis*-18:1 or 18:2, milk yield of *cis*-18:1 and 18:2 were apparently increased by 39 to 49 g or 31 to 47 g, respectively. If these rates are considered as maximum potential levels, then apparent recovery of *cis*-18:1 in this study was approximately half and apparent recovery of 18:2 was approximately one-tenth of the maximum potential. Thus, future research should focus on methods to protect supplemental dietary unsaturated fatty acids from rumen biohydrogenation.

Table 5.1. Fatty acid composition (g/100 g fatty acids) of oil supplements.

Fatty acid	Canola oil	Soybean oil
16:0	4.3	10.6
16:1	0.3	0.1
17:0	-	0.1
17:1	0.3	-
18:0	2.4	3.6
cis-18:1	73.5	20.1
18:2	13.8	56.2
18:3	2.7	8.8
20:0	1.0	0.4
20:5	-	0.2
CLA[1]	1.7	-

[1] Conjugated linoleic acid (*cis*-9-*trans*-11 linoleic acid)

5.2. Ingredients and chemical composition of the control diet and the treatment diets fed to Jersey cows.

	Control diet	Treatment diets
Ingredient, % of dry matter		
Corn, cracked	33.6	29.6
Soybean meal	6.1	6.1
Prolak[1]	1.5	2.1
Corn silage	24.8	24.8
Alfalfa haylage	32.8	32.7
Mineral /vitamin premix[2]	1.2	1.2
Oil[3]	-	3.5
Chemical composition, % of dry matter		
Dry matter	54.5	54.7
Organic matter	95.3	94.8
Crude protein	16.8	16.8
Ether extract	3.0	6.4

[1] Prolak (H. J. Baker & Bro., Inc., Atlanta, GA). Contained 60% CP, 6% Fat, 2% Fiber, 2.69 to 5.75% Ca, and 2.75% P.

[2]Contained 6.5% P, 16.0% Ca, 4.3% NaCl, 2.2% Mg, 3.5% K, 3.2% S, 0.11% Mn, 0.13% Zn, 0.03% Fe, 0.13% Cu, 0.002% I, 0.0003% Co, 0.0005% Se, 110,000 IU vitamin A/kg, 44,000 IU vitamin D3/kg, and 1,350 IU vitamin E/kg.

[3]Canola oil and (or) soybean oil

Table 5.3. Fatty acid content (μg/g of dry matter) of the diet with no supplemented oil (Control) and diets supplemented with 3.5% canola oil (Canola), 3.5% soybean oil (Soybean), or 1.75% canola oil + 1.75% soybean oil (Can-Soy).

Fatty acids	Control	Canola	Can-Soy	Soybean
10:0	4	4	4	4
12:0	65	65	65	65
14:0	221	221	221	221
14:1	32	32	32	32
15:0	110	110	110	110
16:0	6719	7639	8509	9379
16:1	399	438	437	437
17:0	118	122	123	124
17:1	16	24	23	22
18:0	957	1477	1663	1849
cis-18:1	6161	21919	16569	11219
trans-18:1	25	25	25	25
18:2	17845	20795	26394	31993
18:3	5014	5600	6407	7214
20:0	329	327	326	323
20:3	310	530	470	410
CLA[1]	5	7	13	7
22:1	3	5	5	5
20:4	18	25	25	25
20:5	72	100	101	101
22:4	5	6	6	6
22:5	22	31	31	31

[1] Conjugated linoleic acid (*cis*-9-*trans*-11 linoleic acid).

Table 5.4. Ruminal ammonia and volatile fatty acid (VFA) concentrations in Jersey cows fed a control diet (Control) or diets supplemented with 3.5% canola oil (Canola), 3.5% soybean oil (Soybean), or 1.75% canola oil plus 1.75% soybean oil (Can-Soy).

						Probability[1,2]		
	Control	Canola	Can-Soy	Soybean	SE[3]	Control vs all	Canola vs Soybean	Can-Soy vs Canola+Soybean
Ammonia, (mg/dL)	22.4	14.4	18.1	23.4	2.2	0.20	0.02	0.77
Total VFA, (mM)	165.1	136.6	174.1	203.4	27.5	0.85	0.13	0.91
Acetate, (%)	72.1	71.0	71.1	71.5	0.8	0.32	0.65	0.90
Propionate, (%)	14.1	15.6	15.0	15.0	0.4	0.03	0.17	0.42
Isobutarate, (%)	10.8	10.0	11.2	11.0	0.8	0.92	0.50	0.54
IsoValerate, (%)	1.2	1.1	1.1	1.1	0.1	0.18	0.75	0.58
Valerate, (%)	0.9	1.0	0.9	0.9	0.1	0.49	0.28	0.57
Acetate / Propionate	5.1	4.5	4.9	4.8	0.1	0.01	0.08	0.17

[1] Trt = Treatment effect.

[2] Number of cows was four for Canola and five each for Control, Can-Soy, and Soybean.

[3] Standard Error for 5 cows (the value is greater for Canola).

Table 5.5. Concentrations of medium- and long-chain fatty acids (g/100 g fatty acids) in rumen fluid from Jersey cows fed a control diet (Control) or diets supplemented with 3.5% canola oil (Canola), 3.5% soybean oil (Soybean), or 1.75% canola oil plus 1.75% soybean oil (Can-Soy).

	Control	Canola	Can-Soy	Soybean	SE[3]	Probability[1,2]		
						Control vs all	Canola vs Soybean	Can-Soy vs Canola+Soybean
12:0	0.3	0.3	0.4	0.3	0.1	0.63	0.81	0.42
14:0	0.9	1.1	1.0	0.9	0.1	0.29	0.15	0.65
14:1	1.2	1.7	1.4	1.4	0.1	0.03	0.01	0.13
15:0	0.9	1.3	1.2	1.2	0.1	0.01	0.02	0.23
16:0	20.9	25.0	22.8	19.5	0.7	0.51	0.04	0.59
16:1	0.29	0.22	0.28	0.29	0.04	0.77	0.37	0.77
17:0	0.53	0.70	0.66	0.67	0.04	0.05	0.46	0.39
18:0	55.9	53.6	55.8	62.0	1.6	0.61	0.03	0.38
cis-18:1	6.1	4.0	4.6	4.4	0.3	0.01	0.41	0.32
trans-18:1	4.7	3.9	4.1	3.4	0.2	0.03	0.30	0.23
18:2	3.3	3.7	3.2	2.9	0.3	0.99	0.17	0.77
18:3	0.9	1.2	1.2	1.0	0.1	0.02	0.19	0.69
20:0	0.9	0.7	0.9	1.1	0.1	0.35	0.11	0.67
CLA[4]	1.2	1.7	1.8	1.4	0.1	0.06	0.07	0.09
20:3	0.52	0.57	0.63	0.62	0.04	0.07	0.18	0.30
20:5	0.25	0.38	0.23	0.28	0.06	0.61	0.31	0.25

[1]Trt = Treatment effect.
[2]Number of cows was four for Canola and five each for Control, Can-Soy, and Soybean.
[3] Standard Error for 5 cows (the value is greater for Canola).
[4]Conjugated linoleic acid (*cis*-9-*trans*-11 linoleic acid).

Table 5.6. Concentration of fatty acids (μg/mL) in arterial blood plasma from Jersey cows fed a control diet (Control) or diets supplemented with 3.5% canola oil (Canola), 3.5% soybean oil (Soybean), or 1.75% canola oil plus 1.75% soybean oil (Can-Soy).

	Control	Canola	Can-Soy	Soybean	SE[3]	Probability[1,2]		
						Control vs all	Canola vs Soybean	Can-Soy vs Canola+Soybean
14:0	1.9	2.4	1.9	1.8	0.3	0.75	0.15	0.46
14:1	6.6	7.6	5.0	5.3	0.7	0.43	0.03	0.10
15:0	3.7	3.6	2.9	3.1	0.4	0.25	0.37	0.33
15:1	4.7	4.7	2.8	2.9	0.7	0.13	0.07	0.23
16:0	73.7	91.9	86.2	82.7	9.5	0.21	0.47	0.94
16:1	5.0	7.0	5.2	4.9	0.6	0.31	0.04	0.09
17:0	10.9	17.9	13.7	17.9	1.9	0.01	0.99	0.07
17:1	1.8	1.8	1.4	1.2	0.1	0.08	0.02	0.62
18:0	109.9	154.9	142.3	121.6	18.0	0.14	0.19	0.85
cis-18:1	55.1	110.3	70.9	57.2	10.7	0.05	0.01	0.31
trans-18:1	6.1	12.1	12.7	16.2	1.8	0.01	0.12	0.49
18:2	363.9	495.9	429.5	475.1	40.2	0.03	0.71	0.24
CLA[4]	0.3	1.1	0.9	1.6	0.3	0.01	0.19	0.23
18:3	34.4	37.5	41.7	37.7	7.9	0.60	0.99	0.66
20:3	23.7	25.7	20.7	21.8	2.1	0.66	0.20	0.22
20:5	7.4	8.8	7.0	6.8	0.9	0.91	0.12	0.41
20:4	15.9	19.2	15.2	15.4	1.7	0.72	0.10	0.36
22:4	5.2	4.2	3.3	3.4	0.8	0.08	0.34	0.46

[1] Trt = Treatment effect.
[2] Number of cows was five for Soybean and six each for Control, Canola and Can-Soy.
[3] Standard Error for 5 cows (the value is smaller for Control, Canola and Can-Soy).
[4] Conjugated linoleic acid (*cis*-9-*trans*-11 linoleic acid).

Table 5.7. Milk yield, milk composition and milk component yields of Jersey cows fed a control diet (Control) or diets supplemented with 3.5% canola oil (Canola), 3.5% soybean oil (Soybean), or 1.75% canola oil plus 1.75% soybean oil (Can-Soy).

						Probability[1,2]				
	Control	Canola	Can-Soy	Soybean	SE[3]	Control vs all	Canola vs Soybean	Can-Soy vs Canola+Soybean	Time	Time*Trt
Milk, kg/d	22.2	22.7	23.9	24.1	1.7	0.13	0.26	0.34	0.84	0.12
Fat, %	4.7	4.7	4.9	4.8	0.4	0.51	0.37	0.89	0.92	0.33
Lactose, %	5.0	4.8	4.9	4.9	0.1	0.11	0.08	0.37	0.71	0.90
Protein, %	3.7	3.5	3.5	3.5	0.1	0.04	0.10	0.61	0.11	0.09
SNF, %	9.3	9.1	9.2	9.2	0.1	0.17	0.24	0.47	0.73	0.43
Fat, kg/d	1.0	1.1	1.2	1.2	0.1	0.05	0.05	0.50	0.66	0.58
Lactose, kg/d	1.1	1.1	1.2	1.2	0.1	0.27	0.12	0.24	0.72	0.12
Protein, kg/d	0.8	0.8	0.8	0.8	0.1	0.87	0.19	0.22	0.48	0.44
SNF, kg/d	2.1	2.1	2.2	2.2	1.1	0.25	0.12	0.26	0.65	0.26

[1] Trt = Treatment and Time = effect of duration (wk 1 through 5) for Time*Trt interaction.

[2] Number of cows was five each for Control and Soybean, and six each for Canola and Can-Soy.

[3] Standard Error for 5 cows (the value is smaller for Canola and Can-Soy).

Table 5.8. Milk fatty acid composition (g/100 g fatty acids) of Jersey cows fed control diet (Control) or diets supplemented with 3.5% canola oil (Canola), 3.5% soybean oil (Soybean), or 1.75% canola oil plus 1.75% soybean oil (Can-Soy) listed according to their influence on plasma cholesterol when included in the diet of humans[4]

						Probability[1,2]				
	Control	Canola	Can-Soy	Soybean	SE[3]	Control vs all	Canola vs Soybean	Can-Soy vs Canola+Soybean	Time	Time*Trt
Neutral[4]										
4:0	1.8	2.2	4.0	3.3	1.0	0.04	0.17	0.04	0.01	0.17
6:0	2.4	2.0	1.8	2.0	0.3	0.01	0.98	0.24	0.31	0.25
8:0	1.4	1.3	1.2	1.3	0.1	0.09	0.99	0.52	0.04	0.10
10:0	4.7	3.8	4.1	3.7	0.5	0.01	0.83	0.38	0.32	0.30
18:0	11.2	15.1	13.2	14.5	1.2	0.01	0.47	0.03	0.20	0.15
Total	22.1	25.0	23.9	23.7	1.4	0.01	0.18	0.54	0.12	0.02
Hypercholesterolemic[4]										
12:0	4.4	3.4	3.7	3.3	0.4	0.01	0.71	0.14	0.72	0.55
14:0	14.0	11.8	12.4	11.9	1.0	0.01	0.82	0.35	0.27	0.56
16:0	33.9	25.2	26.0	26.7	2.2	0.01	0.29	0.94	0.11	0.02
trans-18:1	2.1	2.1	4.2	4.5	0.7	0.01	0.01	0.03	0.48	0.40
Total	54.4	42.5	46.3	46.4	2.9	0.01	0.81	0.59	0.12	0.04
Hypocholesterolemic[4]										
cis-18:1	15.7	24.6	22.4	22.0	2.4	0.04	0.10	0.57	0.52	0.62
18:2	2.0	2.1	2.4	2.9	0.2	0.01	0.01	0.50	0.59	0.06
18:3	0.61	0.80	0.75	0.75	0.04	0.01	0.04	0.19	0.02	0.11
Total	18.3	27.5	25.6	25.7	2.6	0.01	0.23	0.65	0.61	0.66

(Table 5.8 continues on the next page)

Table 5.8 (Continued).

	Control	Canola	Can-Soy	Soybean	SE[3]	Probability[1,2]				
						Control vs all	Canola vs Soybean	Can-Soy vs Canola+Soybean	Time	Time*Trt
Others										
14:1	1.3	1.2	1.2	1.1	0.1	0.01	0.25	0.35	0.18	0.36
15:0	1.6	1.3	1.2	1.2	0.1	0.01	0.54	0.69	0.74	0.08
16:1	0.38	0.34	0.38	0.37	0.04	0.51	0.38	0.59	0.11	0.60
17:0	0.66	0.55	0.54	0.57	0.04	0.01	0.33	0.41	0.99	0.49
17:1	0.23	0.21	0.19	0.19	0.09	0.01	0.34	0.52	0.90	0.85
CLA[5]	0.51	0.85	0.92	1.27	0.09	0.01	0.01	0.24	0.27	0.05
20:0	0.26	0.40	0.31	0.29	0.04	0.01	0.01	0.05	0.05	0.20
20:3	0.12	0.11	0.13	0.14	0.04	0.93	0.03	0.93	0.96	0.56
20:4	0.12	0.13	0.11	0.12	0.04	0.19	0.66	0.33	0.97	0.99
20:5	0.05	0.02	0.04	0.03	0.04	0.06	0.35	0.06	0.46	0.22
22:5	0.04	0.06	0.08	0.08	0.04	0.13	0.28	0.73	0.75	0.06
Unsat / Saturated[6]	0.23	0.48	0.47	0.48	0.06	0.01	0.98	0.54	0.94	0.54

[1]Trt = Treatment effect and Time = effect of duration (wk 1 through 5) for Time*Trt interaction.

[2]Number of cows was five each for Control and Soybean, and six each for Canola and Can-Soy.

[3] Standard Error for 5 cows (the value is smaller for Canola and Can-Soy).

[4]Berner (1993).

[5]Conjugated linoleic acid (*cis*-9-*trans*-11-linolecic acid).

[6]Ratio of unsaturated fatty acids (total of 14:1, 16:1, 17:1, *cis*-18:1, *trans*-18:1, 18:2, 18:3, CLA, 20:3, 20:4, 20:5, and 22:5) to saturated fatty acids (total of 4:0, 6:0, 8:0, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0).

Table 5.9. Supplemental fatty acid intake and change in yield of fatty acids in milk of Jersey cows fed control diet (Control) or diets supplemented with 3.5% canola oil (Canola), 3.5% soybean oil (Soybean), or 1.75% canola oil plus 1.75% soybean oil (Can-Soy).

	Control	Canola	Can-Soy	Soybean	SE[3]	Probability[1,2]				
						Trt	Canola vs Soybean	Can-Soy vs Canola+Soybean	Time	Time*Trt
Average amounts of supplemental fatty acid intake (g/d)										
Cis-18:1	0	354.7	283.5	94.0						
18:2	0	66.6	205.0	262.9						
Apparent change in yield (g/d)										
MCFA[4]	-	-47.9	-32.9	-92.5	31.8	0.02	0.04	0.04	0.01	0.01
Cis-18:1	-	21.0	23.8	73.8	19.9	0.01	0.01	0.04	0.01	0.11
18:2	-	-2.8	1.3	3.0	2.1	0.01	0.01	0.27	0.01	0.01

[1]Trt = Treatment effect and Time = effect of duration (wk 1 through 5) for Time*Trt interaction.

[2]Number of cows was five each for Control and Soybean, and six each for Canola and Can-Soy.

[3] Standard Error for 5 cows (the value is smaller for Canola and Can-Soy).

[4]MCFA = medium chain fatty acids (total of 12:0, 14:0, and 16:0).

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APPENDIX

Table 1. Statistical procedures for Experiment 1.

Assignment of cows to treatments

Cow	Preliminary period	Period 1	Period 2	Period 3	Period 4
A	No infusion	Sunflower	Fish	Olive	Sesame
B	No infusion	Fish	Sesame	Sunflower	Olive
C	No infusion	Olive	Sunflower	Sesame	Fish
D	No infusion	Sesame	Olive	Fish	Sunflower

ANOVA example

Source	DF	Mean Square	F Value	Pr > F
Treatment	3	4.81	7.17	0.02
Cow	3	1.90	2.82	0.13
Period	3	5.16	7.69	0.02
Error	6	0.67		
Corrected Total	15			

Table 2. Flow of organic matter (OM), crude protein (CP), and ether extract (EE) to the feces, and apparent total tract digestibility of OM and CP in Jersey cows during abomasal infusion of olive oil, sesame oil, sunflower oil, or fish oil.

		Oil infusion				
	Preliminary	Olive	Sesame	Sunflower	Fish	SE
Flow to feces, kg/d						
OM	5.6 ± 0.5	5.6	6.5	6.4	5.5	0.6
CP	1.1 ± 0.1	1.1	1.3	1.2	1.1	0.1
EE	0.93 ± 0.13	0.13	0.14	0.13	0.14	0.41
Apparent digestibility in total tract, %						
OM	69.2 ± 1.5	67.3	62.3	62.5	68.0	4.3
CP	59.2 ± 2.5	56.8	49.0	51.5	55.8	5.2

Table 3. Fatty acid (FA) absorption in the intestine and flow to the feces of Jersey cows infused abomasally with olive oil, sesame oil, sunflower oil, or fish oil

		Oil infusion				
	Preliminary	Olive	Sesame	Sunflower	Fish	SE
FA absorbed, g/d						
16:0	62.8 ± 23.8	84.0	72.3	81.0	86.8	22.6
18:0	260.0 ±120.9	337.8	316.0	348.0	349.5	111.0
cis-18:1	61.8 ± 19.8	206.5	119.8	112.3	106.3	31.6
trans-18:1	26.8 ± 14.3	16.0	40.3	14.5	27.0	14.1
18:2	10.9 ± 3.1	15.8	17.8	29.3	20.3	7.0
18:3	1.7 ± 0.8	2.3	2.1	2.4	3.0	0.8
FA flow to the feces, g/d						
16:0	13.8 ± 4.6	14.5	20.5	17.5	14.8	4.0
18:0	77.5 ± 37.8	55.5	78.8	72.3	80.4	15.4
cis-18:1	13.3 ± 1.5	12.3	18.0	15.5	9.4	3.4
trans-18:1	2.0 ± 0.4	1.9	2.7	2.4	2.7	0.8
18:2	2.4 ± 0.5	2.1	3.4	2.9	2.1	0.9
18:3	0.8 ± 0.3	0.6	0.8	0.8	0.8	0.2

Table 4. Statistical procedures for Experiment 2.

Assignment of cows to treatments

Cow	Period 1	Period 2	Period 3	Period 4
Jersey A	Canola	Control	Sunflower	Olive
Jersey B	Control	Olive	Canola	Sunflower
Jersey C	Sunflower	Canola	Olive	Control

Cow	Period 1	Period 2	Period 3	Period 4
Holstein A	Canola	Olive	Control	Sunflower
Holstein B	Olive	Sunflower	Canola	Control
Holstein C	Sunflower	Control	Olive	Canola

ANOVA example

Source	DF	Mean Square	F Value	Pr > F
Treatment	3	0.22	0.11	0.95
Breed	1	0.15	0.08	0.78
Period	1	0.83	0.43	0.52
Treatment*Breed	3	1.27	0.67	0.59
Error	15	1.90		
Corrected Total	23			

Table 5. Flow of organic matter (OM), crude protein (CP), and ether extract (EE) to the feces, and total tract digestibility of OM and CP in Holstein and Jersey cows in response to abomasal infusion of distilled water (control), canola oil (Canola), olive oil (Olive), or high-oleic sunflower oil (HO-Sun).

Parameter	Treatment averages				Breed averages			P <[1]		
	Control	Canola	Olive	HO-Sun	Holstein	Jersey	SE[2]	Treatment	Breed	T*Breed
Flow with feces, kg/d										
OM	6.1	5.4	5.9	5.7	7.2	4.4	0.5	0.79	0.01	0.69
CP	1.3	1.2	1.2	1.2	1.5	0.9	0.1	0.85	0.01	0.67
EE	0.13	0.14	0.12	0.14	0.15	0.11	0.04	0.65	0.01	0.44
Digestibility in total tract (%)										
OM	66.7	69.2	68.0	69.2	65.6	71.0	1.5	0.61	0.01	0.85
CP	55.8	56.5	60.0	60.9	55.1	61.4	3.3	0.62	0.08	0.68

[1] Effects of treatment (T), breed, or T x breed interactions are considered significant when $P < 0.05$.

[2] Treatment Standard Error.

Table 6. Fatty acid absorption in the intestine and flow to the feces in Holstein and Jersey cows in response to abomasal infusion of distilled water (control), canola oil (Canola), olive oil (Olive), or high-oleic sunflower oil (HO-Sun).

Parameter	Treatment averages				Breed averages			P <[1]		
	Control	Canola	Olive	HO-Sun	Holstein	Jersey	SE[2]	Treatment	Breed	T*Breed
12:0:										
Absorption in intestine (g/d)	1.6	2.3	1.7	1.8	2.1	1.5	0.2	0.27	0.02	0.32
Fecal flow, g/d	0.9	0.8	0.9	0.8	1.0	0.7	0.1	0.72	0.01	0.98
14:0:										
Absorption in intestine (g/d)	6.0	8.2	6.5	6.0	7.6	5.8	0.8	0.27	0.06	0.11
Fecal flow, g/d	3.7	3.5	3.2	3.7	4.3	2.7	0.4	0.75	0.01	0.99
16:0:										
Absorption in intestine (g/d)	95.6	123.7	126.6	104.0	126.1	98.9	12.1	0.25	0.04	0.19
Fecal flow, g/d	31.1	29.2	37.1	31.1	37.9	26.4	3.5	0.44	0.01	0.92
18:0:										
Absorption in intestine (g/d)	246.9	331.9	270.4	266.9	320.3	237.8	31.4	0.29	0.02	0.40
Fecal flow, g/d	77.4	95.3	91.4	76.4	83.1	87.1	17.7	0.83	0.83	0.41
cis-18:1:										
Absorption in intestine (g/d)	60.2[b]	185.6[ab]	195.4[a]	280.4[a]	182.8	178.1	33.3	0.01	0.89	0.06
Fecal flow, g/d	10.4	12.4	16.9	15.9	15.7	12.1	2.2	0.19	0.13	0.69
trans-18:1:										
Absorption in intestine (g/d)	32.2[ab]	50.1[a]	27.9[ab]	18.1[b]	45.3	18.9	6.9	0.03	0.01	0.24
Fecal flow, g/d	8.3	8.9	7.2	8.0	9.2	7.1	1.3	0.85	0.15	0.42
18:2:										
Absorption in intestine (g/d)	39.1	69.7	61.9	69.5	65.8	54.3	8.0	0.05	0.17	0.07
Fecal flow, g/d	7.6	6.1	7.7	7.4	8.4	6.0	1.3	0.80	0.07	0.84

[1] Effects of treatment (T), breed, or T x breed interactions are considered significant when $P < 0.05$.
[2] Treatment Standard Error.

Table 7. ANOVA examples for Experiment 3.

ANOVA used for feed intake, milk yield, milk composition, milk component yields, milk fatty acid composition, and milk fatty acid yield data.

Source	DF	Mean Square	F Value	Pr > F
Treatment	3	38.11	3.93	0.03
COVMK	1	67.87	7.00	0.02
Cow(Treatment)	17	9.69		
TIME	4	8.04	1.92	0.12
TIME*TRT	12	9.01	2.15	0.02
TIME*COVMK	4	7.82	1.87	0.13
Error(TIME)	68	4.18		
Corrected total	109			
Contrasts				
CAB vs all	1	73.03	7.54	0.01
CO vs SO	1	18.57	1.92	0.18
CS vs CO+SO	1	3.86	0.40	0.54
TIME*CAB vs all	4	9.05	2.16	0.08
TIME*CO vs SO	4	12.89	3.08	0.02
TIME*CS vs CO+SO	4	3.06	0.73	0.57

ANOVA used for ruminal ammonia, ruminal volatile fatty acids, ruminal medium- and long-chain fatty acids, and plasma fatty acids.

Source	DF	Mean Square	F Value	Pr > F
TRT	3	3351.70	0.89	0.47
COVMK	1	863.30	0.23	0.64
Error	14	3777.35		
Corrected Total	18			
Contrasts				
CAB vs all	1	144.67	0.04	0.85
CO vs SO	1	9762.70	2.58	0.13
CS vs CO+SO	1	54.20	0.01	0.91

VITA

Name:	Aloka B. P. A. Bandara
Primary school:	Gamini Vidyalaya, Thambuttegama , Sri Lanka
High school:	Central College, Anuradhapura, Sri Lanka
Basic degree:	B.Sc. (Agriculture), University of Peradeniya, Sri Lanka
Graduate degrees:	M.Phil. (Animal Science), University of Peradeniya, Sri Lanka
	Ph.D. (Animal Science/Dairy), Virginia Polytechnic Institute and State University, Blacksburg, Virginia, U. S. A.