MANIPULATION OF RUMINAL FERMENTATION TO ALTER MILK FATTY ACID COMPOSITION IN DAIRY COWS

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ABSTRACT

The objective of this study was to determine the effects of method of barley grain processing (dry-rolled *vs.* pelleted barley) and source of oilseed (ground canola *vs.* ground flaxseed), arranged as a 2 x 2 factorial, on feed intake, ruminal fermentation, nutrient flow to the duodenum, and milk production and composition in dairy cows. Eight Holstein cows (655 ± 69 kg; 83 ± 16 DIM) were used in a replicated 4 x 4 Latin square with 28-d periods. Cows in one square were fitted with ruminal and duodenal cannulae. Cows fed dry-rolled barley consumed 1.8 to 3.5 kg/d more ($P = 0.02$) DM than those fed pelleted barley; however, source of supplemental dietary fat had no effect on DM intake. Ruminal pH was lower ($P = 0.045$) in cows fed pelleted barley compared to those fed dry-rolled barley. Ruminal concentration of acetate was greater ($P = 0.001$), whereas ruminal concentration of propionate tended to be lower ($P = 0.11$), in cows fed dry-rolled barley compared to those fed pelleted barley; consequently, the acetate:propionate ratio was higher ($P = 0.01$) in cows fed dry-rolled barley compared to those fed pelleted barley. Ruminal concentration of total VFA was unaffected ($P > 0.05$) by diet. Source of dietary fat had no effect on ruminal digestion of OM, NDF, ADF or starch; however, ruminal starch digestion was slightly higher in cows fed pelleted barley compared to those fed dry-rolled barley (90.8 *vs.* 89.5%). Total dietary fatty acid intake was higher ($P < 0.05$) in cows consuming dry-rolled barley compared to those fed pelleted barley. Duodenal flow of $C_{18:0}$ was lower, whereas that of $C_{18:2n6c}$ was higher ($P < 0.05$) in cows fed pelleted barley compared to those fed dry-rolled barley. Feeding flaxseed increased duodenal flows of $C_{18:3n3}$, *cis*-9, *trans*-11 and *trans*-10, *cis*-12 conjugated linoleic acid compared to feeding canola. Milk yield was unaffected ($P > 0.05$) by diet; however, milk

fat content was higher (P = 0.004) in cows fed dry-rolled barley compared to those fed pelleted barley. Milk fat content of $C_{18:3}$ was higher (P = 0.005) in cows fed canola compared to those fed flax. Milk fat content of $C_{18:3}$ and *cis*-9, *trans*-11 $C_{18:2}$ were higher in cows fed pelleted barley compared to those fed dry-rolled barley with flax as the source of oilseed, but not with canola (interaction, $P < 0.01$). Milk fat content of saturated fatty acids decreased ($P < 0.001$) and that of polyunsaturated fatty acids increased (P = 0.003) in cows fed pelleted barley compared to those fed dry-rolled barley. In summary, milk fatty acid profiles were altered by method of grain processing and source of oilseed.

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LIST OF ABBREVIATIONS

ADF – acid detergent fibre

CLA – conjugated linoleic acid

DMI – days in milk

DM – dry matter

DMI – dry matter intake

FA – fatty acid

GC – gas chromatography

MFD – milk fat depression

MUFA – monounsaturated fatty acids

NDF – neutral detergent fibre

NEFA – nonesterified fatty acids

NS – not statistically significant

OM – organic matter

PUFA – polyunsaturated fatty acids

SFA – saturated fatty acids

TMR – total mixed ration

UFA – unsaturated fatty acids

WDD – whole duodenal digesta

1.0 GENERAL INTRODUCTION

Bovine milk fat is highly saturated even though diets that are consumed by lactating ruminants are generally unsaturated in nature (Shingfield and Griinari, 2007). In the past few decades, consumers have become increasingly aware of the negative effects of saturated fatty acids, including high cholesterol levels, atherosclerosis and coronary heart disease (Williams, 2000). As a result, there has also been a push to increase the amount of unsaturated fatty acids incorporated into dairy products due to their health benefits and critical roles on early immune and fetal development (Enke et al., 2008). The polyunsaturated fatty acid (PUFA) of utmost interest is conjugated linoleic acid (CLA). Conjugated linoleic acid has been found to reduce carcinogenesis, atherosclerosis, the onset of diabetes and body fat mass (Daley et al., 2005). Ruminant-derived food products are the main source of CLA (Parodi, 2003) and are a natural source of omega-3 PUFA (Dewhurst, 2005) for humans. Through this increasing consumer demand, significant research efforts have been directed towards determining how to increase the amount of PUFA and decrease the amount of saturated fatty acids being supplied in bovine milk. Many of these studies have been aimed at manipulating rumen biohydrogenation of dietary unsaturated FA, such that duodenal flow of these FA is increased.

The process of ruminal biohydrogenation is whereby 70 to 90% of dietary unsaturated fatty acids are converted to saturated fatty acids (Chilliard, 1993). It is facilitated by rumen microbes (Jenkins, 1993) with its end purpose ensuring that the toxic effects of unsaturated fatty acids on bacterial growth is reduced (Harfoot and Hazlewood, 1998). Once ruminal biohydrogenation has occurred, the saturated end products are

transported to the small intestine for absorption and incorporation in milk (Jenkins, 1993). However, some fatty acids are able to escape biohydrogenation and remain in an unsaturated form (Wu et al., 1991), such as CLA. However, in order for the dietary unsaturated fatty acids to reach the small intestine in the form of CLA, the rumen biohydrogenation pathway requires manipulation. Such changes can be achieved through altering rumen bypass ability (Khorasani et al., 1991), changing the rumen environment (Latham et al., 1972) and the feed ingredients used (Knapp et al., 1991).

There are numerous CLA isomers, however there are two that are considered to be the most biologically active, *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA. These isomers have the ability to influence human health and depress milk fat production (Lawson et al., 2001; Bauman and Griinari, 2003). Milk fat depression (MFD) is a phenomenon that occurs from particular diets that are influential in markedly reducing milk fat content and yield, as well as altering the fatty acid profile (Bauman and Griinari, 2001). These diets generally contain a high proportion of rapidly fermentable carbohydrate relative to fibre content (Dewhurst, 2005) and/or highly unsaturated fat supplements (Davis and Brown, 1970). However, in today's milk market, where milk fat is the major marketable component of milk, MFD can lead to a decline in profits for producers.

Common feedstuffs that are available in western Canada, such as barley grain, canola seed and flaxseed, are ideal for incorporation into dairy cow diets. These ingredients are generally fed in a processed form to ensure the availability of all nutrients. Barley grain is a rapidly fermentable carbohydrate source that can be used to manipulate ruminal fermentation characteristics; and flaxseed and canola have differing (Siemens

and Daun, 2005) and attractive fatty acid profiles that make them ideal for the production of biohydrogenation intermediates, such as CLA. When supplied together in a ruminant diet, processed barley grain and oilseeds can work in unison to produce a ruminal environment that is suitable for the production of unsaturated fatty acids that can subsequently be incorporated into milk fat, thus making milk more appealing to consumers. The major focus of this thesis was to investigate how interactions between barley grain processing and source of oilseed alter duodenal fatty acid flow and, subsequently, milk fatty acid composition.

2.0 LITERATURE REVIEW

2.1 Fatty Acid Composition of Ruminant Products

Lipids are a major constituent of milk fat, with triacylglycerols being the most predominant (98%; Grummer, 1991). Phospholipids and sterols are also found in the milk fat profile, however, in much smaller concentrations at 1 and 0.5%, respectively (Kennelly, 1996). The fatty acid composition of milk is highly saturated, regardless of the degree of unsaturation present in the diet (Kennelly, 1996). The chain lengths of fatty acids present in milk also tend to vary greatly. They can be short-chain (4 to 8 carbons), medium-chain (10 to 14 carbons) or long-chain (>16 carbons) (Bauman and Griinari, 2003).

Fatty acid metabolism in the rumen has a major influence on the fatty acid composition of ruminant meats and milk (Jenkins et al., 2008). While ruminant diets generally consist of unsaturated fatty acids, including *cis*-9, *cis*-12 $C_{18:2}$ and $C_{18:2n6c}$, ruminant products such as meat and milk contain much lower concentrations of the aforementioned fatty acids and contain higher amounts of saturated fatty acids (Shingfield and Griinari, 2007). It has been known for some time that tissue lipids of ruminants are more saturated than those of nonruminants (Banks and Hilditch, 1931). This difference in fatty acid content is due to the metabolism of unsaturated fatty acids in the rumen, which is known as rumen biohydrogenation (Shingfield and Griinari, 2007).

2.2 Health Concerns

In the past few decades, consumers have become increasingly health conscious

with regards to the effects of saturated and unsaturated fats on their health. As a result, manipulating the fatty acid content of bovine milk has been of considerable research interest since the 1980s. These research projects have been brought to the attention of numerous governments, which have subsequently put forth action requiring a reduction in total fat, saturated fat and cholesterol found in everyday consumer products (Jenkins and McGuire, 2006). Consumption of diets high in saturated fats has been considered to contribute to high cholesterol levels, atherosclerosis and coronary heart disease (Williams, 2000). On the other hand, PUFA have been associated with health benefits and such critical roles as the involvement on early immune and fetal development (Enke et al., 2008). There are numerous PUFA, including conjugated linoleic acid (CLA) and docosahexaenoic acid (DHA) that have beneficial attributes. CLA refers to numerous positional and geometric isomers of $C_{18:2n6c}$, in which there is a conjugated arrangement of the two double bonds instead of a methylene interruption (Parodi, 1999). Positive health benefits of CLA include reducing carcinogenesis, atherosclerosis, the onset of diabetes and body fat mass (Daley et al., 2005). The main source of CLA (Parodi, 2003) and natural source of omega-3 PUFA (Dewhurst, 2005) for humans is through the consumption of ruminant-derived food products. Attributes of other PUFA, such as DHA, include normal brain function, visual acuity and the prevention of cardiac arrhythmias (Horrocks and Yeo, 1999). Therefore, due to consumers increasing their consumption of unsaturated products, research attempts are focusing on increasing the amounts of unsaturated fatty acids present in milk.

2.3 Origin of Bovine Milk Fat

Milk fat can be derived from numerous sources. Approximately 50% of the fatty acids in milk are derived via *de novo* synthesis in the mammary gland (Bauman and Davis, 1974), 40 to 45% from the dietary source and less than 10% are derived from the mobilization of adipose tissue (Palmquist and Jenkins, 1980). *De novo* synthesis produces mainly short-chain and medium-chain fatty acids (Bauman and Griinari, 2003). This process occurs within the epithelial cells of the mammary gland (Neville and Picciano, 1997). Acetate produced from ruminal carbohydrate fermentation is the chief precursor in the production of these fatty acids. β-hydroxybutyrate, another fermentative product, also donates its four carbon skeleton for the formation of fatty acids produced in the mammary gland (Bauman and Griinari, 2003). Long-chain fatty acids are derived from lipids circulating throughout the body. Furthermore, 16 carbon unit fatty acids can be produced via *de novo* synthesis or via uptake of circulating lipids (Bauman and Griinari, 2003). The mammary gland has the ability to utilize preformed fatty acids and directly incorporate them into milk fat synthesis. Circulating lipoproteins and nonesterified fatty acids (NEFA) are the sources of these preformed fatty acids which originate from the digestive tract and from the mobilization of body fat reserves, respectively (Barber et al., 1997). A typical FA profile of bovine milk is illustrated in Table 2.0. The mammary gland also has the ability to convert $C_{18:1n11t}$, which is an intermediate of ruminal fatty acid biohydrogenation, into *cis*-9, *trans*-11 CLA (Bauman et al., 2003). The mechanism involved is the enzyme delta-9 desaturase, found in the mammary gland, which attaches to the $C_{18:1n11t}$ at its ninth carbon resulting in a more unsaturated product, CLA (Mir et al., 2003).

Table 2.0. Typical fatty acid profile of bovine milk (adapted from Bauman and Griinari, 2003).

Chain Length	Fatty Acid[1]	Molar %
Short-chain	4:0	12
	6:0	5
	8:0	2
Medium-chain	10:0	4
	12:0	4
	14:0	11
Long-chain	16:0	24
	16:1	3
	18:0	7
	18:1	24
	18:2	3
	18:3	1
	>18:3	<1

[1]4:0 – Butyric acid
6:0 – Caproic acid
8:0 – Caprylic acid
10:0 – Capric acid
12:0 – Lauric acid
14:0 – Myristic acid
16:0 – Palmitic acid
16:1 – Palmitoleic/Palmitelaidic acid
18:0 – Stearic acid
18:1 – Oleic/Elaidic/Vaccenic acid
18:2 – Linoleic/Linolelaidic/Rumenic acid
18:3 – α-linolenic acid
>18:3 – DPA/EPA/Arachidonic acid

The activity of this enzyme in the mammary system is responsible for synthesizing approximately 78% of the CLA that is found in cows fed a total mixed ration (TMR) (Griinari et al., 2000) and up to 91% in cows fed a traditional pasture diet (Kay et al., 2004).

Milk fat contains approximately 600 to 700 grams of saturated fatty acids (SFA), 250 to 350 grams of monounsaturated fatty acids (MUFA) and up to 50 grams of polyunsaturated fatty acids (PUFA) per kilogram milk fatty acids (Jensen, 2002). The main PUFA are $C_{18:2n6c}$, CLA and $C_{18:3n3}$ found typically at 20, 5 and 5 g/kg of milk fatty acids, respectively. MUFA is generally made up of two-thirds $C_{18:1n9c}$ (200 g/kg total fatty acids), with the remaining one-third consisting of *cis* and *trans* $C_{18:1}$ isomers (Dewhurst, 2005).

Milk fat concentration and its fatty acid composition can vary tremendously from cow to cow. The major factors that affect milk composition are diet, stage of lactation, genetic potential, age of the animal (Dewhurst, 2005) and season (Heinrichs et al., 1997). These factors will be discussed in greater detail in a later section.

2.4 Lipid Digestion in the Rumen

Rumen biohydrogenation is the process in which 70 to 90% of dietary unsaturated fatty acids are converted to saturated fatty acids (Chilliard, 1993). This process is facilitated by rumen microbes (Jenkins, 1993) and is pertinent in reducing the toxic effects that unsaturated fatty acids have on bacterial growth (Harfoot and Hazlewood, 1998). The initial step of rumen biohydrogenation involves the hydrolysis of the dietary triglyceride into a free fatty acid and a glycerol molecule (Jenkins, 1993). However, the

isomerase enzyme responsible for the hydrolysis will not catalyze this reaction unless there is a free carboxyl group available on the fatty acid (Kepler et al., 1970). Therefore, the rate limiting step in the hydrolysis or biohydrogenation of dietary triglycerides is the accessibility of a free carboxyl group (Jenkins, 1993). The schematic process of lipolysis and biohydrogenation is outlined in Figure 2.0.

The microbial populations that are mainly responsible for rumen biohydrogenation are ciliate protozoa, anaerobic bacteria and anaerobic fungi (Jenkins et al., 2008). Up to half of the rumen microbial biomass may be protozoal (Williams and Coleman, 1992). Microbes contain fatty acids and approximately three-quarters of all the fatty acids present in the rumen may be present in protozoa (Keeney, 1970). As a result, protozoa could be a very important contributor of PUFA, CLA and $C_{18:1n11t,}$ which are then incorporated into meat and milk (Jenkins et al., 2008).

There are two unsaturated fatty acids that are essential to the ruminant, namely $C_{18:2n6c}$ and $C_{18:3n3}$ (Morimoto et al., 2005). During the biohydrogenation of these fatty acids, several important intermediates are formed. Depending on dietary and ruminal factors, these intermediates can accumulate in the rumen, such that larger quantities can then flow post-ruminally and are absorbed at the small intestine whereby they can be incorporated into milk. Table 2.1 illustrates the difference in the fatty acid profiles of a TMR and the subsequent flow to the duodenum. These intermediates of rumen biohydrogenation include CLA isomers (Kepler and Tove, 1967), and $C_{18:1n11t}$, which are produced as the first stable intermediate of biohydrogenation and both are incorporated into milk fat (Harfoot and Hazlewood, 1997). The formation of these two intermediates is illustrated in Figure 2.1. Recent *in vivo* studies have revealed that there are many more

Lipolysis and Biohydrogenation

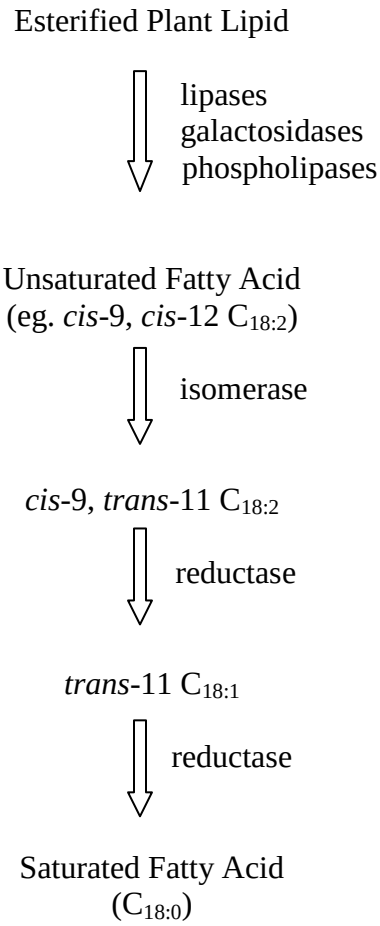

Figure 2.0. Key steps in the conversion of dietary lipids to saturated fatty acids via lipolysis and biohydrogenation in the rumen (Jenkins, 1993).

Table 2.1. Fatty acid intake and duodenal flow in dairy cows fed a low carbohydrate diet (Adapted from Loor et al., 2004).

Fatty Acid	Dietary Intake, g/d	Duodenal Flow, g/d
$C_{12:0}$	0.73	1.69
$C_{14:0}$	2.04	6.32
$C_{16:0}$	68.60	72.20
$C_{18:0}$	7.68	196.50
$C_{18:1n9c}$	56.40	30.90
$C_{18:2n6}$	96.70	26.00
$C_{18:3n3}$	82.10	9.69
Total	316.40	426.60

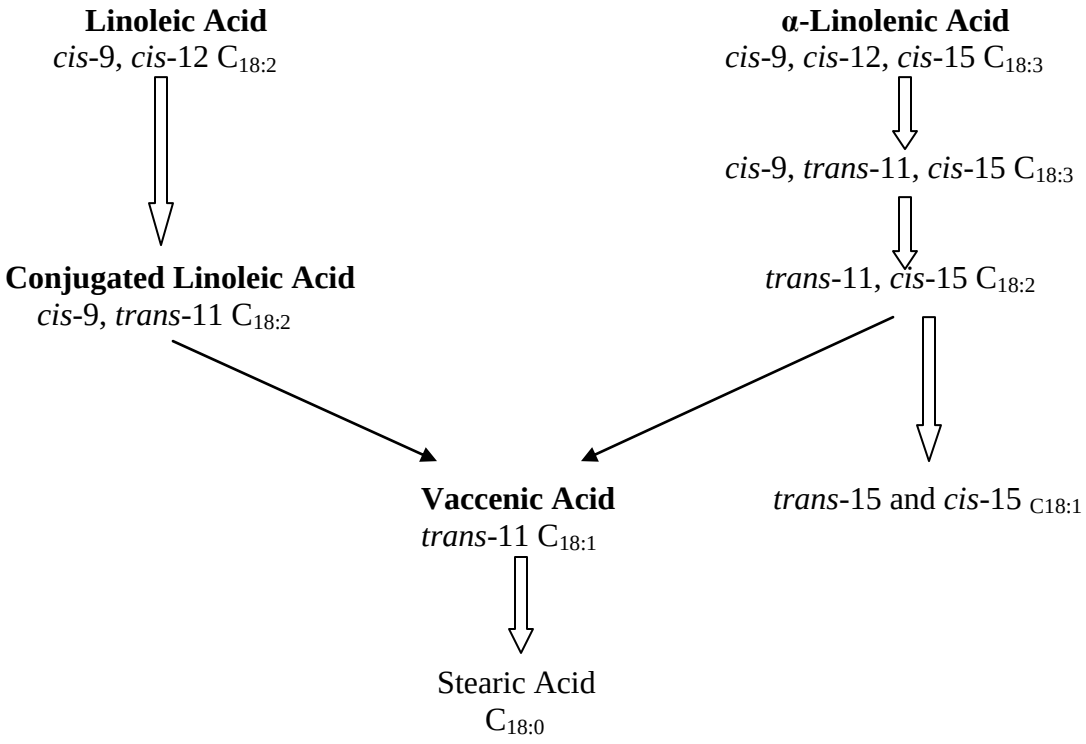

Figure 2.1. Biohydrogenation pathway of linoleic and linolenic acid with important intermediates CLA and Vaccenic Acid (Harfoot and Hazlewood, 1997).

intermediates that exist in ruminal contents than have been accounted for in the past; however, it is difficult to identify the origin of a specific intermediate as the animal's diet consists of an array of fatty acids (Jenkins et al., 2008).

Once biohydrogenation has occurred, the saturated end products, such as $C_{18:0}$, can be transported to the small intestine for absorption and, subsequently, to be deposited into animal tissues and products (Jenkins, 1993). However, some dietary fatty acids are able to escape rumen biohydrogenation (Wu et al., 1991), therefore remaining in an unsaturated form.

There are numerous isomers of CLA; however, the most biologically active ones, *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA, have been the main focus of several studies. This is due to their ability to influence human health and depress milk fat production (Lawson et al., 2001; Bauman and Griinari, 2003). *Cis*-9, *trans*-11 is the major CLA isomer, contributing to approximately 75 to 90% of total CLA found in ruminant fat. However, milk fat does contain most of the other 24 CLA isomers, but these are generally found at a concentration of 1% or less of total CLA (Bauman et al, 2008). Once these PUFA are formed through rumen biohydrogenation, they can be absorbed into the intestine and be transported into the mammary gland and, subsequently, be incorporated into milk fat.

2.5 Factors Affecting Milk Components

Milk fat concentration and its fatty acid composition can vary tremendously from cow to cow. The major factors that affect milk composition are diet, stage of lactation, genetic potential, age of the animal (Dewhurst, 2005) and season (Heinrichs et al., 1997).

2.5.1 Diet

The diet consumed by the cow can greatly influence the composition and yield of milk fat. The type of carbohydrate and fat source included in the ration can be altered in order to produce a ruminal environment that favours the production of unsaturated fatty acids. In order for dietary unsaturated fatty acids to reach the small intestine and mammary gland, manipulation of the ruminal biohydrogenation pathway is key. Such changes can occur through alterations in the ability of fatty acid substrates to pass through the rumen without degradation, and changes in the rumen environment and the feed ingredient used. There are numerous dietary factors that can manipulate the ruminal biohydrogenation pathway.

2.5.1.1 Protected Lipids

Feeding a protected lipid that has been chemically or physically altered to resist microbial biohydrogenation (Khorasani et al., 1991; Ashes et al., 1992) is one method of ensuring unsaturated fatty acids reach the small intestine. Feeding protected lipids is a common practice on dairy farms; however, they are expensive resulting in their limited use by the producer. Therefore, it is of importance to find alternate methods to increase the amount of dietary unsaturated fatty acids reaching the small intestine. This could be achieved by manipulating the ruminal biohydrogenation pathway. Furthermore, heat treatment of oilseeds may protect dietary fat from biohydrogenation via denaturing the protein matrix encapsulating the fat droplets. This would then result in increasing concentrations of PUFA reaching the small intestine (Kennelly, 1996).

Feeding fat supplements requires careful attention due to their individual and specific properties and their potential effects on biological processes (Ashes et al, 1997). There are numerous factors that should be considered when incorporating a fat supplement into a nutrition program. These factors include:

i. The lipid composition and to what degree the lipid is protected, with respect to the proportion of triacylglycerols to free fatty acids and the rate of ruminal lipolysis and the accumulation of *trans*-octadecenoic acids produced via the biohydrogenation pathway;

ii. The type of fatty acids supplemented, especially unsaturated C_{18} fatty acids, as they have the ability to inhibit microbial cellulolytic activity more than saturated fatty acids, which results in the production of *trans* isomers;

iii. The degree of protection or inertness in the rumen; with more protection and inertness, the deleterious effect on ruminal metabolism and milk synthesis decreases;

iv. Digestibility; as the proportion of unsaturated fatty acids increases, there is a positive correlation in intestinal digestion and absorption of long chain fatty acids;

v. The amount incorporated into milk; approximately 50% to 60% of the fatty acids are transferred into milk fat, but this is dependent on the quantity, composition and degree of ruminal digestibility and protection; and

vi. The potential effects on mammary gland lipogenesis. Supplements containing $C_{16:0}$ to $C_{18:0}$ fatty acids decrease synthesis of $C_{6:0}$ to $C_{14:0}$ and with increased

amounts of *trans* $C_{18:0}$ fatty acids, delta-9-desaturase and lipogenesis activity may be inhibited (Ashes et al., 1997).

2.5.1.2 Rumen pH Manipulation

Reducing the pH of the rumen is an effective method used to alter biohydrogenation. One way this can be achieved is by feeding a high grain diet. The lower ruminal pH reduces lipolysis (Latham et al., 1972) by inhibiting the activity of the lipase enzyme responsible for lipid breakdown (Lawson et al., 2001). Consequently, less biohydrogenation occurs, resulting in an increased amount of dietary unsaturated fatty acids reaching the small intestine and the mammary tissues. *In vitro* studies have also demonstrated that the inhibition of lipolysis increases with decreasing incubation pH (Van Nevel and Demeyer, 1996). This, in turn, affects the subsequent biohydrogenation process, ultimately decreasing it. This limiting effect alters the amounts and degree to which dietary unsaturated fatty acids are processed in the rumen in order to produce the fatty acids that are typically found in the milk. As a result, the fatty acid profile is altered and exhibits a much more unsaturated product.

Another method of altering ruminal pH is related to particle size and the rate of fermentation. Finely chopped forages and readily fermentable carbohydrates reduce chewing time and rumination. This depresses ruminal pH (Beauchemin et al., 1994), which thereby potentially reduces rumen biohydrogenation and increases the flow of biohydrogenation intermediates to the proximal duodenum. Feeding processed grain (pelleting, grinding, rolling, steam flaking) also has the ability to reduce rumen pH. Pelleting, grinding and rolling have the ability to change the physical structure of the

grain to varying degrees, thereby increasing fermentable surface area (Mason, 2005). This is especially true for finely ground feed, which is rapidly fermented in the rumen. As a result, there is an increase in the production of volatile fatty acids (VFA), which leads to a lower ruminal pH.

2.5.1.3 Whole Oilseeds

Feeding supplemental fat in the form of whole oilseeds may slow the rate at which fatty acids are released in the rumen, therefore decreasing exposure to rumen microbes responsible for biohydrogenation (Knapp et al., 1991). Oilseeds are a good source of unsaturated fatty acids, therefore the amount of CLA found in milk fat increases as the amount of PUFA, especially $C_{18:2n6c}$, increases in the diet of the lactating cow (Ward et al., 2002). However, the type of oilseed has an effect on the amount of CLA produced. Diets containing flaxseed tend to produce lower amounts of CLA in the milk than diets containing canola (Ward et al., 2002), although the amount produced from flaxseed is more than diets not containing any source of oilseed (Soita et al., 2003). These observations are expected due to canola seed containing significantly more $C_{18:2n6c}$ than flaxseed (Siemens and Daun, 2005).

The proportion of saturated C_{18} that is generally found in the milk after rumen biohydrogenation is decreased when unsaturated fatty acids are fed. Also, incomplete biohydrogenation occurs to a greater extent in cows that are fed rations containing high levels of polyunsaturated fats (Kalscheur et al., 1997). The degree to which unsaturated fatty acids are biohydrogenated is highly correlated to rumen conditions. Factors including diet fatty acid composition, species of ruminal bacteria present and the pH of

ruminal fluid all have the ability to influence the biohydrogenation of unsaturated fatty acids (Kalscheur et al., 1997).

Coupling these two methods of feeding processed grain and source of whole oilseed has the potential to reduce the extent of biohydrogenation and increase the amount of dietary unsaturated fatty acids reaching the small intestine and subsequently incorporated into the milk, and this was a major objective of this thesis research.

2.5.2 Age and Stage of Lactation

The age of the animal at first lactation can have an effect on the level of milk volume produced, as well as fat and protein concentrations (Lin et al., 1988). Generally there is significantly less milk (Ettema and Santos, 2004), fat and protein produced during the first lactation of early-bred heifers (Lin et al., 1988). Milk fat and protein percentage tend to decrease by approximately 0.2% and 0.02% to 0.05%, respectively, each year from the first to fifth lactation of the cow. This is generally due to an increase in the incidence of udder infections (Heinrichs et al., 1997).

Milk components also tend to be affected by stage of lactation. Early-lactating dairy cows are unable to consume adequate DM to meet their nutrient demands for maintenance and milk production. Consequently, early-lactating dairy cows experience a negative energy balance, and they mobilize body tissue, particularly adipose tissue. This results in an influx of nonesterified fatty acids (NEFA) present in the circulating blood and the subsequent incorporation of long-chain fatty acids into milk fat. As a result, *de novo* synthesis is down regulated and the production of short-chain fatty acids is limited (Bauman and Davis, 1974). However, the concentration of these short-chain fatty acids

increases to its maximal proportions (>90%) by week 8 of the lactation curve. In correlation, the inhibition of adipose mobilization is generally completed by the 4th to 6th week of the lactation curve. At peak lactation, milk fat content declines; however, the fatty acid profile of milk fat remains fairly consistent (Garnsworthy and Hugget, 1992). Bormann et al., (2002) found that lactation curves adjusted for test-day fat yield were quite flat with a slight decrease over the entire lactation for first parity cows, whereas there was a steeper slope observed for second and later parities. In dairy ewes, typically, milk fat and protein are at their lowest concentrations when the cow is at peak lactation and tend to increase as the lactation curve progresses, differing from lactose, which is generally at its highest concentration early on and begins to decline as DIM increases (Othmane et al., 2002).

2.5.3 Genetic Potential and Breed Differences

Dairy breeding systems have become more popular and multiple programs can be implemented on the same dairy operation. Such programs need to be cognizant of the full array of traits that are translated into a cow's lifetime performance and the potential of the additive and nonadditive genetic variation that control these traits (McAllister et al., 1994). It has been concluded that genetic correlation of production traits are positive and high; therefore, it is unlikely that milk fat production can be reduced while selecting for increasing milk and protein production (Everett, 1990). There is a marked difference in milk fat, milk fatty acid profile and milk volume amongst the dairy breeds. When stage of lactation (DIM) and parity are taken into account, Holsteins tend to have higher milk production with lower concentrations of fat and protein, whereas Jerseys have the

lowest production level with the highest concentration of fat and protein (Bleck et al., 2009; Parrish et al., 1950). For example, Palmquist and Beaulieu (1992) found, based on the percentage of total milk fatty acids and independent of any potential diet influences, $C_{6:0}$ to $C_{14:0}$ fatty acids were 8 to 42% higher in the Jersey breed compared to Holstein Friesians. However, Ayrshires, Guernseys and Brown Swiss generally have intermediate levels of milk production and fat and protein concentrations (Bleck et al., 2009; Parrish et al., 1950). Pure breeds can be equal or superior to crossbred animals; however, if the crossbred has the right combination of superior traits for the component of interest, that situation is ideal.

2.5.4 Season and Temperature Effects

Time of year has a dramatic effect on milk fat and protein levels. Lactating cows produce a large amount of metabolic heat and accumulate extra heat from radiant energy. This heat production and accumulation in conjunction with an inhibited cooling capacity, the heat load experienced by the cow increases to the point where the internal body temperature rises and production declines (West, 2003). As the temperature begins to cool down in the fall, milk production, fat and protein levels begin to increase again. These changes may be indicative of the cow's feeding patterns, as DMI tends to decline in the summer and increase in the winter (Heinrichs et al., 1997; Warntjes et al, 2008). Prolonged heat waves have been associated with decreases in DMI and, subsequently milk yields (Aharoni et al., 2005) of lactating animals (Collier et al., 2006). A slight increase in temperature can have a numerical effect on DMI, fat and protein yield; however, the differences are not significant. Cows fed during the day throughout the

summer compared to cows fed during the night ate significantly less and yielded less fat and protein (Aharoni et al., 2005). Depressing DMI in combination with heat stress can potentially lead to bouts of ruminal acidosis due to cyclical intakes of high energy feeds (Collier et al., 2006). As a result, rumen biohydrogenation may decrease, resulting in an increase in unsaturated intermediates flowing to the duodenum and their subsequent incorporation into milk fat. Also, altering DMI may cause disproportionate ingestion of feedstuffs causing unbalanced fatty acid intakes which under heat stress conditions can lead to a decrease in $C_{17:0}$ and $C_{18:0}$ production (Warntjes et al., 2008).

2.6 Milk Fat Depression

Milk fat yield and composition can be manipulated by several environmental factors, predominantly through nutrition (Bauman and Griinari, 2001; Shingfield and Griinari, 2007). One example of milk fat manipulation is the occurrence of lowering the overall fat yield. This phenomenon is also known as milk fat depression (MFD), whereby particular diets are influential in markedly reducing milk fat content and yield, as well as altering the fatty acid profile (Bauman and Griinari, 2001). In today's milk market, where milk fat is the major marketable component of milk, MFD can lead to drastically reduced economic returns for producers.

Ruminant diets causing MFD can be divided into two groups (Davis and Brown, 1970). The first group includes diets that contain a large proportion of rapidly degradable carbohydrates and small amounts of fibrous feeds, the most common being a high grain/low forage diet (Dewhurst, 2005). The second group of diets includes dietary supplements that contain highly unsaturated oils.

2.6.1 Forage to Grain Ratio

Diets containing more than 50% of the dry matter intake as readily fermentable starch are the most effective in lowering milk fat (Ashes et al, 1997). However, diets that are considered to have adequate amounts of roughage, but are fed ground, pelleted or very small in particle size, also fall into this category, as the effectiveness of this fiber is inadequate to ensure proper rumen function. Salivation and rumination are decreased leading to a more acidotic environment, thereby leading to a suitable environment for MFD. Dietary forage particle size and the forage:concentrate ratio has the ability to greatly influence ruminal pH. As a result, the ratio may influence ruminal biohydrogenation of supplemental fats, and the subsequent postruminal flow of unsaturated fatty acids and their inclusion into milk fat (Soita et al., 2005).

2.6.2 Fat Supplements

Adding oil supplements directly to the ration can induce MFD, as well as supplementing diets with whole oilseeds or oilseed meal containing polyunsaturated fatty acids (Davis and Brown, 1970; Dewhurst, 2005). Generally during MFD, the concentrations of biohydrogenation intermediates tend to increase. Milk fat concentration and yields also decrease linearly as the amount of unsaturated fat supplementation in the diet increases (Harvatine and Allen, 2006). This is due mainly to the increasing amount of *trans*-10, *cis*-12 CLA that is produced via ruminal biohydrogenation (Harvatine and Allen, 2006) and its biological activity in reducing lipogenic activity (Baumgard et al., 2002).

2.6.3 Milk Fatty Acid Profile Changes

The reduction of milk yield also affects the type of fatty acids produced. However, the most noticeable decline is in the amount of fatty acids derived through *de novo* synthesis (Davis and Brown, 1970). With this marked decline, there is a substantial increase in the production of *trans* $C_{18:1}$ fatty acids. As a result, there is a shift towards a milk composition favoring more long chain fatty acids and fewer short and medium chain fatty acids.

2.6.4 Milk Fat Depression Theories

Numerous theories have been proposed over the last 50 years to elucidate the mechanisms of MFD; however, only a single theory is now widely accepted in the scientific community (Bauman and Griinari, 2001). The major theories that have been suggested as causative mechanisms of MFD are discussed below.

2.6.4.1 Rumen Production of Acetate and Butyrate Theory

Reductions in acetate and β-hydroxybutyrate production in the rumen, thereby limiting substrates that are available for *de novo* fatty acid synthesis, is thought to be a contributing factor to decrease milk fat synthesis. This theory suggests that a decrease in the production of acetate and butyrate, due to feeding low fibre diets, in the rumen will ultimately limit milk fat synthesis thereby contributing to milk fat depression. However, an increase in the amount of propionate produced is the major influence in skewing the acetate:propionate ratio (Bauman et al., 1971). Consequently, these alterations ultimately

change the ratios of butterfat and lactose precursors and affect the amount of milk fat produced (Oldham and Emmans, 1988). Therefore, this theory is now generally disregarded.

2.6.4.2 Glucogenic-Insulin Theory

Increased propionate and glucose production, which is responsible for stimulating insulin secretion, thereby causing fatty acids to be preferentially partitioned towards adipose tissue rather than the mammary gland, is also thought to have an effect of mild fat depression. This theory, originally proposed by McClymont and Vallance (1962), suggests that there is a competition for nutrients between mammary glands and nonmammary tissues and their differences in tissue response to the availability of insulin. Low fiber diets tend to increase the energy intake and reduce secretions of milk fat. As a result, insulin levels in circulating blood are elevated and, due to insulin-induced pathways, nutrients are diverted from the mammary gland to adipose tissue, thereby causing a shortage of milk fat precursors (Bauman and Griinari, 2003). This theory is not thought to be major, but cannot absolutely be discounted.

2.6.4.3 Methylmalonic Acid Theory

The inhibition of *de novo* fatty acid synthesis by methylmalonate arising from decreasing vitamin B_{12} and increasing propionate synthesis possibly contributes to milk fat depression. This theory postulates that increasing amounts of propionate delivered to the liver decreased the activity of methylmalonyl CoA mutase and, thereby, produces a processing bottleneck leading to the accumulation of methylmalonic acid. The

accumulated acid then enters the bloodstream where, upon reaching the mammary gland, impairs milk fat synthesis (Frobish and Davis, 1977). Acceptance of this theory is also generally disregarded.

2.6.4.4 Trans Fatty Acid Theory

The direct inhibition by *trans* fatty acids derived from incomplete rumen biohydrogenation (Shingfield and Griinari, 2007) is the most current theory accepted as the mechanism contributing to milk fat depression. This theory that MFD occurs due to incomplete rumen biohydrogenation of dietary unsaturated fatty acids is continually receiving more support with more information arising from more researchers conducting relevant studies. Such a deviation in the biohydrogenation pathway is then responsible for producing specific *trans*-fatty acids, which have been associated with having a role in MFD (Bauman and Griinari, 2001).

Altering the rumen biohydrogenation of dietary unsaturated fatty acids seems to be a prerequisite for developing MFD, which leads to the aforementioned increase in *trans* $C_{18:1}$ fatty acids in milk fat. This alteration is generally induced by feeding diets that are supplemented with either plant or fish oils (Bauman and Griinari, 2001). However, diet-induced MFD is more closely correlated with changes in milk fat *trans* $C_{18:1}$ isomer profiles rather than solely due to an increase in the amount of milk fat *trans* $C_{18:1}$ (Griinari et al., 1998).

As a result, it can be concluded that MFD is derived from two conditions. These include: 1) an alteration in rumen microbial processes; and 2) the presence of unsaturated fatty acids. Due to these conditions, products of rumen bacteria are produced via the

diet-induced shifts through rumen microbial processes and the intake of unsaturated fatty acids (Bauman and Griinari, 2001).

The biohydrogenation theory is the most recent to explain the mechanisms of how MFD occurs. The theory is based on the ideology that ruminant diets that are responsible of MFD also alter ruminal lipid metabolism, which results in the formation of specific biohydrogenation intermediates in the rumen that can directly inhibit milk fat synthesis (Shingfield and Griinari, 2007). Numerous studies have been conducted in order to determine the isomer responsible for MFD. Results have identified that subjecting dairy cows to infusions of *trans*-10, *cis*-12 CLA for five days reduced milk fat content and yield by 42 and 48%, respectively (Baumgard et al., 2002). Once infusions ceased, milk fat levels returned to normal (Baumgard et al., 2000). Also, in contrast, *cis*-9, *trans*-11 CLA had no effect on the fat content or yield of bovine milk (Bauman et al., 2008). Therefore, the intermediate that has been observed, without a doubt, to inhibit milk fat synthesis is *trans*-10, *cis*-12 CLA. The fatty acid profile is also altered by *trans*-10, *cis*-12 CLA. Baumgard et al. (2002) found that the majority of the fatty acids produced via *de novo* synthesis decreased in concentration, whereas the concentration of the preformed fatty acids present increased.

Gene expression in the mammary system is also affected by *trans*-10, *cis*-12 CLA, whereby many enzymes involved in lipogenesis such as fatty acid synthetase (FAS), acetyl CoA carboxylase (ACC) and stearoyl CoA desaturase (SCD) are down regulated by 40, 39 and 48%, respectively (Baumgard et al., 2002). Decreasing the activity of these enzymes is strongly correlated with the changes that are occurring in the fatty acid profile during MFD. This correlation relates to FAS and ACC being the key

enzymes in *de novo* synthesis (Baumgard et al., 2002). The decrease in the amount of milk fat produced due to *trans*-10, *cis*-12 CLA infusions can be attributed to the 42 and 41% decline in the glycerol phosphate acyltransferase (GPAT) and acylglycerol phosphate acyltransferase (AGPAT) concentrations, respectively, which are involved in milk fat triglyceride synthesis (Baumgard et al., 2002). Other studies have suggested that there may be two other CLA isomers (Bauman et al., 2008) that work in conjunction and may also be responsible for depressing milk fat (Shingfield and Griinari, 2007). However, due to the limited amount of studies conducted in this area, such results should be interpreted cautiously.

2.7 Dairy Ration Feedstuffs

In western Canada, barley grain is the most common cereal/carbohydrate source fed to dairy cows. It is commonly fed in either a dry-rolled or pelleted form. Processing of the grain is imperative to ensure that the nutrients contained in the starch portion of the kernel can be digested and utilized efficiently for energy by the animal. Protein sources commonly included in dairy rations are generally related to whole canola seed and flaxseed. Usually, they are further processed to ensure the availability of all nutrients.

Canola seed is one of many oilseeds that have been extensively researched as a source of protein and supplemental fat in ruminant diets. However, it has an almost impenetrable pericarp, and for effective digestion of the protein, lipid and carbohydrate portions of the seed by ruminants, canola must further be processed (Wang et al., 1997). Whole canola seed contains a lipid content of approximately 43% (Canadian Grain Commission, 2009 www.grainscanada.gc.ca/canola/export-exportation/ceqd-dqec-

eng.htm), of which more than 85% consists of fatty acids of 18 carbons in length. Of these fatty acids, the most predominant is $C_{18:1}$, contributing to more than 60% of the total fatty acids in the seed (Ackman, 1990). Due to the fat content in the seed, when incorporated into dairy diets, it is generally considered to be a supplement in order to supply excess energy. When included in livestock rations, the economic merit of canola is proven due to its high nutrient supplying power and the increase in animal production.

Flaxseed is not included in dairy rations as commonly as canola, but is gaining popularity due to its competiveness in supplying a nutrient profile similar to canola. Flaxseed contains, on average, 45% oil; however, the oil content can vary due to the growing conditions and moisture availability (Canadian Grain Commission, 2008 www.grainscanada.gc.ca/Quality/flaxcdn/flaxq-e.htm). Crop years that tend to be hotter and drier produce flax seed that has a lower oil content. However, the effect on the protein content varies inversely with the oil content, therefore protein content increases as oil levels decrease.

Canola seed and flaxseed have differing fatty acid profiles, especially with regard to the amount of $C_{18:2n6c}$ and $C_{18:3n3}$. Canola seed contains 22% $C_{18:2n6c}$ and 12% $C_{18:3n3}$, whereas flaxseed contains 15% $C_{18:2n6c}$ and 59% $C_{18:3n3}$ (Siemens and Daun, 2005). The fatty acid profiles of these oilseeds make them ideal for the production of biohydrogenation intermediates through the rumen biohydrogenation pathway. Canola seed and flaxseed are also practical for use in western Canadian dairy operations. However, there are limited comparative studies conducted with canola and flax as supplemental fat sources. Many research projects in the past have studied barley processing independently or source of oilseed independently; however here is a lack of

information from studies investigating the interactions of barley processing and source of oilseed on ruminal characteristics and milk production. Therefore, the study conducted was of great importance to the Western Canadian dairy industry, with respect to the possible economic and human health benefits for consumers.

2.8 HYPOTHESIS

The hypothesis for this thesis research was that manipulating ruminal biohydrogenation via barley grain processing (dry-rolled *vs.* pelleted) and feeding oilseeds varying in fatty acid content (ground canola seed *vs.* flaxseed) would alter the profiles of fatty acids flowing to the proximal duodenum and, subsequently, milk fatty acid profiles in lactating dairy cows.

2.9 OBJECTIVES

The overall objective of this proposed study was to investigate how interactions between barley grain processing (dry rolling *vs.* pelleting) and source of oilseed (canola *vs.* flaxseed) affect:

1. Feed intake;
2. Ruminal fermentation characteristics;
3. Duodenal nutrient and fatty acid flows; and
4. Milk production and composition, and fatty acid profiles.

3.0 MATERIALS AND METHODS

3.1 Animals and Experimental Design

Eight multiparous Holstein cows (655 ± 69 kg; 83 ± 16 days in milk) were used in a replicated 4 x 4 Latin square design trial with a 2 x 2 factorial arrangement of dietary treatments. One square consisted of 4 cows that were fitted with ruminal and duodenal cannulae and were used in a metabolic study. Ruminal cannulae were 10 cm in centre diameter (Cannula #1C, Bar Diamond, Parma, ID). Duodenal cannulae were simple T-shaped and were inserted proximal to the common bile and pancreatic duct, approximately 10 cm distal to the pylorus. Throughout the experiment, animals were housed individually in tie-stalls at the Greenbrae Dairy Research Facility (University of Saskatchewan). Each experimental period consisted of 14 d of dietary adaptation and 14 d of data collection. Cows were cared for and handled in accordance with the Canadian Council of Animal Care regulations, and their use in the experiment was approved by the University of Saskatchewan Animal Care Committee (UCACS Protocol No. 20040048).

3.2 Experimental Treatments and Feeding Management

Experimental diets were combinations of barley grain processing (dry-rolling or pelleting) and two sources of dietary supplemental fat (ground canola seed or ground flaxseed) as follows: a) dry-rolled barley + ground canola seed; b) dry-rolled barley + ground flaxseed; c) pelleted barley + ground canola seed; and d) pelleted barley + ground flaxseed. The diets were formulated to be isonitrogenous and isolipidic (Table 3.0). Dry-rolled barley was prepared by passing whole barley grain through large rollers (23 x 58

Table 3.0. Ingredient and chemical composition of total mixed rations with dry-rolled or pelleted barley containing canola or flax as supplemental fat sources.

	Canola		Flax	
Item	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley
Ingredients, % DM				
Barley silage	32.46	32.46	32.48	32.48
Alfalfa hay	17.24	17.24	17.26	17.26
Barley grain	29.81	29.81	29.39	29.39
Canola seed	8.13	8.13	-	-
Flax seed	-	-	8.30	8.30
Soybean meal	3.55	3.55	3.75	3.75
Molasses	0.40	0.40	0.40	0.40
Canola meal	3.49	3.49	3.49	3.49
Corn gluten meal	0.96	0.96	0.96	0.96
Wheat dried distillers grains	1.78	1.78	1.78	1.78
Dynamate[1]	0.18	0.18	0.18	0.18
Salt, Co-I[2]	0.33	0.33	0.33	0.33
Mineral-vitamin supplement[3]	1.66	1.66	1.66	1.66
Analyzed Chemical composition				
DM, %	57.4	58.1	57.4	58.4
Starch, % of DM	23.0	22.6	24.7	23.83
CP, % of DM	16.6	16.6	16.5	16.0
NDF, % of DM	35.9	35.2	36.0	35.8
ADF, % of DM	20.8	20.8	21.1	21.1
Crude fat, % of DM	5.3	5.3	5.2	5.3
Particle Size, % TMR DM				
> 19 mm	7.00	5.56	8.31	8.17
8 - 19 mm	57.74	62.62	52.42	58.92
1.1 - 8.8 mm	30.69	27.35	34.71	28.75
< 1.18 mm	4.57	4.47	4.56	4.17

[1]Contained 18% K, 11% Mg, and 22% S.
[2]Salt, cobalt and iodine mix.
[3]Contained (per kg of premix; DM basis): 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1000 IU of vitamin E, 16% Ca, 8.5% P, 6.3% Na, 4.5% Mg, 2,100 mg Zn, 1,500 mg Mn, 535 mg Cu, 12 mg Se, 45 mg I.

cm). For pelleting, whole barley grain was ground through a 6.25 mm screen in a hammer-mill and then pelleted using a California pellet mill. Both dry-rolled and pelleted barley were from the same source. Whole flaxseed and canola seed were ground through a hammermill with screen size of 2.78 mm and 1.19 mm, respectively. Cows were fed for ad libitum intake twice daily at 0900 and 1600 h. Feed was offered as a total mixed ration (TMR). The forage:concentrate ratio of the TMR was 50:50. The forage component of the TMR was a mixture of barley silage and chopped alfalfa hay.

3.3 Sample Collection

The weight of feed offered and refused was recorded daily throughout the 14 d collection period. Samples of TMR and orts were collected three times weekly during the data collection period (d 15 to d 28) and stored at -20^{o}C for later determination of chemical composition. Particle size distribution of TMR (as fed) were determined using the 4-screen Penn State Particle Size Separator (PSPS) as described by Kononoff et al. (2003) on d 23 to d 25. Briefly, sub-samples of TMR were manually sieved through three screens of >19 mm, >8 mm and >1.18 mm, with particles <1.18 mm being collected in a bottom pan. The sample remaining on the top of each of the three screens and bottom pan was subsequently weighed in order to determine the TMR particle distribution (Kononoff et al., 2003).

Cows were milked twice daily starting at 0430 and 1530 h, and milk yield was recorded during the 14 d data collection period. Milk samples were collected into plastic vials with or without preservative (2-bromo-2-nitropropane-1-2-diol) on d 25, 26, and 27 from the morning and afternoon milkings. Daily milk samples from the two milkings for

each cow were pooled based on milk yield. Pooled milk samples with preservative were sent to the Provincial Milk Testing Laboratory (Saskatchewan Ministry of Agriculture, Regina, SK). Pooled milk samples without preservative were frozen at -20^{o}C until further analysis for fatty acid composition.

Total tract nutrient digestion, ruminal fermentation characteristics and nutrient flow to the duodenum were determined using the 4 cannulated cows in one square. Total tract nutrient digestion was based on total collection of feces and urine between d 15 and d 19 of each experimental period. Feces were collected into large steel trays which were positioned over the gutter behind each stall. Daily fecal output for each cow was determined by weighing and feces were then mixed thoroughly before 2.5% of the daily output was sampled and stored at -20°C for later chemical analysis. Total urine output was collected using indwelling Bardex Foley bladder catheters (26 Fr, 75 cc ribbed balloon, lubricious-coated; C. R. Bard Inc., Covington, GA). Catheters were inserted at 0900 h on d 14, and were then connected to urine collection tubing on d 15. Urine was collected into 20 L Carboy polyethylene containers.

Starting on d 25 of each experimental period, ruminal pH was measured continuously for three consecutive days using the Lethbridge Research Center Ruminal pH Measurement System (Dascor, Escondido, CA, USA) (Penner et al., 2006). Ruminal pH readings were taken every 30 seconds, stored in a data logger (model M1b-pH-1KRTD, Dascor, Escondido, CA, USA) and downloaded daily. Calibration of ruminal pH electrodes was conducted daily (Penner et al., 2006).

To quantify digesta flow to the duodenum, $YbCl_3$ (Siddons et al., 1985) and CrEDTA (Udén et al., 1980) were used as digesta markers for the solid and liquid phases,

respectively. Briefly, just before marker infusions into the rumen were initiated on d 22, samples of whole ruminal contents and whole duodenal digesta were taken from each cow to determine background concentration of Cr and Yb. At the beginning of the infusions, priming doses equal to one-half of the daily dose of Yb and Cr were administered via the ruminal cannula. Thereafter, between d 22 and d 28, marker solutions prepared in distilled water and containing Yb (2.2 g/d) and Cr (2.7 g/d) were then continuously infused using an automatic peristaltic pump (model 205U; Watson Marlow, Cornwall, England) into the rumen at a constant rate of 1,000 mL/d. Sampling of whole ruminal contents and whole duodenal digesta began at 0700 h on d 27 and was conducted every 2 h, for approximately 20 min, until 0500 h on d 28, to represent the 24 h-feeding cycle. At each sampling, 300 mL of whole duodenal digesta (WDD) was taken and kept frozen at -20^{o}C for later analysis. At the same time-points as for the duodenal sampling, 1,000 mL of ruminal contents were collected by manually taking 250 mL of ruminal contents (solid and fluid fractions) from the cranial ventral, caudal ventral, central and cranial dorsal rumen through the cannula. Rumen fluid samples were obtained by squeezing ruminal contents through four layers of cheesecloth. Two 5.0 mL aliquots of strained ruminal fluid were mixed with 1.0 mL of metaphosphoric acid (25% wt/vol) and stored at -20°C for later determination of ruminal volatile fatty acids (VFA).

4.0 Sample Analyses

4.1 Chemical Composition of TMR, Feces and WDD

The weekly TMR and fecal samples were composited by cow for each period and

then dried in an oven at 55°C for 48 h to determine dry matter (DM) content (AOAC, 1990). Dried TMR samples were then ground through a 1 mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England). Dried fecal samples were ground through a 1 mm screen using a ZM-1 Retsch (Brinkmann Instruments Ltd., ON, Canada). The TMR and fecal analytical DM content were determined by oven drying at 135°C for 2 h (AOAC, 1990, method no. 930.15). The TMR and fecal crude protein (CP) content was determined using the macro-Kjeldahl procedure using a Kjeltec 2400 auto-analyzer (AOAC, 1990, method no. 984.13). The dried whole duodenal digesta (WDD) samples were analyzed for CP by using the LECO FP-528 analyzer (LECO Corporation, St. Joseph, MI). The TMR and fecal neutral detergent fibre (NDF) and acid detergent fibre (ADF) (AOAC, 1990, method no. 973.18) contents were determined in sequence using a digestion procedure (ANKOM 200, ANKOM Technology Corp., Fairport, NY) that utilized sodium sulphite and alpha-amylase for NDF as described by Van Soest et al. (1991). Starch content of the TMR, feces and duodenal digesta was determined using a total starch assay kit (Megazyme, Bray Co., Wicklow, Ireland). The crude fat content of the TMR, fecal and duodenal digesta was determined using ether to extract the fat as described by AOAC (1990, method no. 920.39).

4.2 Yb and Cr Analysis

Whole duodenal digesta (WDD) samples were thawed at room temperature and composited by animal and period, before being separated into 2 phases for determination of Yb and Cr marker concentration. Liquid (LDD) and solid (SDD) duodenal digesta phases were separated by filtering through double-layered cheesecloth. Samples of

WDD, LDD and SDD were freeze-dried and then ground through a 1-mm screen. The concentrations of Cr and Yb in LDD and SDD were determined by atomic absorption spectrophotometry (Perkin Elmer 2300, Perkin-Elmer Corp, Norwalk, CT) and atomic emission spectroscopy (Varian Spectra 220 Atomic Absorption Spectrometer, Varian, Mulgrave, VIC3170, Australia), respectively, after sample digestion by the method described by Lopez et al. (1988) and Vicente et al. (2004). Concentrations of Yb and Cr in LDD and SDD were then used to physically recombine DM from the freeze-dried LDD and SDD in the correct proportions to reconstitute the duodenal true digesta (DTD) reaching the duodenum using the double-marker method (France and Siddons, 1986).

4.3 Milk Composition and Fatty Acid Profiles

Pooled milk samples with preservative were sent to the Provincial Milk Testing Laboratory (Saskatchewan Ministry of Agriculture, Regina, SK) for milk protein, fat and lactose analyses using a near infrared analyzer (Bentley 2000) according to AOAC (1990, method no. 972.16). Milk urea nitrogen was analyzed using a Beckman analyzer (Beckman Instruments, California, USA).

Pooled milk samples without preservative were thawed at 38^{o}C in a water bath according to AOAC (1990) method 925.21. Fat was extracted using a technique based on Bligh and Dyer (1959). A 5 mL aliquot of milk was mixed with 25 mL of chloroform and 50 mL of methanol (MeOH) for 2 min in a stoppered round bottom Erlenmeyer flask. To this solution, 25 mL of chloroform was added and mixed for 30 sec, which was followed by the addition of 25 mL of double distilled water. This solution was mixed for 30 sec and 25.5 mL of chloroform was then added and subsequently mixed for 1 min.

The solution was filtered through a Whatman 9 cm #1 filter paper using a Buchner funnel with slight suction into a 250 mL side-arm Erlenmeyer flask. The filtered solution was then transferred into a 250 mL separatory funnel and approximately 1.0 g tert-butylhydroquinone (antioxidant) (Sigma-Aldrich, Inc.) crystals were added to the funnel before it was capped with a glass plug and allowed to sit and separate for a minimum of 12 h. The chloroform layer was filtered into a graduated cylinder through a #1 filter paper containing roughly 1 g of anhydrous sodium sulphate to absorb any excess water. The volume of chloroform and the amount of fat were recorded. An aliquot of 5 mL of the chloroform was transferred to an aluminum weigh boat which was previously pre-weighed and dried overnight in an oven at 100^{o}C. The chloroform was allowed to completely evaporate in a fumehood and the weigh boat was then placed in a 100^{o}C oven for 1 h. The remaining chloroform in the graduated cylinder was transferred into a round bottom flask which was subsequently evaporated using a rotovapor (Brinkmann R110, Rexdale, ON). The fat was transferred into a round bottomed screw-top test tube and any remaining chloroform was evaporated under a stream of nitrogen. The fat sample was flushed with nitrogen, capped and stored at -20^{o}C until methylation.

Fatty acids were methylated using sodium methoxide ($NaOCH_3$) as a catalyst, and quantified using GC (Hewlett-Packard, model 5890 Series II, Palo Alto, CA, USA). The GC was equipped with a flame-ionization detector, a 100-m CP Sil 88 column (Chrompack Inc., Middelburg, The Netherlands), an autosampler and a computer software system (Hewlett-Packard ChemStation A.07). Methylated lipids were analyzed using GC in a split-less mode (0.3 s), with inlet and detector temperatures set at 250^{o}C and a temperature program which began at 45^{o}C (hold 4 min) and incorporated a

temperature rise at a rate of 13°C/min to 175°C (hold 27 min) and another subsequent temperature increase at a rate of 4°C/min to 215°C (hold 35 min). The FAME were identified and quantified by comparison with FAME standards from Nu Check Prep (Elysian, MN).

4.4 TMR and Duodenal Fatty Acid Profiles

Fatty acid composition of TMR and WDD was determined as described by O'Fallon et al. (2007). Briefly, 1.0 mL of $C_{13:0}$ (internal standard), 0.7 mL of 10 *N* potassium hydroxide (KOH) and 5.3 mL methanol (MeOH) were added to an aliquot of TMR (1.0 g) or duodenal digesta (0.5 g) in a 16 x 125 mm screw-cap Pyrex culture tube. Tubes were incubated at 55°C in a water bath for 1.5 h, with vigorous manual shaking for 5 sec every 20 min in order to permeate, dissolve and hydrolyze the sample. Samples were then cooled to room temperature (20°C) and 0.58 mL of 24 *N* H_2SO_4 was added. The solutions were mixed by vortexing which resulted in a precipitate of potassium sulphate (K_2SO_4). For synthesis of fatty acid methyl esters (FAME), samples were then incubated at 55°C in a water bath for 1.5 h, with vigorous manual shaking for 5 sec every 20 min. Sample tubes were then cooled to room temperature, followed by the addition of 3.0 mL of hexane, and then vortexed. Vortexed tubes were then centrifuged for 5 minutes at 1,800 x *g* in a Beckman J6-MC centrifuge, and the hexane layer, containing the FAME, was placed into a gas chromatography (GC) vial. The vial was sealed and stored at -20°C until analysis by GC (Agilent 6890, Mississauga, ON) using a SP-2560, 100 m x 0.25 mm x 0.20 μm column. Methylated lipids were analyzed using GC in a split-less mode and a temperature program beginning at 100°C (hold 5 min) and

incorporating a temperature rise at a rate of 3°C/min to 240°C (hold 40 min) and another subsequent temperature increase at a rate of 5°C/min to 245°C (hold 10 min). The FAME were identified and quantified by comparison with standards (Supelco 37 Component FAME Mix, Bellefonte, PA).

4.5 Ruminal Volatile Fatty Acid Analysis

Ruminal VFA concentrations were analyzed based on the methods of Erwin et al., 1961). Ruminal fluid samples were thawed, centrifuged at 18,000 x *g* for 15 min and filtered through a 0.45-µm membrane. A 0.9 mL portion of the filtered supernatant was mixed with 0.1 mL of 10 mg/mL crotonic acid as an internal standard. Ruminal VFA were separated and quantified by GC (Agilent 6890, Mississauga, ON) using a ZB-FFAP, 30 m x .32 mm x .25 µm column. Volatile fatty acids were analyzed using GC in a split-less mode and a temperature program beginning at 100°C (hold 1 min) and incorporating a temperature rise at a rate of 8°C/min to 160°C (hold 0 min) and another subsequent temperature increase at a rate of 20°C/min to 200°C (hold 1 min). Quantification of VFA was determined based on comparing the area and elution times of the standard samples with the samples of interest.

4.6 Calculations and Statistical Analysis

The ruminal pH data that was recorded daily was averaged for each minute and summarized daily as minimum pH, mean pH and maximum pH. The degree and incidence of ruminal acidosis was determined from the pH data using three pH thresholds i.e., 5.8, 5.5 and 5.2. Ruminal acidosis was considered to occur when ruminal pH was <

5.8 (total ruminal acidosis). The pH profiles were further classified as mild RA when 5.8 > ruminal pH > 5.5, severe RA when 5.5 > ruminal pH > 5.2, and acute RA when ruminal pH < 5.2 (Penner et al., 2006). The duration (min/d) and total area (pH x min) that ruminal pH was below each threshold was calculated. Furthermore, the number of daily episodes that occurred in each level of ruminal acidosis was documented. An episode was defined to begin when ruminal pH was below the pre-defined threshold and ended when the ruminal pH was equal to or above the threshold (Penner et al., 2006).

Apparent digestion of nutrients in the rumen was calculated as follows: Nutrient apparently digested in the rumen = nutrient intake – duodenal flow of nutrient, where nutrient intake, digestion, and flow to the duodenum are expressed in kg/d. Organic matter truly digested in the rumen (OMTDR) was determined after correcting for microbial OM flowing to the duodenum using the following equation: OMTDR = OM intake – (duodenal OM flow – microbial OM flow), where OMTDR, OM intake, duodenal OM flow and microbial OM flow are expressed in kg/d.

All data were analyzed using PROC MIXED (SAS, 2004). The statistical model that was used for DM intake, milk yield and composition, nutrient digestibilities and nutrient flow to the duodenum included the following terms: method of barley processing and source of supplemental fat, which were considered fixed, the interaction between method of barley processing and source of supplemental fat, the random effects of period and cow, and the residual error. Data on ruminal pH and ruminal concentration of VFA were analyzed accounting for repeated measures. Data for these repeated measures were analyzed by including in the statistical model a REPEATED model statement, as well as terms for time (hour or day) and interactions (method of barley processing x time, source

of supplemental fat x time, and time x method of barley processing x source of supplemental fat). The variance-covariance error structure with the lowest Akaike's and Bayesian information criteria was used. When processing x time or oilseed x time interactions were significant , the slice option for the LSMEANS statement in PROC MIXED was used to determine which time period means were different. Significance for all models was declared at $P \leq 0.05$, and trends are discussed for $0.05 < P \leq 0.10$. When there was a significant interaction between method of barley processing and source of supplemental fat, least square means were separated by Tukey's HSD test (SAS, 2004).

5.0 RESULTS AND DISCUSSION

5.1 Diet Characteristics

The experimental diets used in this study were formulated to meet the nutrient requirements of dairy cows producing 32 kg/d of milk with 3.6% fat (NRC, 2001). Treatments were formulated to be isonitrogenous and isolipidic (Table 3.0). Mean crude fat content of the diets was 5.3% (Table 3.0), which falls within the desired range of <6% total dietary fat. Most ruminant diets generally contain 2 to 5% lipids, of which approximately one-half are fatty acids (Doreau and Ferlay, 1994). Dietary fat levels exceeding 6 to 7% reduce fibre digestion and, consequently, lower DM intake in dairy cows (NRC, 2001); hence it was desired to maintain total dietary fat below 6% of DM. Barley grain was used as the principal energy and carbohydrate source as it is easily available in western Canada. Barley is commonly fed on-farm in either a dry-rolled or pelleted form. Barley processing (i.e. dry-rolling *vs.* pelleting) was performed to alter ruminal fermentation of carbohydrates. The reduction in particle size when barley was pelleted was expected to shift carbohydrate digestion from post-ruminal sites to the rumen, thereby increasing ruminal energy availability. Previous *in situ* studies in our laboratory clearly indicated a higher soluble starch fraction, a higher degradation rate of the degradable starch fraction and a higher effective starch degradability of pelleted barley when compared with dry-rolled barley (Kiran and Mutsvangwa, 2007).

Whole canola seed and whole flaxseed were chosen as the supplemental fat sources for the experimental diets for two major reasons. Firstly, both oilseeds are readily available and are common ingredients in dairy cow rations in western Canada and

the northern United States. Secondly, canola seed and flaxseed differ in their unsaturated fatty acid profiles. Table 5.0 shows the fatty acid profiles for canola seed and flaxseed used in the current study. Canola seed is high in $C_{18:1}$ and $C_{18:2}$, whereas flaxseed is high in $C_{18:3}$. In addition, these oilseeds differed in their iodine values, (calculated from individual fatty acid composition; AOCS, 1989), with mean values of 112 to 115 and 190 for canola seed and flaxseed, respectively. Due to these differences in fatty acid profiles, it was expected that the oilseeds would differently impact the rumen microbes and, subsequently, ruminal fermentation.

The TMR fatty acid profiles for the four treatment diets were very similar, except for their $C_{18:1}$ and $C_{18:3}$ fatty acid content (Table 5.1). This is because the ingredients included were identical with the exception of the oilseed present. As expected based on the fatty acid profiles of supplemental oilseeds, TMR containing canola seed were high in $C_{18:1n9c}$, $C_{20:1}$ and $C_{20:2}$, whereas TMR containing flaxseed was higher in $C_{18:3n3}$ concentration.

5.2 Ruminal Fermentation Characteristics

Cows fed pelleted barley had a minimum ($P < 0.01$) and mean ($P = 0.05$) ruminal pH that was lower than cows fed dry-rolled barley (Table 5.2). This difference in ruminal pH was expected because more extensive processing of barley would result in a more rapid and extensive ruminal carbohydrate fermentation (Kiran and Mutsvangwa, 2007), thus increasing the risk of ruminal acidosis. Similarly, Yang et al. (2000) reported a lower ruminal pH in cows fed medium- or flat-rolled barley compared to those fed coarsely-rolled barley. Ruminal pH levels over a 24- hr period for each treatment are

Table 5.0. Fatty acid profiles of canola seed and flaxseed (Beauchemin et al., 2009).

Fatty Acid	Canola (g/100 g of FA)	Flax (g/100 g of FA)
Palmitic ($C_{16:0}$)	4.40	5.90
Stearic ($C_{18:0}$)	1.80	3.90
Oleic ($C_{18:1n9c}$)	56.50	16.70
Linoleic ($C_{18:2n6c}$)	22.10	19.90
α-Linolenic ($C_{18:3n3}$)	11.10	52.80

Table 5.1. Total mixed ration (TMR) fatty acid (FA) composition (expressed as mg FA/100 mg total detected FA) containing dry-rolled or pelleted barley and utilizing canola or flaxseed as supplemental fat sources.

	Canola		Flax	
Fatty acids	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley
$C_{6:0}$	0.08	0.07	0.09	0.09
$C_{8:0}$	0.10	0.08	0.12	0.16
$C_{12:0}$	0.10	0.12	0.12	0.11
$C_{14:0}$	0.35	0.42	0.39	0.40
$C_{15:0}$	0.12	0.14	0.13	0.13
$C_{15:1\ (cis)}$	0.37	0.50	0.45	0.46
$C_{16:0}$	11.50	12.60	13.50	13.50
$C_{16:1}$	0.31	0.32	0.20	0.18
$C_{17:0}$	0.14	0.15	0.15	0.15
$C_{17:1\ (cis)}$	0.07	0.08	0.06	0.07
$C_{18:0}$	2.08	2.29	2.87	2.97
$C_{18:1n9c}$	42.00	31.40	15.70	15.70
$C_{18:2n6c}$	27.70	33.50	26.10	25.00
$C_{18:3n3}$	9.22	10.20	35.80	37.50
$C_{20:0}$	0.56	0.62	0.29	0.30
$C_{20:1}$	0.99	1.05	0.37	0.36
$C_{20:2}$	2.61	3.26	1.04	1.09
$C_{22:0}$	0.48	0.53	0.36	0.38
$C_{20:4n6}$	0.06	0.07	0.07	0.06
$C_{23:0}$	0.11	0.14	0.14	0.14

Table 5.2. Ruminal pH in dairy cows (n = 4) fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources.

	Canola		Flax			*P* value[1]		
Variable	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley	SEM	BP	OS	BP x OS
Minimum pH	5.65	4.92	5.30	5.06	0.11	<0.01	0.52	0.13
Mean pH	6.13	5.73	5.99	5.77	0.11	0.05	0.66	0.48
Maximum pH	6.59	6.67	6.74	6.67	0.13	0.92	0.79	0.78
Total RA[2]								
Duration min/d	140.0	827.2	414.4	741.8	106.2	<0.01	0.42	0.19
Area, pH x min	26.6	380.2	104.5	337.7	79.9	0.02	0.81	0.55
Mild RA[3]								
Daily Episodes	5.1	5.7	4.9	6.8	1.2	0.25	0.68	0.54
Duration min/d	139.8	349.4	199.3	277.9	49.7	<0.01	0.89	0.16
Area, pH x min	26.9	194.1	80.5	178.0	29.9	<0.01	0.51	0.31
Severe RA[4]								
Daily Episodes	0.3[b]	7.3[a]	2.1[b]	5.0[a]	0.65	<0.01	0.85	<0.01
Duration min/d	60.7	256.2	146.3	220.5	43.6	0.02	0.40	0.17
Area, pH x min	10.8	100.1	22.5	105.9	25.8	0.03	0.71	0.82
Acute RA[5]								
Daily Episodes	0.7	3.0	0.5	4.4	1.1	0.05	0.56	0.78
Duration min/d	6.0	221.7	11.8	243.5	74.8	0.06	0.88	0.96
Area, pH x min	0.0	86.0	1.4	53.9	29.9	0.13	0.72	0.70

[1]BP = method of barley processing (dry-rolled *vs.* pelleted); OS = source of supplemental fat (canola *vs.* flax); BP x OS = interaction.
[2]Total Ruminal Acidosis (RA) = pH ≤ 5.8
[3]Mild RA = 5.8 ≥ pH ≥ 5.5
[4]Severe RA = 5.5 ≥ pH ≥ 5.2
[5]Acute RA = pH ≤ 5.2
[a-c]Means within rows with different superscripts differ ($P < 0.05$).

presented in Figure 5.0. There is a pronounced decline in pH after feeding at approximately 0900 and 1600 h. These decreases are due to the degradation of rapidly fermentable starch in the rumen. Cows fed pelleted barley not only spent on average 500 min more ($P < 0.01$) throughout the day in a state of total ruminal acidosis than cows fed dry-rolled barley, but the extent of the acidosis was also more significant ($P = 0.02$) in cows fed pelleted barley, as the area under the predetermined threshold of 5.8 was on average 299 pH x min larger. The increase in time spent and the varying degree of acidosis experienced by cows fed pelleted barley compared to those fed dry-rolled barely was expected as increasing the digestibility of the grain tends to result in a more dramatic drop and gradual return to optimal ruminal pH (Yang et al., 2001). Cows fed pelleted barley rations also spent more ($P < 0.01$) time in a state of mild ruminal acidosis compared to those fed dry-rolled barley.

There was a barley processing x oilseed source interaction in the number of daily episodes of severe ruminal acidosis (Table 5.2). An episode was defined as to begin when the ruminal pH crossed below the pre-determined threshold and end when the pH returned to meet or exceed that threshold again. Cows fed pelleted barley with canola experienced 7.0 more ($P < 0.01$) episodes of severe ruminal acidosis than cows fed dry-rolled barley with canola; on the other hand, cows fed pelleted barley with flax experienced 2.9 more ($P < 0.01$) episodes of severe ruminal acidosis than cows fed dry-rolled barley and flax. Individual and total concentrations of volatile fatty acids (VFA) were greater ($P < 0.05$) in cows fed dry-rolled barley compared with those fed pelleted barley; consequently, the acetate:propionate ratio was higher ($P < 0.001$) in cows fed dry-rolled barley compared with those fed pelleted barley (Table 5.3). As the rate of ruminal

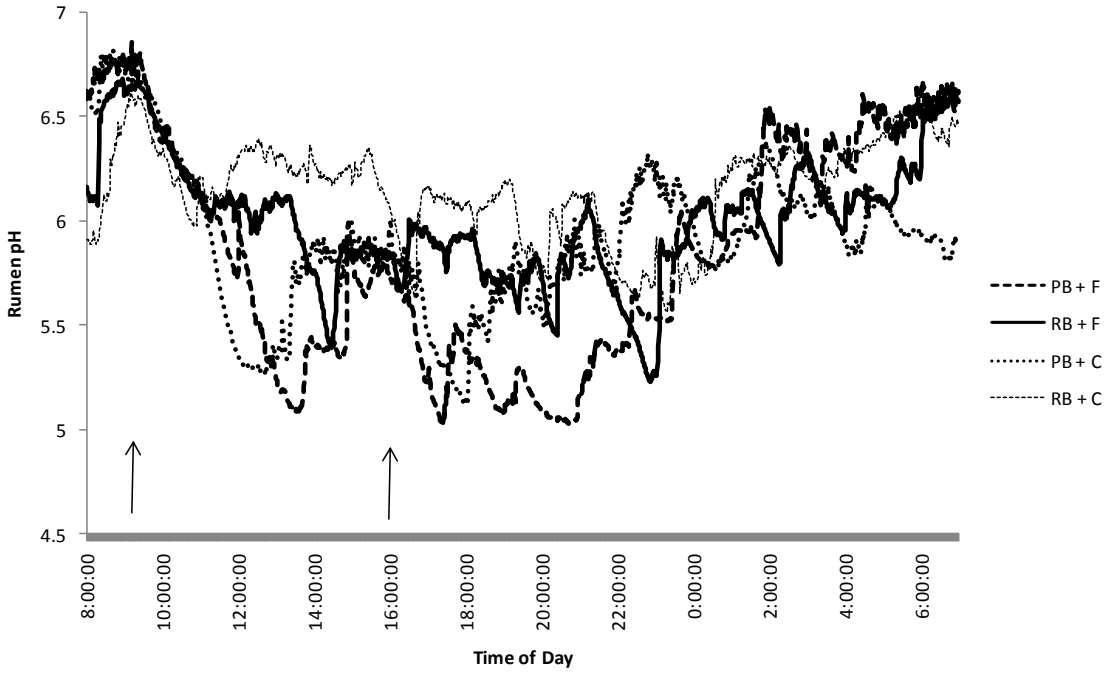

Figure 5.0. Rumen pH levels of the 4 dietary treatments over a 24 hour time period (PB + F: pelleted barley + flax; RB + F: dry-rolled barley + flax; PB + C: pelleted barley and canola; RB + C: dry-rolled barley + canola, arrows indicate time of TMR feeding; $n = 4$).

Table 5.3. Ruminal fermentation characteristics in dairy cows (n = 4) fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources.

	Canola		Flax			*P* value[1]		
Item	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley	SEM	BP	OS	BP x OS
Volatile fatty acids, m*M*								
Acetate	90.0	74.0	85.8	73.6	1.4	<0.001	0.13	0.20
Propionate	31.1	35.7	28.3	37.3	2.1	0.007	0.50	0.31
Isobutyrate	0.38	0.28	0.34	0.26	0.02	<0.001	0.08	0.37
Butyrate	19.0[a]	15.4[b]	17.7[b]	16.1[b]	0.7	<0.001	0.41	0.002
Isovalerate	0.96	0.66	1.05	0.63	0.07	<0.001	0.91	0.76
Valerate	1.93	2.58	1.71	2.96	0.31	0.005	0.72	0.21
Total VFA	143.3	128.5	134.9	130.6	3.4	0.007	0.35	0.13
Acetate:propionate ratio	3.0[a]	2.4[b]	3.2[a]	2.2[c]	0.13	<0.001	0.05	0.006

[1]BP = method of barley processing (dry-rolled *vs.* pelleted); OS = source of supplemental fat (canola *vs.* flax); BP x OS = interaction.
[a-c]Means within rows with different superscripts differ ($P < 0.05$).

starch degradation is expected to differ with method of grain processing, it is reasonable to expect differences in ruminal VFA patterns, particularly acetate and propionate, when barley grain is fed dry-rolled or pelleted. There were no time x method of barley processing interactions for the two major ruminal VFA; however, there was a time x method of barley processing interaction for valerate (P = 0.01) because ruminal concentrations of valerate were similar at feeding, but were higher at all other sampling times in cows fed dry-rolled compared with those fed pelleted barley (Figure 5.1). In addition, there was a time x method of barley processing interaction for isovalerate (P < 0.001) because ruminal interaction for isovalerate were higher at all sampling times during the first 20 h after the first feeding in cows fed dry-rolled barley compared with those fed pelleted barley, but were similar thereafter (Figure 5.2). There were no effects of source of supplemental fat on ruminal VFA; however, there was a time x source of supplemental fat interaction (P < 0.01) for butyrate because ruminal butyrate concentrations were higher at 8 h after the first feeding, but tended (P < 0.10) to be lower at 16 and 20 h after the first feeding, in cows fed canola seed compared with those fed flaxseed (Figure 5.3).

In addition, there was a time x source of supplemental fat interaction (P = 0.04) for isobutyrate as ruminal isobutyrate concentrations tended (P < 0.10) to be higher at 12 and 20 h after the first feeding in cows fed canola seed compared with those fed flaxseed (Figure 5.4).

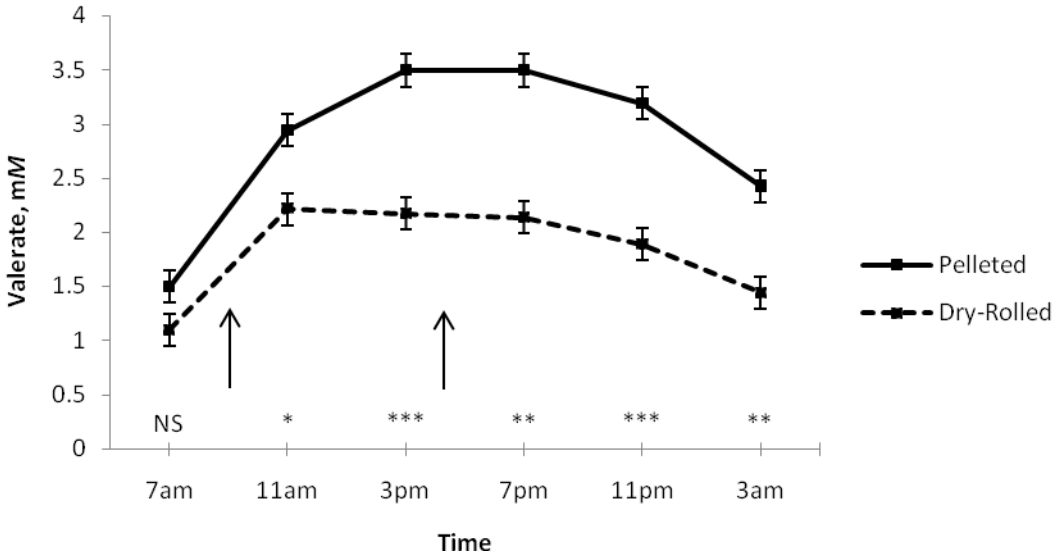

Figure 5.1. Time x method of barley processing interaction for valerate in dairy cows (*n* = 4) fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources. (NS : $0.5 < P < 0.1$; * : $P < 0.05$; ** : $P < 0.01$; *** : $P < 0.001$; arrows indicate time of TMR feeding).

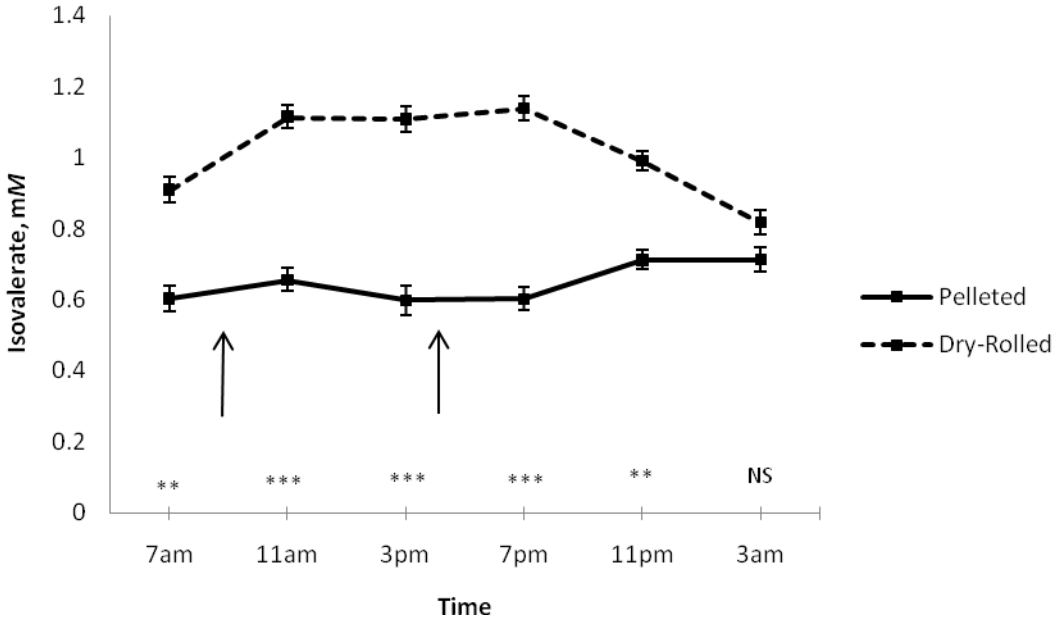

Figure 5.2 Time x method of barley processing interaction for isovalerate in dairy cows (*n* = 4) fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources. (NS : 0.5 < P < 0.1; ** : P < 0.01; *** : P < 0.001; arrows indicate time of TMR feeding).

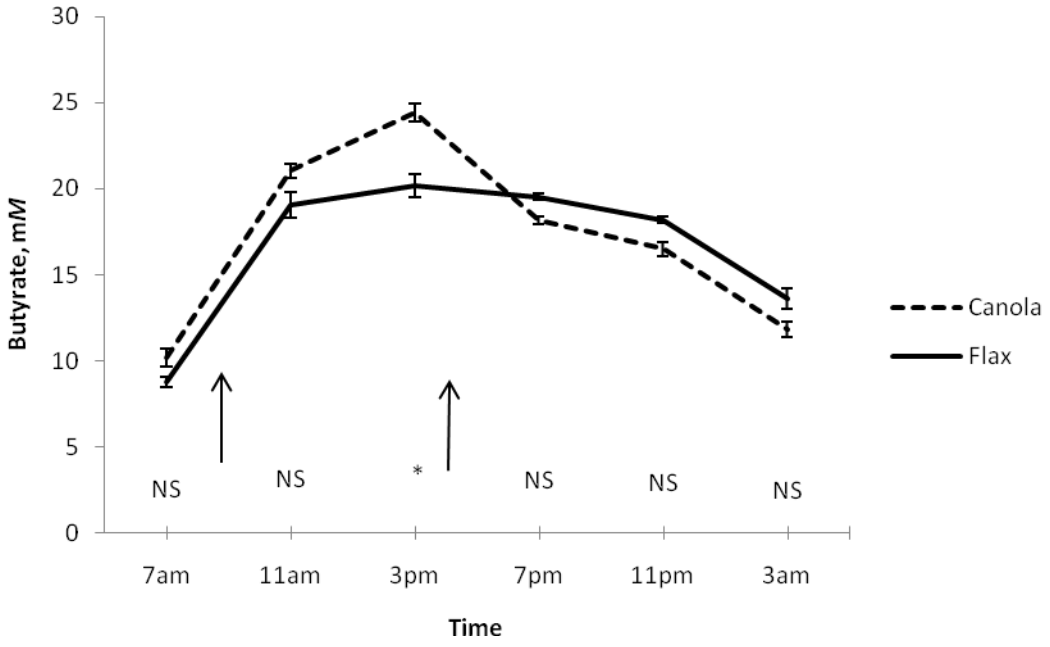

Figure 5.3 Time x source of supplemental fat interaction for butyrate in dairy cows (*n* = 4) fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources. (NS : $0.5 < P < 0.1$; * : $P < 0.05$; arrows indicate time of TMR feeding).

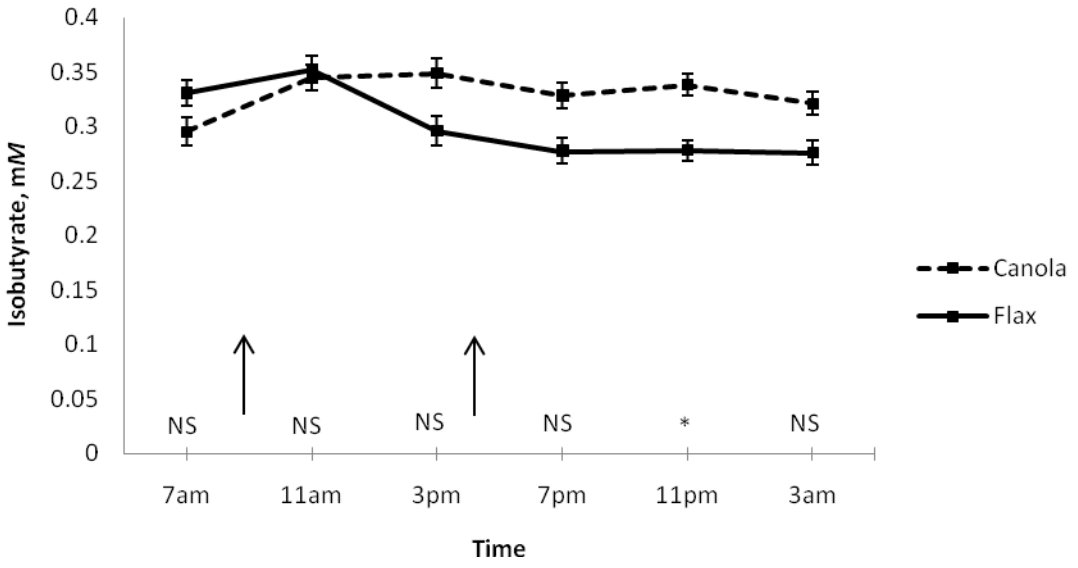

Figure 5.4 Time x source of supplemental fat interaction for isobutyrate in dairy cows (*n* = 4) fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources. (NS : 0.5 < P < 0.1; * : P < 0.05; arrows indicate time of TMR feeding).

5.3 Dry Matter Intake and Total Tract Digestibilities

Dry matter intake was on average 2.25 kg/d higher (P = 0.04) in cows consuming dry-rolled barley diets than cows fed pelleted barley diets (Table 5.4). This depression in DMI with more extensive barley processing is likely mediated via increased acidity in the rumens of cows fed pelleted barley. Dry matter intake may be a contributing factor and account for the difference in the number of severe ruminal acidosis episodes experienced. This may be associated due to the fact that cows fed dry-rolled barley + flax and pelleted barley + canola ate more than cows fed dry- rolled barley + canola and pelleted barley + flax. Animal variation may also have had an effect on ruminal pH. Individual cows may be genetically predisposed to a lower pH or they may have a tendency to sort the smaller concentrate particles more aggressively (DeVries et al., 2008). The lower DMI in cows consuming pelleted barley compared with those fed dry-rolled barley diets is in agreement with results reported by Yang et al. (2000). These researchers observed a negative quadratic response in DMI of dairy cows, whereby cows fed diets with increasing degrees of processed barley, DMI decreased. In feedlot cattle, Hironaka et al. (1992) observed lower DMI and a higher incidence of digestive disturbances when thinly-rolled or medium-rolled barley diets were fed compared with coarsely rolled or whole barley diets. In contrast, Yang et al. (2001) reported higher DMI in cows fed flatly rolled barley compared with those fed coarsely rolled barley. However, the decrease in mean ruminal pH (a reduction of 0.13 pH units) observed with more extensive barley grain processing in that study was not as severe as that observed in the present study (a reduction of 0.31 pH units; Table 5.2). As a result, this could explain the discrepancy in results.

Table 5.4. Dry matter intakes (DMI) and total tract nutrient digestibilities in dairy cows (n = 4) fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources.

	Canola		Flax			*P* value[1]		
Item	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley	SEM	BP	OS	BP x OS
DMI, kg/d	22.3	21.1	23.8	20.5	1.0	0.04	0.65	0.34
Nutrient digestibility, %								
DM	69.2	67.5	68.3	68.4	2.7	0.78	0.99	0.75
Starch	89.8	98.5	89.8	99.0	1.2	<0.0001	0.85	0.85
Crude fat	88.1	88.6	86.7	87.1	1.3	0.8	0.31	0.95
ADF	45.7	37.6	43.3	43.7	4.9	0.44	0.71	0.40
NDF	47.3	42.7	45.8	47.1	4.2	0.71	0.74	0.50
OM	70.6	69.2	69.7	70.3	2.7	0.88	0.97	0.73

[1]BP = method of barley processing (dry-rolled *vs.* pelleted); OS = source of supplemental fat (canola *vs.* flax); BP x OS = interaction.

Source of dietary supplemental fat did not affect DMI. Mean DMI was 22 kg/d, or approximately 3.4% of mean BW, and this falls within the expected range for dairy cows producing 32 kg/d of milk (NRC, 2001). Therefore, it is unlikely that feeding supplemental fat decreased DMI. These results are in agreement with those of Ward et al. (2002) in which DMI was similar in cows fed barley-based diets that contained canola or flax as a supplemental fat source.

Total tract digestibilities of DM, crude fat, ADF, NDF, and OM were unaffected ($P > 0.05$) by method of barley processing or source of oilseed; however, total tract starch digestibility was higher ($P < 0.0001$) in cows fed pelleted barley compared with those fed dry-rolled barley. In contrast, Yang et al. (2001) reported higher DM and OM digestibilities in cows fed flatly rolled barley compared with those fed coarsely rolled barley; however, in agreement with observations in the present study, ADF and NDF digestibilities were not altered. When barley is fed to ruminants in either a whole or crushed form, at least 90% of the starch is fermented in the rumen (Ørskov, 1986), which was evident in this study. However, total tract starch digestibility was on average 8.9% lower ($P < 0.0001$) in cows fed diets containing dry-rolled barley compared to cows fed pelleted barley. This is comparable to Theurer et al. (1999) who observed lower total tract starch digestibility in cows fed dry-rolled sorghum grain versus steam-flaked sorghum grain. These results can be attributed to observations whereby there is a 25% reduction in the amount of starch that escapes the rumen when pelleted grain is fed compared to when it is rolled (Nocek and Tamminga, 1991). The lack of effect of barley grain processing on ADF and NDF digestion is somewhat surprising because pelleting barley increased ruminal acidity when compared with dry-rolling. This is possibly due to

a higher hindgut fermentation of ADF and NDF in cows fed pelleted barley compared with those fed dry-rolled barley, thus compensating for any depression in ruminal fibre digestion that might have occurred. Across dietary treatments, the estimates of total tract digestibilities of ADF and NDF are numerically lower than has been previously observed in cows fed diets with similar ingredient compositions (Yang et al., 2000). Lower ADF and NDF digestibilities observed in the present study could be attributed to depressed ruminal pH. Cows fed dry-rolled barley had numerically higher ADF and NDF digestibilities than those fed pelleted barley, which relates to lower ruminal pH in those cows fed pelleted barley. In a previous study (Yang et al., 2000) reporting higher ADF and NDF digestibilities, mean ruminal pH was 0.17 to 0.43 pH units higher when compared with mean ruminal pH values observed in the present study. Across all diets, mean ruminal pH ranged from 5.73 to 6.13 (mean of 5.91), which is lower than the ruminal pH of 6.2 which has been suggested as the minimum ruminal pH for optimum fibre digestion (Van Soest, 1994). Previously, in vitro studies indicated that populations of the principal fibre-digesting bacteria such as *Fibrobacter* spp. and *Ruminococcus albus* decline rapidly when pH falls below 6.0 (Russell and Wilson, 1996). In addition, total dietary fat in the present study was high (>5%; DM basis) and composed mainly of unsaturated fatty acids. In general, supplemental fat sources that are high in unsaturated fatty acids can have inhibitory effects on ruminal fibre digestion **(**Schauff et al., 1992; Pantoja et al., 1994), thus partially explaining the lower fibre digestibilities observed in the present study when compared with another study (Yang et al., 2000) in which total dietary fat contents were lower.

5.4 Nutrient Intake, Duodenal Flow and Rumen Digestion

Cows fed pelleted barley ate significantly less (P = 0.02) DM compared to those fed dry-rolled barley. Intakes of NDF (P = 0.007), ADF (P = 0.03), and EE (P = 0.0008) were higher, and that of starch tended (P = 0.08) to be higher, in cows fed dry-rolled barley compared to those fed pelleted barley, reflecting observed differences in DM intake; however, source of supplemental fat had no effect ($P \geq 0.36$) on intakes of these nutrients (Table 5.5). Nutrient DMI of cows varied from total tract DMI in Table 5.4 albeit the same animals were used. This difference is attributed to total tract digestibility collections were only measured for 5 days of the treatment period, whereas nutrient flow collections included data from all 14 days of the treatment period. Duodenal flows of DM ($P \geq 0.14$), OM ($P \geq 0.12$), NDF ($P \geq 0.71$), and ADF ($P \geq 0.47$) were unaffected by method of barley grain processing or source of supplemental fat. The amount of dietary DM apparently digested in the rumen tended (P = 0.06) to be higher in cows fed dry-rolled barley compared to those fed pelleted barley; however, when expressed as a proportion of DM intake, apparent ruminal digestibility of DM was unaffected (P = 0.15) by method of barley grain processing. In parallel with observed changes in ruminal DM digestibility, amounts of OM apparently (P = 0.08) or truly (P = 0.09) digested in the rumen tended to be higher in cows fed dry-rolled barley compared to those fed pelleted barley; however, there were no differences in apparent (P = 0.23) or true (P = 0.28) ruminal OM digestibility when expressed as a proportion of OM intake. There was a greater increase in duodenal OM flow in cows fed pelleted barley compared to those fed dry-rolled barley with canola (+0.54 kg/d) than with flax (+0.17 kg/d) (interaction, P = 0.04). Source of supplemental fat had no effect on apparent ruminal digestibility of DM,

Table 5.5. Nutrient intake, rumen disappearance and duodenal flow in dairy cows (n = 4) fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources.

	Canola		Flax			*P* value[1]		
Item	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley	SEM	BP	OS	BP x OS
Dry matter (DM)								
Intake, kg/d	24.6	21.1	24.5	22.7	0.93	0.02	0.41	0.36
Duodenal flow, kg/d	14.5	13.9	15.5	14.7	0.56	0.23	0.14	0.90
Apparently digested in the rumen								
kg/d	10.1	7.3	9.1	8.0	0.87	0.06	0.86	0.32
% of DM intake	40.4	33.4	36.7	34.9	2.77	0.15	0.70	0.37
Organic matter (OM)								
Intake, kg/d	22.5	19.5	22.8	21.0	0.86	0.02	0.30	0.45
Duodenal flow, kg/d								
Total OM	14.4	13.5	15.4	14.5	0.60	0.16	0.12	0.94
Microbial OM	1.62	2.18	2.02	2.25	0.15	0.01	0.07	0.39
Apparently digested in the rumen								
kg/d	9.85	8.15	9.30	8.75	0.80	0.18	0.97	0.47
% of OM intake	42.7	41.4	40.6	41.6	2.50	0.95	0.70	0.66
Truly digested in the rumen								
kg/d	11.4	10.3	11.3	11.1	0.76	0.41	0.65	0.55
% of OM intake	49.7	52.9	49.5	53.0	2.58	0.22	0.99	0.96
Starch								
Intake, kg/d	5.65	4.80	6.00	5.38	0.37	0.08	0.24	0.77
Duodenal flow, kg/d	0.58	0.40	0.68	0.48	0.05	0.003	0.07	0.77
Digested in the rumen								
kg/d	5.08	4.40	5.38	4.90	0.38	0.17	0.32	0.80
% of starch intake	89.9	90.8	89.1	91.1	1.22	0.11	0.79	0.56

[1]BP = method of barley processing (dry-rolled *vs.* pelleted); OS = source of supplemental fat (canola *vs.* flax); BP x OS = interaction.

Table 5.5. Nutrient intake, rumen disappearance and duodenal flow in dairy cows (n = 4) fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources (continued).

	Canola		Flax			*P* value[1]		
Item	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley	SEM	BP	OS	BP x OS
NDF								
Intake, kg/d	8.63	7.38	8.85	8.10	0.34	0.007	0.10	0.35
Duodenal flow, kg/d	5.05	5.08	5.03	4.83	0.38	0.81	0.71	0.76
Digested in the rumen								
kg/d	3.63	2.28	3.85	3.25	0.34	0.02	0.12	0.30
% of NDF intake	41.8	31.7	43.1	39.9	3.48	0.09	0.20	0.34
ADF								
Intake, kg/d	4.95	4.30	5.15	4.75	0.22	0.03	0.13	0.53
Duodenal flow, kg/d	3.03	2.93	3.00	2.83	0.18	0.47	0.74	0.84
Digested in the rumen								
kg/d	1.93	1.40	2.13	1.93	0.23	0.14	0.14	0.47
% of ADF intake	38.7	32.3	41.3	40.3	3.59	0.33	0.19	0.48
Ether Extract (EE)								
Intake, kg/d	1.28	1.12	1.29	1.14	0.05	0.008	0.66	0.89
Duodenal flow, kg/d	1.02	1.35	1.34	1.27	0.15	0.32	0.38	0.16
Apparently digested in the rumen								
kg/d	0.26	-0.24	-0.04	-0.12	0.18	0.07	0.50	0.16
% of EE intake	21.1	-25.0	-3.59	-11.2	14.2	0.04	0.61	0.10

[1]BP = method of barley processing (dry-rolled *vs.* pelleted); OS = source of supplemental fat (canola *vs.* flax); BP x OS = interaction.

OM, NDF and ADF, when expressed as absolute amounts ($P \geq 0.12$) or as a proportion of intake ($P \geq 0.19$). Apparent ruminal digestibility of NDF, when expressed in absolute amounts or as a proportion of NDF intake, was higher ($P = 0.02$) or tended to be higher ($P = 0.09$), respectively, in cows fed dry-rolled barley compared to those fed pelleted barley. These negative effects of more extensive barley grain processing on ruminal fibre digestion were not surprising because pelleting increased ruminal acidity when compared to dry-rolling.

Cows fed dry-rolled barley consumed 12 to 18% more ($P = 0.08$) starch compared to those fed pelleted barley, in parallel with the observed changes in DM intake. Surprisingly, apparent ruminal starch digestibility, when expressed in absolute amounts ($P = 0.17$) or as a proportion of starch intake ($P = 0.11$), was unaffected by method of barley grain processing. Because pelleting decreases particle size, it had been anticipated that cows fed pelleted barley would exhibit a greater extent of ruminal starch disappearance compared to those fed dry-rolled barley. The lack of effect of barley grain processing on ruminal starch digestion is not consistent with the changes in ruminal acidity that were observed. Compared to cows fed pelleted barley, those fed dry-rolled barley tended ($P = 0.08$) to have a higher starch intake, and had a higher ($P = 0.003$) duodenal flow of starch, and the combination of these two factors may have reduced the impact of pelleting on ruminal starch digestion. Also, it is noteworthy that cows fed dry-rolled barley digested 10 to 15% (in absolute amounts) less starch in their rumens compared to those fed pelleted barley. In agreement with the present study, Yang et al. (2000) did not observe any effects of grain processing on ruminal starch digestion in dairy cows fed barley grain processed to varying degrees, ranging from coarsely-rolled to

flat-rolled; however, Yang et al. (2001) reported a higher ruminal starch digestion when dairy cows were fed flat-rolled barley compared to coarsely-rolled barley. Across dietary treatments, approximately 90% of dietary starch intake was digested in the rumen.

In parallel with DM intakes, daily intakes of EE were higher ($P = 0.008$) in cows fed dry-rolled barley compared to those fed pelleted barley; however, source of supplemental fat had no effect ($P = 0.66$) on EE intakes. Duodenal flow of EE was similar ($P \geq 0.32$) across dietary treatments, and ranged from 80% of dietary EE intake in cows fed dry-rolled barley with canola, to 104 to 121% in cows fed the other diets. These data indicate that there were both positive and negative balances of EE across the rumen, resulting from variability in the amounts of *de novo* fatty acid synthesis in the rumen (Bock et al., 1991) and ruminal metabolism and absorption of fatty acids (Jesse et al., 1992).

5.5 Fatty Acid Intake and Duodenal Flow

Cows fed diets containing dry-rolled barley consumed more ($P = 0.005$) unsaturated fatty acids and had a higher ($P = 0.02$) total intake of fatty acids than cows fed pelleted barley (Table 5.6), reflecting observed differences in DMI. However, barley processing did not have an effect ($P = 0.61$) on the amount of saturated fatty acids that cows consumed. Higher consumption levels of $C_{18:0}$, of approximately 1.35 times more ($P = 0.001$) and $C_{18:3n3}$, of approximately 3.8 times more ($P < 0.0001$), were also observed in cows fed flaxseed compared to cows fed canola. Cows consuming diets containing canola consumed approximately 2.5 times more ($P < 0.0001$) $C_{18:1n9c}$ than cows fed flax. This value is consistent with results obtained by Loor and Herbein (2003),

Table 5.6 Fatty acid (FA) intake (expressed as g FA/day) of dairy cows (n = 4) fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources.

	Canola		Flax			P value[1]		
Fatty acid intake, g/d	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley	SEM	BP	OS	BP x OS
$C_{6:0}$	0.92	1.04	1.03	0.99	0.07	0.54	0.67	0.31
$C_{8:0}$	1.29	0.86	1.51	1.82	0.31	0.85	0.09	0.27
$C_{12:0}$	1.16	1.37	1.40	1.34	0.14	0.61	0.48	0.36
$C_{14:0}$	4.26	4.54	4.75	4.50	0.38	0.96	0.57	0.50
$C_{15:0}$	1.49	1.51	1.58	1.48	0.12	0.78	0.82	0.62
$C_{15:1\ (cis)}$	4.51	5.53	5.40	5.23	0.77	0.59	0.71	0.46
$C_{16:0}$	140.2	138.0	163.1	152.4	11.1	0.57	0.13	0.71
$C_{16:1}$	3.77	3.48	2.36	2.05	0.27	0.29	<0.001	0.97
$C_{17:0}$	1.71	1.68	1.81	1.72	0.15	0.70	0.64	0.82
$C_{17:1\ (cis)}$	0.90	0.88	0.73	0.75	0.11	0.99	0.21	0.85
$C_{18:0}$	25.4	24.9	34.6	33.4	1.89	0.66	0.001	0.87
$C_{18:1n9c}$	514.6[a]	416.8[b]	189.4[c]	177.1[c]	16.3	0.01	<0.0001	0.03
$C_{18:2n6c}$	340.1	376.5	315.6	282.3	59.3	0.98	0.34	0.57
$C_{18:3n3}$	113.7	112.3	433.7	424.8	16.9	0.77	<0.0001	0.83
$C_{20:0}$	6.83	6.80	3.54	3.35	0.51	0.84	<0.0001	0.88
$C_{20:1}$	12.1	11.5	4.40	4.04	0.93	0.58	<0.0001	0.87
$C_{20:2}$	31.4	35.1	12.5	12.2	4.70	0.73	0.002	0.68
$C_{20:4n6}$	0.76	0.80	0.84	0.67	0.09	0.53	0.78	0.28
$C_{22:0}$	5.85	5.82	4.35	4.25	0.46	0.90	0.009	0.94
$C_{23:0}$	1.31	1.55	1.74	1.60	0.19	0.79	0.23	0.35
Unsaturated[2]	972.9	804.9	943.1	886.7	29.9	0.005	0.41	0.10
Saturated[3]	180.1	177.4	209.9	197.8	14.0	0.61	0.11	0.74
Total	1225	1059	1194	1123	42.0	0.02	0.70	0.29

[1]BP = method of barley processing (dry-rolled *vs.* pelleted); OS = source of supplemental fat (canola *vs.* flax); BP x OS = interaction.
[2]Unsaturated = $C_{16:1} + C_{18:1} + C_{18:2} + C_{18:3}$
[3]Saturated = $C_{6:0} + C_{8:0} + C_{12:0} + C_{14:0} + C_{16:0} + C_{18:0} + C_{20:0}$
[a-c]Means within rows with different superscripts differ ($P < 0.05$).

who found that feeding cows diets high in $C_{18:1n9c}$ and/or $C_{18:2n6c}$ resulted in intakes of $C_{18:1n9c}$ that were double compared to cows fed a diet that had a low inclusion of $C_{18:1n9c}$ and/or $C_{18:2n6c}$. However, there was no difference (P = 0.34) in the amount of $C_{18:2n6c}$ consumed between cows fed diets containing flaxseed or canola. These observations reflect the differences in the dietary fatty acid profiles, which are illustrated in Table 5.1.

The total amount of fatty acids reaching the proximal duodenum was not affected by grain processing (P = 0.16) or source of oilseed (P = 0.39), but cows fed pelleted barley rations had a much larger flow of unsaturated (P = 0.005) and significantly less (P = 0.02) flow of saturated fatty acids. Higher flows of saturated fatty acids and lower amounts of unsaturated fatty acids reaching the duodenum reflect cows fed dry-rolled barley having a higher rumen pH, which leads to more complete biohydrogenation of dietary fatty acids compared to those cows that have a lower rumen pH (Kalscheur et al., 1997). A more acidic rumen environment gives rise to a higher likelihood of a decline in lipolysis and the subsequent microbial activity related to biohydrogenation (Van Nevel and Demeyer, 1996). There was a larger amount of total fatty acids reaching the duodenum compared to the amount consumed. This difference can be attributed to the ability of rumen microbes to synthesize fatty acids (Jenkins et al., 2008). There was a larger amount of $C_{18:0}$ flowing to the duodenum from all dietary treatments relative to the intakes recorded (Table 5.7). This difference is due to the biohydrogenation of unsaturated fatty acids, especially $C_{18:2n6c}$ and $C_{18:3n3}$, in the rumen (Harfoot and Hazlewood, 1997). However, cows fed pelleted barley had considerably less $C_{18:0}$ (P = 0.01) reaching the duodenum than cows fed dry-rolled barley, reflecting reduced rates of ruminal biohydrogenation of unsaturated FA due to the more acidic ruminal environment

Table 5.7. Fatty acid flow to the duodenum (expressed as g FA/day) in dairy cows (n = 4) fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources.

	Canola		Flax			*P* value[1]		
Fatty acids, g/d	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley	SEM	BP	OS	BP x OS
$C_{4:0}$	3.27	3.64	3.93	5.11	0.40	0.09	0.03	0.34
$C_{6:0}$	0.34	0.66	0.54	0.81	0.32	0.37	0.60	0.94
$C_{12:0}$	3.11	3.53	3.64	3.74	0.20	0.23	0.10	0.45
$C_{14:0}$	10.10	13.20	11.40	14.40	0.75	<0.01	0.14	0.94
$C_{15:0}$	11.60	12.70	15.00	15.30	1.45	0.63	0.07	0.83
$C_{15:1\ (cis)}$	2.16	2.37	2.17	2.87	0.20	0.05	0.23	0.25
$C_{16:0}$	176.80	178.00	223.60	220.80	14.1	0.96	0.01	0.89
$C_{16:1}$	5.86	6.02	7.77	6.16	0.71	0.33	0.18	0.24
$C_{17:0}$	7.77	8.91	9.93	10.30	1.14	0.53	0.16	0.73
$C_{18:0}$	1059.40	797.60	1080.00	661.20	104.1	0.01	0.59	0.47
$C_{18:1n9t}$	84.00	197.10	143.90	284.60	26.4	0.001	0.02	0.61
$C_{18:1n9c}$	81.20	133.80	94.50	96.30	17.2	0.15	0.50	0.17
$C_{18:2n6c}$	44.90	60.00	41.00	65.90	7.65	0.03	0.90	0.54
CLA[2] $_{(c9\text{-}t11)}$	1.30	1.28	1.73	1.32	0.12	0.10	0.07	0.13
CLA $_{(t10\text{-}c12)}$	1.21	1.72	3.88	3.96	0.89	0.75	0.02	0.82

[1]BP = method of barley processing (dry-rolled *vs.* pelleted); OS = source of supplemental fat (canola *vs.* flax); BP x OS = interaction.
[2]CLA = conjugated linoleic acid
[3]Unsaturated = $C_{16:1} + C_{18:1} + C_{18:2} + C_{18:3}$
[4]Saturated = $C_{6:0} + C_{12:0} + C_{14:0} + C_{16:0} + C_{18:0} + C_{20:0}$

Table 5.7. Fatty acid flow to the duodenum (expressed as g FA/day) in dairy cows (n = 4) fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources (continued).

	Canola		Flax			*P* value[1]		
Fatty acids, g/d	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley	SEM	BP	OS	BP x OS
$C_{18:3n3}$	7.00	10.70	20.90	46.60	7.81	0.09	0.01	0.19
$C_{20:0}$	20.50	17.10	13.40	11.60	0.99	0.03	<0.0001	0.46
$C_{20:1}$	3.40	6.33	1.24	2.71	0.41	<0.0001	<0.0001	0.88
$C_{20:2}$	4.84	9.87	3.62	4.24	2.09	0.21	0.14	0.32
$C_{20:3n3}$	1.35	1.35	1.70	1.49	0.24	0.66	0.32	0.66
$C_{22:0}$	9.14^{ab}	8.94^{ab}	9.76^{a}	3.86^{b}	1.26	0.04	0.11	0.05
$C_{22:5\ (DPA)}$	9.95	7.32	13.70	9.12	0.99	0.01	0.02	0.35
$C_{23:0}$	2.41	2.46	3.01	2.41	0.23	0.27	0.27	0.19
Unsaturated[3]	224.30	408.90	309.80	512.80	51.6	0.005	0.10	0.86
Saturated[4]	1270.20	1010.10	1332.50	912.40	117.4	0.02	0.89	0.51
Total	1552	1487	1714	1493	92.3	0.16	0.39	0.42

[1]BP = method of barley processing (dry-rolled *vs.* pelleted); OS = source of supplemental fat (canola *vs.* flax); BP x OS = interaction.
[2]CLA = conjugated linoleic acid
[3]Unsaturated = $C_{16:1} + C_{18:1} + C_{18:2} + C_{18:3}$
[4]Saturated = $C_{6:0} + C_{12:0} + C_{14:0} + C_{16:0} + C_{18:0} + C_{20:0}$[a-c]Means within rows with different superscripts differ ($P < 0.05$).

when compared to cows fed dry-rolled barley. Duodenal flow of $C_{18:1n9c}$ was numerically higher (P = 0.15) in cows fed pelleted barley compared to cows fed dry- rolled barley, even though there was a larger amount of $C_{18:1n9c}$ consumed by cows fed dry-rolled barley compared to those fed pelleted barley. On the contrary, cows fed pelleted barley had larger (P = 0.03) amounts of $C_{18:2n6c}$ flowing to the duodenum. Cows fed diets containing flax had significantly larger duodenal flows of $C_{18:3n3}$ (P = 0.01) and *trans*-10, *cis*-12 CLA (P = 0.02) compared to cows fed canola; however, *cis*-9, *trans*-11 CLA flows only tended (P = 0.07) to be higher in cows fed flaxseed compared to cows fed canola. These observations contradict a study conducted by Loor et al. (2004), where they observed that *trans*-10, *cis*-12 CLA flow to the duodenum to be unaffected by the inclusion of linseed oil compared to those control animals without. However, when "normal" rates of lipolysis have been altered, the extent of biohydrogenation is reduced resulting in an increase in the level of free $C_{18:2n6c}$ found in the rumen. *Trans*-10, *cis*-12 CLA had numerically higher (P = 0.75) duodenal flows in cows fed pelleted barley compared to those fed dry-rolled barley; whereas *cis*-9, *trans*-11 CLA had numerically lower duodenal flows in cows fed pelleted barley compared to dry-rolled barley. These results are expected as it has been demonstrated that a more acidic ruminal pH will alter ruminal biohydrogenation pathways such that *cis*-9, *trans*-11 CLA will be higher at a higher pH and *trans*-10, *cis*-12 CLA will be higher at a lower pH (Troegeler-Meynadier et al., 2003).

5.6 Milk Yield and Composition

Milk yield was not affected by method of barley processing or source of supplemental fat (Table 5.8). In other studies, milk yield increased by 8 to 10% when dry-rolled corn or sorghum was replaced with steam-flaked grains (Theurer et al., 1999) or as the processing index of barley increased (Yang et al., 2000). The large milk yield SEM is atypical and may be indicative of an individual cow outlier. This number may be more representative if the individual squares were analyzed separately, but due to no significant difference between the squares, means were combined. Milk fat content ($P < 0.01$) and milk fat yield ($P = 0.04$) were higher in cows fed dry-rolled barley than in cows fed pelleted barley. Milk fat percentages were 3.39% and 2.83%, and milk fat yields were 1.18 and 1.01 kg/d for cows fed dry-rolled barley and pelleted barley, respectively. The milk fat depression observed in cows fed pelleted barley is likely attributable to the ruminal pH depression and associated changes in ruminal biohydrogenation pathways. In vitro studies indicate that low pH altered biohydrogenation pathways of unsaturated fatty acids, leading to an accumulation of $C_{18:1n11t}$ and conjugated linoleic acid (CLA) isomers (Van Nevel and Demeyer, 1996). Unfortunately the fatty acid protocol was unable to separate $C_{18:1n11t}$ from the other $C_{18:1}$ *trans* isomers. These unsaturated fatty acids are potent inhibitors of *de novo* synthesis of short-chain fatty acids in the mammary gland (Bauman and Griinari, 2001). Therefore, the milk fat depression can be attributed to *trans*-10, *cis*-12 CLA. Barley grain processing and source of supplemental dietary fat did not affect milk protein content; however, milk protein yield tended to be higher ($P = 0.12$) in cows fed diets supplemented with flaxseed. Barley grain processing and source of supplemental dietary fat did not affect milk lactose yield or milk lactose content. Yang

Table 5.8. Effects of barley grain processing (BP) or supplemental dietary fat (OS) on milk production and composition in dairy cows (n = 8).

	Canola		Flax			P value[1]		
Item	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley	SEM	BP	OS	BP x OS
Milk Yield, kg/d	33.42	36.28	36.52	36.35	2.35	0.60	0.54	0.56
Fat, %	3.34	2.94	3.44	2.72	0.11	<0.01	0.72	0.33
Fat, kg/d	1.11	1.05	1.24	0.97	0.07	0.04	0.73	0.17
Protein, %	2.85	2.97	2.99	3.10	0.08	0.34	0.23	0.94
Protein, kg/d	0.94	1.06	1.08	1.09	0.05	0.23	0.12	0.29
Lactose, %	4.13	4.23	4.20	4.26	0.6	0.36	0.57	0.78
Lactose, kg/d	1.38	1.53	1.54	1.53	0.09	0.50	0.46	0.46
Somatic Cell Count, ‘000	262	670	193	210	188	0.32	0.32	0.45
Milk Urea Nitrogen, mg/dL	6.35[a]	4.87[b]	6.13[a]	5.78[a]	0.16	<0.01	0.16	0.03

[1]BP = method of barley processing (dry-rolled *vs.* pelleted); OS = source of supplemental fat (canola *vs.* flax); BP x OS = interaction.
[a-c]Means within rows with different superscripts differ (P < 0.05).

et al. (2000) observed higher milk protein contents in cows fed more extensively processed barley compared to those fed coarsely-rolled barley. Similar improvements in milk protein content have been reported in dairy cows fed steam-flaked compared to dry-rolled sorghum or corn (Theurer et al., 1999), a response likely attributable to increased duodenal flow of metabolizable protein. There was no significant effect of oilseed on DMI, milk yield and composition. These observations reflect the findings of Ward et al. (2002), who found no effect of canola and flax on feed intake, milk yield, milk fat yield or milk fat percentage. They also found that the milk protein content was similar in cows consuming diets containing flax and canola. However, cows consuming flax had increased milk and protein yields, but did not differ in fat yield, which is similar to ewe data obtained by Zhang et al. (2006) and Kalscheur et al. (1997), who found that cows fed supplemental fat experienced a decrease in milk fat percent, milk fat production and milk protein production.

There was a grain processing x oilseed source interaction on the concentration of milk urea nitrogen. Cows consuming dry-rolled barley diets had higher milk urea nitrogen concentrations ($P < 0.01$) than cows consuming pelleted barley diets, on average a difference of 0.92 mg/dL; however there was a greater difference in the milk urea nitrogen concentrations ($P < 0.01$) between cows fed dry-rolled and pelleted barley diets with canola compared to cows fed flax diets.

5.7 Milk Fatty Acid Composition

The amount of saturated fatty acids incorporated into milk fat was much lower ($P < 0.001$) in cows fed pelleted barley compared to cows fed dry-rolled barley (Table 5.9).

Table 5.9. Milk fatty acid (FA) composition (expressed as mg FA/100 mg total detected FA) in dairy cows (n = 8) fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources.

	Canola		Flax			P value[1]		
Fatty acids	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley	SEM	BP	OS	BP x OS
$C_{6:0}$	1.56	1.43	1.68	1.61	0.05	0.17	0.05	0.66
$C_{7:0}$	0.02	0.01	0.02	0.03	0.003	0.60	0.34	0.07
$C_{8:0}$	0.79	0.74	0.91	0.86	0.02	0.11	<0.01	0.95
$C_{9:0}$	0.01	0.02	0.02	0.03	0.002	0.01	0.07	0.99
$C_{10:0}$	1.54	1.46	1.87	1.74	0.06	0.24	<0.01	0.78
$C_{12:0}$	1.88	1.79	2.26	2.15	0.07	0.30	<0.01	0.88
$C_{13:0}$	0.06	0.06	0.07	0.09	0.01	0.23	0.06	0.22
$C_{14:0}$	7.56	7.24	8.31	7.98	0.16	0.16	<0.01	0.98
$C_{14:1\ (trans)}$	0.40	0.45	0.49	0.54	0.02	0.06	<0.01	0.96
$C_{14:1\ (cis)}$	0.68	0.67	0.71	0.87	0.05	0.28	0.12	0.24
$C_{15:0}$	0.89	0.81	0.94	1.05	0.04	0.79	0.02	0.13
$C_{16:0}$	20.25	19.88	21.24	20.33	0.40	0.27	0.22	0.64
$C_{16:1\ (trans)}$	0.28	0.26	0.27	0.27	0.02	0.71	0.99	0.76
$C_{16:1\ (cis)}$	1.37	1.60	1.42	1.71	0.03	<0.001	0.11	0.49
$C_{17:0}$	0.57	0.40	0.26	0.26	0.06	0.32	0.02	0.32
$C_{17:1}$	0.19	0.22	0.14	0.21	0.02	0.12	0.29	0.65
$C_{18:0}$	22.57	20.98	21.35	16.62	1.89	0.81	0.07	0.74
$C_{18:1(trans\ 9\ to\ 11)}$	5.39	6.66	5.24	7.56	0.41	<0.01	0.53	0.38
$C_{18:1(cis\text{-}9)}$	29.44	30.14	26.23	27.02	0.73	0.49	<0.01	0.96
$C_{18:2\ (t,t)}$	0.47	0.70	1.23	1.36	0.12	0.32	<0.01	0.79
$C_{18:2\ (c,c)}$	1.68	1.72	1.79	2.65	0.25	0.23	0.17	0.27

[1]BP = method of barley processing (dry-rolled *vs.* pelleted); OS = source of supplemental fat (canola *vs.* flax); BP x OS = interaction.

Table 5.9. Milk fatty acid (FA) composition (expressed as mg FA/100 mg total detected FA) in dairy cows (n = 8) fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources (continued).

	Canola		Flax			P value[1]		
Fatty acids	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley	SEM	BP	OS	BP x OS
CLA[2] (c9-t11)	0.80[c]	0.94[b]	1.01[b]	1.92[a]	0.08	<0.001	<0.001	<0.01
CLA (t10-c12)	0.04	0.10	0.11	0.10	0.03	0.50	0.36	0.35
$C_{18:3}$ (gamma)	0.25	0.23	0.16	0.16	0.02	0.73	<0.01	0.67
$C_{18:3}$	0.71[c]	0.77[c]	1.47[b]	2.30[a]	0.08	<0.01	<0.0001	<0.01
$C_{20:0}$	0.11	0.23	0.32	0.10	0.07	0.66	0.70	0.10
$C_{20:1}$	0.14	0.15	0.06	0.09	0.02	0.44	<0.01	0.65
$C_{20:4}$	0.12	0.10	0.10	0.12	0.01	0.98	0.77	0.03
$C_{20:5}$ (EPA)	0.08	0.08	0.11	0.11	0.01	0.88	<0.01	0.88
$C_{22:0}$	0.05	0.05	0.11	0.09	0.01	0.78	<0.01	0.50
$C_{22:5}$ (DPA)	0.08	0.08	0.09	0.09	0.01	0.86	0.41	0.80
$C_{22:6}$ (DHA)	0.01	0.02	0.03	0.02	0.01	0.76	0.45	0.46
Short-Chained[3]	5.77	5.42	6.72	6.35	0.17	0.16	<0.01	0.97
Medium-Chained[4]	32.01	31.31	33.65	33.00	0.49	0.35	0.03	0.97
Long-Chained[5]	61.46	58.88	58.93	59.44	1.53	0.64	0.66	0.49
Monounsaturated[6]	37.57	37.56	34.36	37.97	1.24	0.33	0.44	0.32
Polyunsaturated[7]	3.95[c]	4.29[bc]	5.76[b]	8.14[a]	0.26	<0.01	<0.0001	0.02
Saturated[8]	56.26	52.57	57.94	51.38	0.81	<0.001	0.83	0.23

[1]BP = method of barley processing (dry-rolled *vs.* pelleted); OS = source of supplemental fat (canola *vs.* flax); BP x OS = interaction.
[2]CLA = conjugated linoleic acid
[3]Short-Chained = $C_{6:0} + C_{8:0} + C_{10:0} + C_{12:0}$
[4]Medium-Chained = $C_{14:0} + C_{14:1} + C_{15:0} + C_{16:0} + C_{16:1} + C_{17:0}$
[5]Long-Chained = $C_{18:0} + C_{18:1} + C_{18:2} + C_{18:3} + C_{20:0}$
[6]Mononunsaturated = $C_{14:1} + C_{16:1} + C_{18:1}$
[7]Polyunsaturated = $C_{18:2} + C_{18:3}$
[8]Saturated = $C_{6:0} + C_{8:0} + C_{10:0} + C_{12:0} + C_{14:0} + C_{16:0} + C_{18:0} + C_{20:0}$
[a-c]Means within rows with different superscripts differ ($P < 0.05$).

Lower inclusion of these saturated fatty acids into milk fat would be expected due to the smaller flow rates to the duodenum. The levels of $C_{18:0}$ in milk was at a much lower level than were found in duodenal samples. However, unlike duodenal flows, $C_{18:0}$ incorporation into milk fat was unaffected by barley processing but rather tended to be lower ($P = 0.07$) in diets containing flax. Numerically there was a lower concentration ($P = 0.81$) of $C_{18:0}$ in the milk of cows fed pelleted barley which correlates to the duodenal fatty acid profile. Cows fed pelleted barley ($P = 0.001$) and flax ($P = 0.02$) had higher levels of $C_{18:1n9t}$ flowing to the duodenum. This fatty acid is not of great biological importance, but due to the limitation of not having the ability to separate and measure $C_{18:1n11t}$, this fatty acid does contribute to the measurement of $C_{18:1\ (trans\ 9\ to\ 11)}$ therefore contributing to higher amounts found in the milk of cows fed pelleted barley rations ($P < 0.01$). Wonsil et al. (1994) found that with increasing *trans* $C_{18:1}$ concentrations flowing to the duodenum, milk fat percentage was found to decrease in a linear fashion. This observation is comparable to the results found in this study where cows fed pelleted barley had larger amounts of *trans* $C_{18:1}$ in duodenal digesta, resulting from decreased ruminal pH, which was then incorporated into the mammary gland where the isomer was responsible for milk fat depression in those cows. It is reasonable to assume that cows that were fed pelleted barley rations and experienced a reduction in ruminal pH, experienced lower rates of biohydrogenation, allowing for the accumulation of intermediates, such as $C_{18:1n9t}$, and the subsequent flow to the proximal duodenum as rates of ruminal biohydrogenation were not specifically measured. Cows consuming flax had 45% more $C_{18:2}$ and significantly more ($P < 0.0001$) $C_{18:3(c,c)}$ than those than cows consuming canola. Such observations are in agreement with Ward et al. (2002), who

found that diets containing flax increased $C_{18:2}$ and $C_{18:3}$ in milk 39 and 157%, respectively, compared to the average in diets containing canola. Zhang et al. (2006) also observed a significant increase in the concentrations of $C_{18:2n6c}$ and $C_{18:3n3}$ in ewe milk when animals were fed flaxseed enriched diets.

There was a barley processing x oilseed type interaction on the amount of $C_{18:3}$ produced in milk. Cows fed diets with flax had higher levels of $C_{18:3}$ ($P < 0.01$) in milk than cows fed diets containing canola; however, there was a significantly greater difference in the levels of $C_{18:3}$ ($P < 0.02$) between cows fed dry-rolled and pelleted barley diets with flax compared to cows fed canola rations. This interaction can be explained due to the higher levels of $C_{18:3}$ in flaxseed and the decrease in rumen biohydrogenation, thereby resulting in greater amounts of $C_{18:3}$ flowing to the duodenum and being incorporated into the milk fat of cows fed pelleted barley. There was a barley processing x source of oilseed interaction on the amount of *cis*-9, *trans*-11 CLA incorporated into the milk. Cows fed pelleted barley diets had higher ($P < 0.001$) levels of *cis*-9, *trans*-11 CLA than cows fed dry-rolled barley. This result is uncharacteristic as it would be thought that due to the lower ruminal pH cows exhibited due to barley processing, the amount of *cis*-9, *trans*-11 CLA would be lower than the amount of *trans*-10, *cis* -12 CLA in the milk (Troegeler-Meynadier et al., 2003**).** Cows fed flax having more *cis*-9, *trans*-11 CLA than cows fed canola is in accordance to results obtained by Collomb et al. (2004), who found that by adding 1 kg of rapeseed, 1 kg of linseed and 1.4 kg of linseed to the control diet, *cis*-9, *trans*-11 CLA content increased by 37%, 19% and 83%, respectively. Contrasting results have been observed by Ward et al. (2002), who found cows consuming canola had an average 23% more CLA than cows consuming

diets containing flax. There was also a barley processing x source of oilseed interaction on the amount of polyunsaturated fatty acids present in milk fat. Cows fed pelleted barley had higher levels of milk PUFA than cows fed dry-rolled barley; however cows fed flax had much higher (P = 0.02) levels of milk PUFA than cows fed canola. This interaction can be linked to the increased amount of *cis*-9, *trans*-11 CLA, as it is included in the formula determining the total amount of PUFA. There was no effect of barley processing (P = 0.50) or source of oilseed (P = 0.36) on the amount of *trans*-10, *cis* -12 CLA produced in milk. There was no effect of barley processing (P = 0.50) or source of oilseed (P = 0.36) on the amount of *trans*-10, *cis* -12 CLA. This observation could also be related to the increase in *trans* intermediates produced in the rumen through incomplete biohydrogenation of $C_{18:3n3}$. Milk fat percentages in cows fed pelleted barley and flax diets were lower. These results are indicative of a milk fat depression resulting from a decreased ruminal pH thereby increasing the presence of unsaturated biohydrogenation intermediates, their passage to the duodenum and incorporation into milk fat. This is evident based on the observations that cows fed pelleted barley had a lower ruminal pH causing an increase in the amount of *trans*-10, *cis*-12 CLA and $C_{18:1n11t}$ reaching the duodenum which leads to a MFD. However, there was no difference in the amount of *trans*-10, *cis* -12 CLA in milk fat and was unaffected by barley processing and source of oilseed. This observation suggests that the larger (P < 0.01) amounts of *trans* $C_{18:1}$ produced in cows fed pelleted barley compared to cows fed dry-rolled barley can contribute as one of the main factors responsible for the MFD observed and not necessarily all due to the presence of *trans*-10, *cis* -12 CLA.

6.0 GENERAL CONCLUSIONS

Feeding rations to dairy cows consisting of western Canadian feedstuffs has great potential to allow producers to reach niche markets due to the ability to increase the level of milk CLA. In the present study, a more acidic ruminal environment in cows fed pelleted barley compared with those fed dry-rolled barley is consistent with a shift in the site of barley starch digestion.

Results from this study indicated that feeding pelleted barley reduced rumen pH to a point where rumen biohydrogenation was significantly decreased. As a result, the amount of unsaturated fatty acids reaching the duodenum and their subsequent incorporation into milk fat, either through direct absorption from the small intestine, or through *de novo* synthesis in the mammary glad, dramatically increased. However, a decrease in milk fat was observed in cows fed pelleted barley. This result could be seen as detrimental to the dairy producers of western Canada due to milk fat being their key pricing component.

Decreasing the amount of butterfat produced not only decreases a producer's revenue, for that specific milk component, but the solid:nonfat ratio is also altered. This occurs by increasing the ratio and increasing the market availability of skim milk powder. This potentially floods an already over-supplied market. As a result there is a decline in consumer demand for an over supplied product, ultimately leading to a decrease in the price paid to the producer for his/her milk.

Cows experiencing a milk fat depression not only affect producers and their bottom line, but the health of cow is compromised. Due to the decreasing ruminal pH, rumen papillae are damaged, resulting in a rather incapable nutrient absorptive surface.

Consequently, cows are unable to utilize dietary nutrients and vitamins to the best of their ability, potentially resulting in malnourishment. Also, cows that spend a large amount of time in an acidotic state tend to not only find physiological problems related to their rumens, but feet as well. Cows experiencing ruminal acidosis for extended periods of time will also develop ulcerations in the soles of their hooves causing lameness and debilitated locomotion. Therefore, walking for food and water becomes challenging and can also lead to a state of malnourishment.

Research shows that cows fed mixed rations, especially when lipid supplements are included in the diet, have been observed to have numerous $C_{18:1}$ and $C_{18:2}$ isomers in the duodenal contents and can vary in amount depending on forage:concentrate ratio (Lee et al., 2006). The presence of these fatty acids is likely correlated with the fatty acid profile of the supplement and their subsequent pathways followed during rumen biohydrogenation. Results from this study also indicated that feeding flaxseed increases the percentage of CLA present in the milk fat and gives rise to more rumen biohydrogenation intermediates that can be converted into CLA through mammary enzymatic processes.

In conclusion, having the ability to manipulate rumen biohydrogenation by feeding processed barley and increasing the amount of unsaturated fatty acids being incorporated into milk is a practical and potentially economical production choice for dairy producers. However, the economic benefits of supplying a health-enriched product must outweigh the negative animal health consequences, or at least take into consideration the potential health risks that these animals may be challenged with.

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