EFFECT OF LIPID SUPPLEMENTATION ON RUMINAL EPITHELIAL MEMBRANE FATTY ACID COMPOSITION AND SHORT-CHAIN FATTY ACID ABSORPTION

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ABSTRACT

Inclusion of lipid into diets increases the energy density and, depending on the type of lipid, may alter the fatty acid (**FA**) composition of tissues. Effects of dietary lipid on the digestive and immune function gastrointestinal tract have been evaluated, but effects on how dietary FA affect short-chain fatty acid (**SCFA**) absorption have not been investigated. The objective of this study was to determine the effect of dietary lipid supply and lipid type on the FA composition of the ruminal epithelium and absorption of SCFA. Twenty-one Holstein steers (194.1 ± 26.77 kg) were randomly assigned to the control (**CON**; 2.2% ether extract) or 1 of 2 lipid supplementation treatments (5% ether extract) utilizing saturated (**SAT**) or unsaturated sources and protected fat (**UNSAT**). After 30 d, calves were killed and samples of ruminal digesta, blood, and ruminal tissue were collected for FA analysis, and ruminal tissue was used for ex vivo measurement of acetate, propionate, and butyrate uptake and flux. Data were analyzed as a randomized complete block design using the mixed model of SAS with the fixed effect of treatment and the random effect of block. Calves fed SAT and UNSAT had greater ($P < 0.01$) concentration of total FA in ruminal fluid than CON. Feeding UNSAT increased the monounsaturated ($P < 0.001$) and polyunsaturated ($P = 0.002$) FA content in ruminal fluid relative to SAT and CON. The concentration of FA in the ruminal epithelium did not differ among treatments but there was a tendency ($P = 0.069$) for SAT calves to have more total FA and saturated FA ($P = 0.053$) than UNSAT. Moreover, UNSAT calves had greater ($P = 0.006$) omega-3 FA concentration in the ruminal epithelium than CON and SAT calves. Calves fed SAT had greater ($P = 0.038$) total propionate uptake with greater passive diffusion ($P = 0.015$) than CON and UNSAT. Calves fed SAT also had greater total butyrate uptake ($P = 0.008$). However, there were no differences for acetate, propionate, or butyrate flux among treatments. Thus, it is concluded that the provision of dietary lipid alters the FA composition of the ruminal epithelium and the uptake of propionate and butyrate with the greatest response when saturated lipid sources are provided.

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LIST OF ABREVIATIONS

SAT = diet containing saturated lipid

UNSAT = diet containing unsaturated lipid

CON = control treatment

$SCFA^-$ = short-chain fatty acid, non-associated state

SCFA = short-chain fatty acids

H-SCFA = short-chain fatty acids, associated state

LCFA = long-chain fatty acids

PUFA = polyunsaturated fatty acids

FA = fatty acids

FFA = free fatty acids

CLA = conjugated linoleic acid

EPA = eicosapentaenoic acid

DHA= docosahexaenoic acid

A/P = acetate propionate ratio

DMI = dry matter intake

ADG = average daily gain

BW = body weight

SCD = stearoyl CoA desaturase

MCT = monocarboxylate transporters

NHE = Na^+/H^+ exchange

CD36 = fatty acid translocase

FATP = fatty acid transport protein

FABP = fatty acid binding protein

FAT = fatty acid transporter

ACTB = beta actin

GAPDH = glyceraldehyde-3-phosphate dehydrogenase

RPLP0 = 60S acid ribosomal protein P0

ATP = Adenosine triphosphate

VLDL = very low density lipoprotein

GP – glycoprotein

PUFA – polyunsaturated fatty acid

ω = omega

Δ = delta

C16:0 = Palmitic acid

C18:0 = Stearic acid

C20:0 = Eicosanoic acid

C22:0 = Docosanoic acid

C18:3N3 = alfa-linolenic fatty acid

1.0 GENERAL INTRODUCTION

The use of dietary fat in ruminants can be an effective strategy to increase the energy density of the diet. For ruminants, dietary fatty acids are first modified by the rumen microbiota and can have specific and potent effects on the subsequent digestion and utilization of other nutrients. While fatty acid supply can alter tissue metabolism, ruminal lipid metabolism has a major impact on the profile of fatty acids available for absorption and tissue utilization (Lock et al., 2006).

Feeding lipid to dairy cows can increase milk production (Palmquist and Jenkins, 1980), especially in early lactation when the amount of energy consumed is not sufficient to meet the energy requirements (Palmquist and Jenkins, 1980). Increasing the dietary lipid concentration can also benefit cows with high milk yields (Mattias et al., 1982) and supplementation may reduce the risk of metabolic disorders such as ketosis (Kronfeld et al., 1980).

Dietary lipid is also a determinant of membrane structure and is a modulator of the biological activity of subcellular membranes and processes (Clandini et al., 1991). Supplementation of lipid modulates the composition of cell membrane lipids which translates to altered movement of nutrients across the membrane due to changes in membrane permeability (Scott, 1993; Maddock et al., 2006; Calder, 2012).

While the microbes in the rumen alter dietary fatty acids, microbial fermentation of carbohydrates in the rumen yields short-chain fatty acids (**SCFA**). Short-chain fatty acids are absorbed across the reticulo-ruminal epithelium and are the main source of energy for ruminants (Bergman, 1990). On top of providing energy, the absorption of SCFA is one of the primary mechanisms for the regulation of ruminal pH (Gäbel et al., 2002). Pathways for SCFA absorption across the reticulo-ruminal epithelium include transporters facilitating ion exchange and simple passive diffusion (Aschenbach et al., 2011). Interestingly, it appears that passive diffusion may be one of the most responsive pathways adapting when cattle are exposed to dietary change (Schurmann et al., 2014). While it is known that dietary lipids can modulate the fatty acid composition of muscle and adipose tissue, it is not clear if the composition of the ruminal epithelium is affected

by dietary lipid source or whether lipid membrane composition may affect SCFA absorption.

2.0 LITERATURE REVIEW

2.1 Overview of ruminal fermentation

The forestomach of ruminants is the primary site for microbial fermentation of feeds and the subsequent production and absorption of short-chain fatty acids (**SCFA**; Gäbel et al., 2002). The primary SCFA include acetate, propionate, and butyrate. The importance of SCFA should not be understated as they have been reported to be the main source of energy for ruminants and it is estimated that they provide up to 75% of the metabolizable energy (Bergman, 1990). Thus, diets that are greater in fermentability have the potential to increase both the production and absorption of SCFA (Sehested et al., 2000). However, feeding diets that are highly fermentable also increase the risk for ruminal acidosis (Penner et al., 2007).

Ruminal pH is a balance between acid production and the removal of acid from the rumen. Acid removal strategies include proton removal via buffering processes (salivary and ruminal epithelial bicarbonate; Dijkstra et al., 2012), removal of proteins with the absorption of SCFA, and with passage of H^+ out of the rumen (Allen, 1997). As noted, saliva is an important mechanism for proton removal as it contains bicarbonate and phosphate buffers (Aschenbach et al., 2010). Saliva is also a source of liquid that dilutes hydrogen ions and increases the passage rate through the omasal orifice (Allen, 1997).

SCFA absorption contributes to stabilization of ruminal pH and strategies to enhance SCFA absorption not only serve as a mechanism to increase energetic supply but also to reduce risk for ruminal acidosis (Penner et al., 2009). Strategies to increase SCFA absorption have been studied during the past years and they include adaptation to diets with a greater fermentability (Penner et al., 2010) and increase in absorptive surface area (Bannink et al., 2008). Uppal et al. (2003) found that feeding a diet that had a moderate to high fermentability contributed to increased rates of SCFA absorption when compared to diets with low fermentability. It should be noted that diets with greater fermentability also increase the risk for ruminal acidosis. Given that SCFA absorption contributes to provision of energy and stabilization of ruminal pH, strategies to maximize ruminal SCFA absorption have merit.

2.2 Short-chain fatty acids and their absorption

Short-chain fatty acids are a major product of fermentative digestion by microorganisms in the forestomach of ruminants (Gäbel and Sehested, 1997). Short-chain fatty acids by definition are organic fatty acids (**FA**) with 1 to 6 carbons and contain a carboxylic acid and a small hydrocarbon chain. The molar proportions of acetic, propionic, and butyric acids in ruminal fluid range from 45 to 70%, 14 to 40%, and 5 to 20%, respectively (Bergman, 1990). The production of large amounts of SCFA can cause an equimolar release of protons due to dissociation of SCFA in the rumen (Gäbel and Ashcenbach, 2006). The dissociation of the SCFA is the driving factor for a decrease in ruminal pH (Aschenbach et al., 2010). The dissociation equilibrium is defined by the pKa value (where pKa = −log(Ka)). At the pKa of an individual compound, 50% will be present in the acid (dissociated phase) and 50% will be in the non-dissociated phase. Short-chain fatty acids have a pKa of approximately 4.8, and as such release a proton when pH is above 4.8 and bind to protons when pH decreases below 4.8 (Aschenbach et al., 2010). Thus, SCFA stabilize ruminal pH between 3.8 and 5.8, thereby acting as weak acids (Krause and Oetzel, 2006).

The majority of the SCFA produced in the rumen are directly absorbed across the epithelium of the reticulo-rumen (Phillipson and McAnally, 1942; Barcroft et al., 1944). It has been estimated that 50 to 85% of the SCFA that are produced in the rumen are also absorbed by the rumen epithelium and around 15 to 50% of the SCFA produced in the rumen passes to the omasum and is absorbed prior to reaching the abomasum (Aschenbach et al., 2010). The concept of pre-gastric absorption is supported by regional SCFA concentrations, with concentrations up to 170.8 m*M* in the rumen with only 6.4 m*M* in the duodenum (Pederzolli, 2016)

Protons can be removed by SCFA absorption and a number of factors influence the rate and pathway for SCFA absorption. The relationship between ruminal pH and absorption is controversial. Dijkstra et al. (1993) found that propionic and butyric acid absorption rates were greater with low initial pH. However, that study used the washed reticulo-ruminal technique and they observed marked increases in the incubation buffer pH by the end of the absorption measurement period. More recent studies have reported a decrease in the absorption rate (Schwaiger et al., 2013; Penner et al, 2009; Wilson et al.,

2012) when tissues were exposed to low pH either in vivo or ex vivo. While the studies differ in their findings, a reduction in absorption rate in association with low ruminal pH may help to reduce intracellular acidification of the ruminal epithelial cells and systemic acidification (metabolic acidosis). While the effect of low ruminal pH is debatable, the rate of absorption and metabolism of each SCFA is related to chain length, where butyrate is more lipophilic than propionate, and propionate more lipophilic than acetate. These rates of lipophilicity are inversely related with their production rates and concentrations in the rumen (Masson and Phillipson, 1951).

Early reports had suggested that absorption of SCFA occurred exclusively by passive diffusion (Bugaut, 1987), although it is now clear that this is not the case (Aschenbach et al., 2010). Nevertheless, some SCFA are absorbed via passive diffusion and as indicated above, passive diffusion may be one of the most responsive pathways. Graham et al. (2007) indicated that passive diffusion was the primary mechanism for SCFA absorption although they did not conduct any studies evaluating functional pathways. Absorption of SCFA via passive diffusion will result in the release of a H^+ inside the cell, which will act to acidify the cell (Muller et al., 2000). To counteract intracellular acidification, upregulation of Na^+/H^+ exchangers (**NHE**) and monocarboxylate transporters (**MCT**) has been reported (Muller et al., 2000). Schurmann et al. (2014) found that passive diffusion was the most responsive pathway for SCFA absorption induced by dietary change. Passive diffusion occurs when SCFA are in the protonated form (**H-SCFA**) as H-SCFA have greater permeability (Walter and Gutknecht, 1986; Gäbel et al., 2002) than when dissociated (**$SCFA^-$**). Given that chain length affects permeability (Walter and Gutknecht, 1986), it is not surprising that butyrate transport relies on passive diffusion to a greater extent than propionate and acetate (Gäbel and Aschenbach, 2006). In fact, the proportion of butyrate transported via passive diffusion is about 72% while the proportion of acetate absorbed via passive diffusion is about 28% (Beauchemin & Penner, 2009). It should be noted that passive diffusion of SCFA represents transcellular movement rather than paracellular movement (Sehested, 1999).

While it is often stated that absorption of SCFA proceeds with passive diffusion, it is unlikely that passive diffusion can explain a significant proportion of the basolateral

efflux (Dengler et al., 2013). This is because H-SCFA will dissociate in the cytosol resulting in H^+ + $SCFA^-$. Dissociation of H-SCFA in the cytosol can decrease intracellular pH (Figure 1). Fortunately, a reduction in intracellular pH also stimulates the activity of NHE (Muller et al., 2002). It is been shown that NHE are the major cell-alkalinizing mechanism to recover pH after cell acidification (Kenyon et al., 1997).

Given that SCFA anions are not lipophilic (Walter and Gutnecht, 1986), even passive diffusive uptake of H-SCFA must partially rely on transporter-mediated pathways such as MCT (Graham et al., 2007) to facilitate basolateral efflux (Dengler et al., 2013). Monocarboxylate transporters have 14 family members; however, only MCT-1, -2, -3, and -4 catalyze proton-coupled transport of metabolically important monocarboxylates (Halestrap and Meredith, 2004). In the rumen and large intestine of goats, MCT4 has been detected (Koho et al., 2005) with the order of abundance equating to rumen > large intestine > caecum > abomasum > small intestine (Kirat, 2006). In addition to regional localization, MCT-4 is predominantly localized on basolateral membranes of the rumen. Basolateral localization suggests that MCT-4 plays an essential role in SCFA efflux across cell membranes and MCT-4 may be involved in basolateral efflux (Kirat et al, 2006). Kirat et al. (2006) also suggested presence of MCT1 in the ruminant gastrointestinal tract further suggesting that MCT may play an important role in association with passive diffusive apical uptake.

In addition to passive diffusion, SCFA- can be transported via facilitated transporters (Aschenbach et al., 2010). The main pathway for $SCFA^-$ absorption has been identified to occur via anion exchange, namely $SCFA^-/HCO_3^-$ exchange (Bilk et al., 2005).

The $SCFA^-/HCO_3^-$ transport mechanism also serves to stabilize ruminal pH as it provides HCO_3^- to the rumen. However, removal of HCO_3^- may further challenge intracellular pH (Kenyon et al., 1997; Müller et al., 2002). The challenge to intracellular pH may be extensive as up to 50% of the SCFA can be absorbed by the $SCFA^-/HCO_3^-$ pathway (Aschenbach et al., 2009). The presence of bicarbonate for absorption in exchange with SCFA is important, especially for acids with less lipophilicity, such as acetate. The cellular bicarbonate supply is provided via Na^+/HCO_3^- co-transport (Archenbach et al., 2010), rather than through intracellular carbonic anhydrase activity (Sehested et al., 1999).

Another mechanism for SCFA absorption is through a nitrate-sensitive pathway (Aschenbach et al., 2009). However, not much is known about this mechanism other than SCFA absorption is inhibited in presence of nitrate whether HCO_3^- is present or absent (Aschenbach et al., 2009). Hence, understanding how diets may affect the rate and pathway of SCFA absorption is important. It is important to note that passive diffusion appears to be the most responsive pathway and strategies to increase passive diffusion may be the most likely approach to increase SCFA absorption.

2.3 Role of lipids in diets for ruminants

Lipids can be neutral (fatty acids, alcohols, glycerides, and sterols), or polar (glycerophospholipids and glyceroglycolipids) and the classification of lipids are based on their properties at room temperature (oils are liquid and fats are solid), their polarity (polar and neutral lipids), their essentiality for humans and animals (essential and nonessential fatty acids), or their structure (simple or complex; Akoh and Min, 2002). With respect to long-chain unsaturated fatty acids, nomenclature is based on the number of carbons and the number and the position of double bonds (Lobb and Chow, 2000). The letters omega (ω) and delta (Δ) are used to identify the position of the double bonds. Omega is used to indicate how far a double bond is from the terminal methyl carbon according to chain length and delta is used to designate the presence and position of double or triple bounds in the hydrocarbon chain counting from the carboxyl carbon (Lobb and Chow, 2000). There is another classification similar to the "ω" which is called "n" (Davidson and Cantrill, 1985). The 'n' classification refers to the position of the first double bond. The geometric configuration or systematic nomenclature identify the stereo-isomers and differentiate the cis-polyunsaturated fatty acids from the trans-isomers. Classifying the cis- and trans- isomers is important due to their differential characteristics (Davidson and Cantrill, 1985).

Lipids are commonly utilized in ruminant diets in response to their high caloric value. Lipid supplementation is also known to increase energy intake and efficiency in lactation (Chilliard, 1993). However, high inclusion rates may result in negative outcomes (Chilliard, 1993; Doreau and Ferlay, 1994). Lipids may modify digestion and absorption of nutrients in the rumen thereby altering the concentration and composition of fatty acids in milk, meat, and fat (Grummer, 1991). The dietary lipid content usually consists of < 3

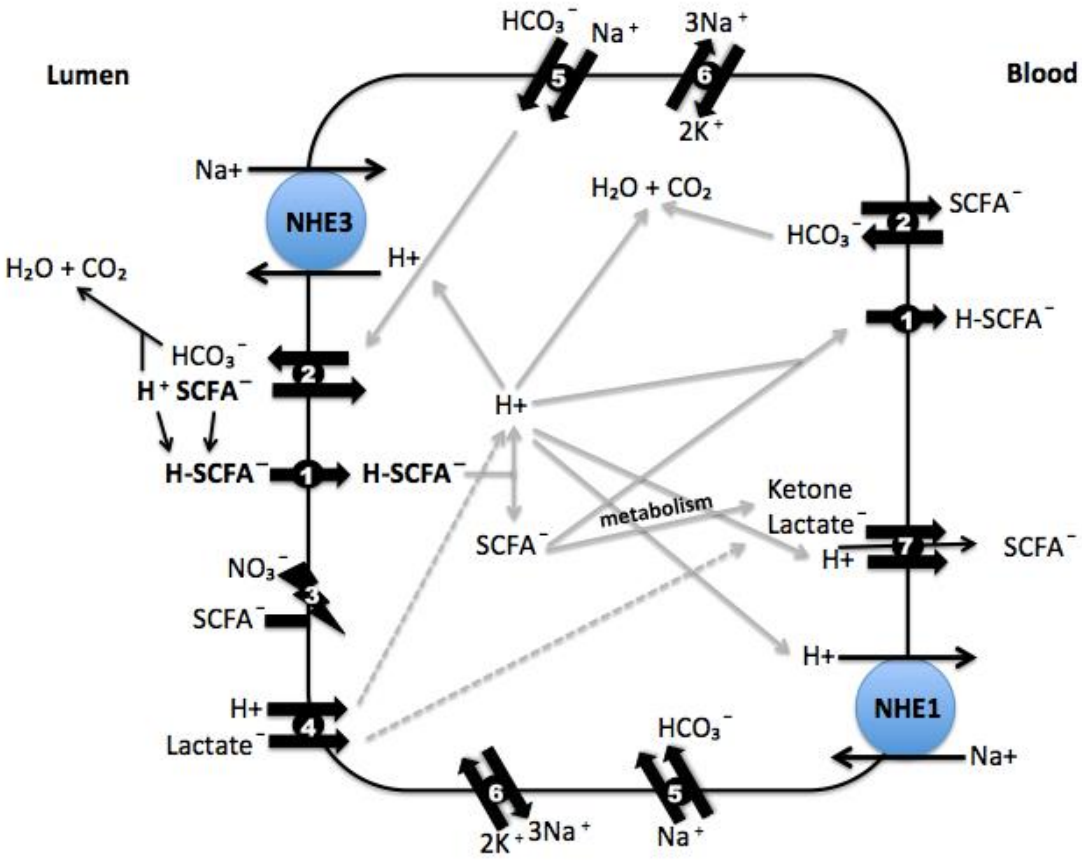

Figure 1.1. Mechanisms of SCFA absorption by the ruminal epithelium. 1) Passive diffusion of short-chain fatty acid in the undissociated form and the subsequent dissociation in the cytosol. 2) Anion exchangers that facilitate SCFA- exchange with bicarbonate. 3) Nitrate-sensitive SCFA transport. 4) Lactate enters the cell via the monocarboxylate transporter (MCT) in co-transport with a proton. 5) Sodium coupled bicarbonate transport facilitates the import HCO3- and Na+ from arterial circulation. 6) Na+/K+ ATPase at the basolateral membrane drives Na+ transport consuming ATP. 7) Lactate and products from the metabolism of SCFA such as ketones can be exported in co-transport with a H+ via MCT. The NHE1 and NHE3 facilitate the removal of protons to avoid cell acidification utilizing a Na gradient. Adapted from Aschenbach et al. (2011).

% of ruminant diets with the lipid sources coming from forage, grains, and oilseeds (Palmquist and Jenkins, 1980). The general recommendation when providing added lipid is that total dietary ether extract should not exceed 6 to 7% of dietary DM (Jenkins, 1993; Doreau et al., 1997; NRC, 2001)

Dietary lipid sources can originate from animal origin such as tallow and fish oils, or from vegetable sources such as flaxseed and palm oil (Chilliard, 1993). The mechanisms for how lipids interfere with and are affected by ruminal fermentation are complex and result in large differences between the initial fatty acid profile of the diet and the final composition of the lipids leaving the rumen. Modification of fatty acids in the rumen is due to lipolysis and biohydrogenation (Jenkins, 2008) and will be discussed later in this chapter. Within source, forms of lipid include phospholipids, triglycerides, and glycolipids. Fresh forages contain around 4 to 6% ether extract with glycolipids as the major lipid class (Harfoot, 1981). Lipid supplements such as Ca-salts are composed by free fatty acids, and by-products contain predominantly triglycerides.

2.3.1 Lipolysis and Biohydrogenation

Shortly after dietary lipid is consumed, ester linkages found in triglycerides, phospholipids, and glycolipids are hydrolyzed by extra-cellular microbial enzymes (lipases). Hydrolyzation results in the release of free fatty acids (**FFA**), glycerol, mono- and di-glycerides (Jenkins, 1993; Figure 2). The glycerol released is rapidly metabolized by rumen bacteria to SCFA, such as propionate (Bauman et al., 2003). Triglyceride hydrolysis occurs rapidly (Jenkins, 2008) and it is estimated that more than 75% of the total lipid is released as free fatty acids for linseed oil when incubated with ruminal contents from sheep (Garton et al., 1958). Others have reported similar results for the rate and extent of glycolipid hydrolysis (Dawson et al., 1974).

Identification of species important for lipid digestion in the rumen has been initiated. For example, lipase from *Butyrivibrio fibrisolvens* has been shown to be effective at hydrolyzing phospholipids and *Anaerovibrio lipolytica* is known to produce two hydrolytic enzymes; a cell-bound esterase and an extracellular lipase (Harfoot, 1978). *Anaerovibrio lipolytica* is also capable of hydrolyzing tri- and di-glycerides (Buccioni et al., 2012). The release of FFA can also occur from hydrolysis of galactolipids and

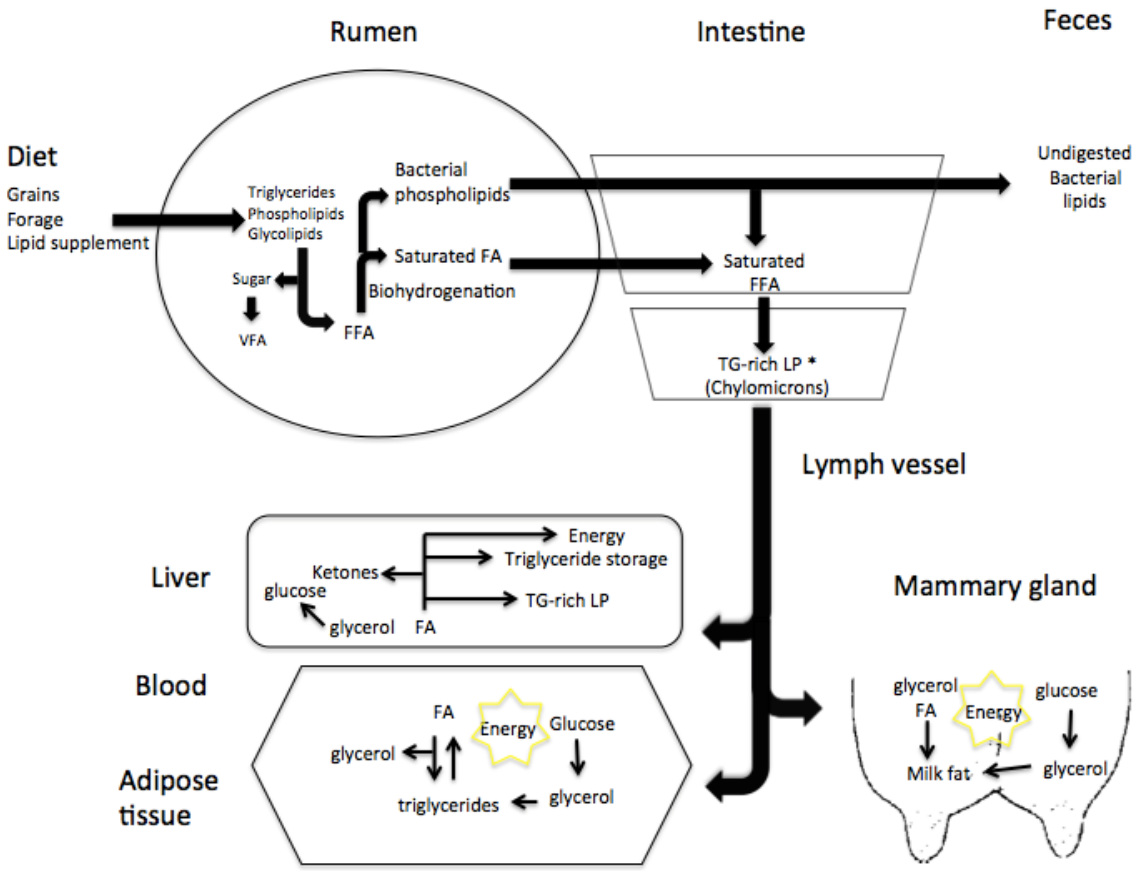

Figure 1.2. Lipid metabolism in ruminants. Lipids enter in the rumen as triglycerides, phospholipids, and glycolipids and are hydrogenated resulting in a release of free fatty acids. The free fatty acids will be modified by microbial biohydrogenation that consists of the conversion of unsaturated to saturated fatty acids. Fatty acids reach the intestine and are esterified into triacylglycerol and phospholipids that are incorporated into chylomicrons (Triglycerides, FFA, and cholesterol) are carried through the lymph vessel ultimately reaching the liver. Fatty acids are used by the liver as a source of energy or are stored. Peripheral tissues may also use triacylglycerol and phospholipids as energy source or incorporated into to milk fat.

phospholipids in response to the action of other types of bacteria with *galactosidase a*nd *phospholipase* activity, respectively (Jenkins, 1993). Hydrolysis of the esterified fatty acids is the first step that leads to another process called biohydrogenation. The lipolysis step is important because it releases free fatty acids that contain a free carboxyl group. The carboxyl group is used in an isomerization reaction that utilizes the cis-12 double bond in unsaturated fatty acids and converts the cis orientated isomer to a trans isomer (Jenkins, 1993). This pathway is used as a protective mechanism by ruminal microbes to reduce the toxicity of unsaturated fatty acids (Jenkins, 1993). This isomerization process is also followed by rapid hydrogenation to yield more saturated fatty acids (Jenkins, 1993).

The principal FA that are hydrogenated in the rumen are linoleic and linolenic acids, with the proportions of hydrogenation varying between 70 and 95%, and 85 and 100%, respectively (Beam et al., 2000). Eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6) fatty acids are normally included in rumen-protected lipid supplements: however, there is some controversy about the actual rate of hydrogenation of these fatty acids in the rumen (Bauman, 2003). Gulati et al. (1999) suggested, based on in vitro studies, that there is a small amount of biodyhrogenation of EPA and DHA fatty acids in the rumen while Scollan (2001) found that almost all of the EPA and DHA were biohydrogenated in vivo, but at a slower rate than when compared to linoleic and linolenic acids.

The presence of double bonds in fatty acids increases the rate of microbial activity in the ruminal environment, likely through isomerization and hydrogenation reactions (Beam, 2000). Bacteria can incorporate and synthesize FA with 15C and 17C and their synthesis are mainly from SCFA (Doreau, 1997). Protozoa and fungi can also incorporate and synthesize FA (Emmanuel, 1974, Kemp et al. 1984). Fatty acids can be stored as free FA in cytosolic droplets. When large amounts of FA are fed to ruminants, they can escape hydrogenation and are normally rich in linoleic acid (Bauchart et al., 1993).

The inhibition or partial inhibition of rumen biohydrogenation is a process that has been studied to improve the capacity of ruminants to incorporate unsaturated fatty acids into meat or milk. Studies have reported that greater inclusion rates of starch and less fiber promotes shifts in biohydrogenation (Offer et al., 1999; Offer et al., 2001). The

effect of increasing starch and decreasing fiber on the partial inhibition of biohydrogenation is thought to be due to inhibition of microbial species in association with low ruminal pH (Kalscheur et al., 1997). Forage conservation methods can also change the extent of biohydrogenation, with biohydrogenation being greater for fresh or ensiled grass than dried hay (Boufaïed et al., 2003). Ionophores such as monensin, nigericin, and tetronasin affect biohydrogenation. Ionophores can also increase cis- and trans- C18:1, and cis-cis-18:2 (Fellner et al. 1997).

2.4 Lipid digestion in intestine

The mechanism for lipid digestion and absorption in the duodenum is similar for ruminants and non-ruminants. Thus, the primary differences between ruminants and monogastrics are related to the nature of the lipid leaving the rumen. For ruminants, lipid sources entering the small intestine can occur as fatty acids or triacylglycerols (Doreau and Chilliard, 1997). Lipids leaving the rumen are very similar to that entering the small intestine, as there is minimal modification occurring within the omasum and abomasum (Noble, 1980). However, the lipid entering the small intestine is more saturated for ruminants than monogastrics and consists primarily of palmitic and stearic acids (Bauman, 2002). Intestinal digestibility and absorption of fatty acids in ruminants is dependent on factors such as the amount of fatty acids reaching the intestine (Lock et al, 2006). Boerman et al. (2015) reported that increasing total FA duodenal flow reduces total apparent intestinal FA digestibility. But, chain length also seems to influence this response with C16:0 digestibility not being affected with increasing flow. In ruminants, there is a continuous flow of fatty acids into the duodenum with the majority of lipids reaching the duodenum as free fatty acids. This is in contrast to monogastrics where the majority is esterified (Lock et al., 2006).

Fatty acids are present in the small intestine as a free fatty acid attached to feed particles or bacteria (Doreau and Chilliard, 1997). Bile supplies bile salt and lecithin, the pancreatic juice supplies an enzyme to convert lecithin to lysolecithins that are used to desorb the FA from feed particles or bacteria and allow lipid solubilization in a micellar phase (Doreau and Chilliard, 1997). If lysolecithin is limited, a reduction in FA digestibility occurs as FA flow increases. Freeman (1984) reported that lysolecithin is an

effective amphiphile for C18:0, increasing absorption as the flux of FA into micelles also increases. C18:0 digestibility also can be reduced if its flow exceeds the capacity of either the lecithin in bile or the phospholipase excreted from the pancreas (Freeman, 1984). The formation of micelles allows for lipid absorption in the small intestine and is a key factor involved in efficient fatty acid absorption (Doreau and Chilliard, 1997). Saturated, mono-unsaturated and polyunsaturated fatty acids and phospholipids need to be solubilized inside the micelle before they are able to infiltrate inside the micelle increasing its hydrophobic core and improving absorption (Lock et al., 2006). Once micelles are formed they facilitate transfer of water-insoluble lipids across the intestinal epithelial cells of the jejunum. In the jejunum, the acidic environment at the surface of the brush border membrane is important for micelle dissociation (Caspary, 1992). The uptake of the lipid from the micelle is determined by the rate of penetration through the hydrophobic layer at the surface of the membrane and diffusion through the lipid bilayer (Caspary, 1992). It was assumed that the penetration of the FA in the membrane was via simple passive diffusion; however, Stremmel (1997) reported that the FA uptake occurs via a cytosolic protein that binds FA. Once FA is taken up, they are esterified into triacylglycerol and phospholipids. In enterocytes, triacylglycerol and phospholipids are incorporated into chylomicrons and very low density lipoproteins (VLDL; Bauchart, 1993) and then transported by the lymph in most of the cases (Figure 2). However, VLDL can also be transported directly to the portal vein.

There is variation in the intestinal digestibility in a range to 55 to 92% (Doreau and Chilliard, 1997) depending on FA intake. Boerman et al. (2015) reported that increasing FA intake by 500 g reduced FA digestibility by 4.25%. The micelle formation could depend on the proportion of different FA or the production of biliary salts, depending on the nature of the FA (Doreau, 1992). The digestibility also depends on chain length, not differing between C16 and C18 FA, but it seems to be lower for C20 and C22 (Doreau and Chilliard, 1997). Otherwise, C18 FA digestibility differs in the quantity of double bounds where, 0, 1, 2 or 3 double bounds have 77, 85, 83 and 76% of digestibility, respectively (Doreau and Ferlay, 1994). However, measurement of unsaturated fatty acid digestibility has a low accuracy as only small amounts of C18:3 reaches the duodenum due to microbial conversion to isomers (Doreau and Chilliard,

1997). There is no significant long-chain fatty acid absorption in the large intestine, suggesting the fecal FA flow in the large intestine is greater than ileal FA flow (Doreau & Ferlay, 1994)

2.5 Fatty acid transport across membranes

Esterified fatty acids are carried by chylomicrons and by VLDL located at the surface of capillaries. Long-chain fatty acids are released and bound by circulating albumin and they are taken up by tissues and will be utilized for various cellular pathways (Hajri and Abumrad, 2002). In dairy cows, for example, the FA composition of milk has two different origins. They can be synthesized de novo from acetyl-CoA, to form SCFA and MCFA (Chilliard and Ferlay, 2004). Alternatively, they can be taken up from arterial circulation which is mediated by lipoprotein lipase residing in the capillary walls (Chilliard and Ferlay, 2004). For FA to be transported into cells, they must cross the plasma membrane barrier. Fatty acids are lipophilic indicating that they can easily cross the lipid bilayer membrane and this transfer is believed to be mediated via passive diffusion or may be facilitated by proteins integrated within the cell wall (Hajri and Abumrad, 2002).

The FA in arterial circulation provides a FA source for peripheral tissues with the uptake by tissues being dependent on their energy demand (Turcotte et al., 1992). In skeletal muscle and heart, long-chain fatty acids (**LCFA**) provides the majority of the energy needed with β-oxidation and the citric acid cycle (Koonen et al., 2005). Fatty acid transfer is passively and rapidly transferred through the membrane lipid and regulation of uptake depends on different factors such as the molar ratio of FA to albumin and the cellular FA metabolism (Hamilton et al., 2001). However, various membrane proteins have also been identified that facilitate the cellular uptake of FA, and are generally referred to “fatty acid transporters”. These transporters allow transfer of the FA into cells by acting as acceptors (Schwenk, 2010).

The FA transporters, such as fatty acid translocase (**CD36**), fatty acid binding protein (**FABP**) and fatty acid transporter protein (**FATP**) differ in molecular mass. These transporters help to organize FA within specific membrane domains facilitating FA transport across the membrane (Schwenk, 2010). To reach the interior of the muscles,

LCFA need to cross the plasma membrane barrier. Transfer of LCFA between membranes is facilitated by binding with soluble FABP (Koonen et al., 2005). Fatty acid binding protein is also important to the movement of LCFA from sarcolemma through the cytoplasm or the mitochondrial membrane, where acyl-CoA synthetase will be present (Koonen et al., 2005). Acyl-CoA synthetase is responsible to convert LCFA into acyl-CoA that will be available for triacylglycerol synthesis or β-oxidation, main source of energy for cellular metabolism (Lopaschuk, 2001). Furthermore, it has been suggested that LCFA transmembrane movement is also related to fatty acid translocase (**FAT**)/CD36 and fatty acid transport protein (Schaffer, 2002).

Past research has shown that CD36, FABP, and FATP are co-expressed in skeletal muscle and heart and are known to have different effects on FA utilization (Nickerson, et al., 2009). In vivo, greater expression of all transporters increase FA transport, but CD36 and FATP4 are more effective. However, CD36 and FABP are related and are essential for FA oxidation (Schwenk, 2010). Therefore, FATP as well as CD36 are present in the mitochondrial membrane, increasing FA oxidation available to promote cellular metabolism (Bezaire, 2006). The membrane transporters have other functions that can be related (FATP, CD36) or not related (FABP, CD36) to FA metabolism (Koonen et al., 2005). For example, FABP is identical to mitochondrial aspartate aminotransferase (Berk et al., 1990), but it is not clear why two totally different functions can be measured (Koonen et al., 2005). Additionally, CD36 is known as glycoprotein (GP), and is responsible for binding of modified and native lipoproteins and anionic phospholipids (Silverstain et al., 1989)

Translocation of CD36 from endosomes to the cell membrane is a mechanism that is known to increase FA uptake, and this occurs concomitantly with glucose uptake that is increased with translocation of GLUT4 to the apical membrane from intracellular compartments (Bonen, et al., 2000, Luiken, et al., 2003, Karlsson at al., 2009) and in response to insulin and exercise (Koonen et al., 2005). The translocation of CD36 is rapid and reversible. Muscle contraction is another body function that is known to increases translocation of the other two transporters, FABP and FATP, to the cell membrane (Jain et al., 2009) and may be related to increase FA uptake. The transporters, or membrane proteins, are essential and integral parts of the FA metabolism system. Fatty acid uptake

has been studied extensively in heart, muscle, and adipose tissue. More research is needed for other parts of the body.

2.6 Effect of lipid on ruminal digestion

Although lipid supplementation is often used to increase the energy density of diets, feeding too much lipid can have negative effects on ruminal fermentation. There are a few theories that help explain the mechanism of fermentation inhibition by lipids. Firstly, it is speculated that lipids may coat feeds by forming a lipid bilayer over the feed particles (Devendra and Lewis, 1974). This lipid bilayer may inhibit microbial attachment to feeds and prevent access of extracellular enzymes to their substrate. Secondly, lipids are thought to have direct antimicrobial activity, where lipid modifies the ruminal population and also decrease the calcium needed for microbial function (Devendra and Lewis, 1974). It is likely that both theories play a role in disrupting ruminal fermentation. Nevertheless, the main effect of lipid on rumen digestion is a disruption in the fermentation process, decreasing ruminal digestibility (Jenkins, 1993) such as for structural carbohydrates where digestion can be decreased by more than 50% (Ikwuegbu and Sutton, 1982). Others have also reported a reduction for DM and fiber digestion (Bock et al., 1991).

While high inclusion rates of lipid certainly can have an effect on ruminal fermentation, the amount of fat added to the diet (Doreau and Chilliard, 1997), and the nature of fat also influences the response. Past work has shown that the negative effect is greater for polyunsaturated fatty acids than saturated fatty acids (Jenkins, 1993). The amount of soluble Ca in the diet may increase calcium salts in the rumen (Palmquist et al., 1986), and decrease concentration of ionized Ca in ruminal fluid. The formation of Ca-salts may reduce Ca availability and thereby be a limiting factor preventing or limiting bacterial attachment to particles (Ferlay and Doreau, 1995). The lipid in the rumen can also have a negative effect on bacterial growth that may consequently reduce fiber digestion (Doreau and Chilliard, 1997).

Additional dietary lipid can also alter ruminal protein metabolism. The addition of linseed oil has been reported to decrease ammonia concentration and increase nitrogen flow to the duodenum (Ikwuegbu and Sutton, 1982). Jenkins (1990) reported similar

effects when corn oil or lecithin was added to the diet of steers (Jenkins, 1990). The greater flow of N to the duodenum and reduced ammonia concentrations can be explained by an increase in the efficiency of microbial protein formation due to a decrease in a protozoa predation (Ikwuegbu and Sutton, 1982).

Dietary lipid can also decrease methane, hydrogen, and volatile fatty acid production, and reduce the acetate:propionate (**A:P**) ratio (Jenkins, 1993). Chalupa et al. (1984) studied the influence of LCFA on ruminal fermentation. It was reported that unsaturated FA added as a free FA, specifically the C18 family, decreased SCFA concentration in the rumen, also decreasing the A:P ratio. Oleic acid reduced the A:P ratio by 54%. However, in this same study, lauric acid also decreased SCFA concentration by 69% and palmitic acid decreased the A:P ratio by 23%. Chalupa et al. (1984) also observed that when LCFA were added as calcium salts or triglycerides, there was no difference in SCFA production or the A:P ratio. They concluded that when FA are added to the diet as free fatty acids (**FFA**) or triglycerides there is a drastic decrease in SCFA concentration and the A:P ratio; however, fatty acids that are fed as a calcium salt do not induce such deleterious effects on ruminal fermentation. Thus, ruminal fermentation inhibition can be minimized, or even eliminated, by feeding calcium salts of fatty acids, hydrogenated fats, or encapsulated fats (Jenkins, 1993).

Protecting lipids is used to limit the impact of microbial modulation of dietary lipids and also to protect the ruminal environmental against negative effects arising from lipids, such as decreased microbial function (Devendra and Lewis, 1974) or reduced ruminal digestibility (Jenkins, 1993). Protection can occur by physical or chemical treatments (Doreau and Chilliard, 1997). A coating method was the first methodology established for lipid protection and used more than 30 years ago by Ashes et al. (1979). This method allowed the protection to be disrupted in the abomasum due to lipase activity and as such it was considered a partial protection (Ashes et al., 1979). Mastication of the product also could affect protection. Association of Ca and FA, also known as Ca-salts, are inert in the rumen environment. However there is concern that Ca-salts might be hydrogenated when exposed to low pH (Ferlay et al., 1993; Enjarbert et al., 1994; Van Hevel and Demeyer, 1996). However, more research is needed to better understand the process of lipid digestion in the rumen content.

2.7 Fatty acid supplementation and tissue composition

One factor determining the quantity and quality of lipid to be supplemented for dairy or beef cattle, besides price, is the effect of the supplementation approach on the final product, in other words, on milk and meat fatty composition. Consumers of animal products are concerned about the relationship between meat and milk quality and health especially because the Department of Health (1994) has stipulated that saturated fatty acid of the human diet should be limited and unsaturated fatty acid intake, such as polyunsaturated fatty acids (**PUFA**), should increase. Omega-3 FA are known to be good for human health, and studies have suggested that provision of Omega-3 FA may, among others, decrease risk for coronary heart disease, and improve brain function and visual development (Dijck-Brouwer et al., 2005). However, meat and milk products arising from ruminants generally have low PUFA concentrations due to a high concentration of saturated fatty acids leaving the rumen in response to biohydrogenation.

All mammalian cell membranes, such as the rumen, consist of a lipid bilayer with protein and enzymes embedded within the membrane. The cell membrane composition is known to be responsive to dietary composition (Spector, 1985). Phospholipids are the main components of cellular membranes. Supplementation with fish oil results in modification in FA profiles of the human inflammatory cells, increasing EPA and DHA in plasma lipids, platelets, erythrocytes, leukocytes, colonic tissue, cardiac tissue and liver tissue and this increment occurs as a replacement with any n-6 PUFA, such as arachidonic acid (Calder, 2012).

Feeding Omega-3 PUFA, such as linseed and fish oil may increase the proportion of unsaturated fatty acids in the tissue of meat animals, but more improvement is observed when fed formaldehyde-treated or protein-encapsulated lipids (Ashes, 1992). Milk from cows fed formaldehyde-protected linseed oil contained 20% more 18:3 n3 FA compared to cows fed unprotected linseed oil, the explanation is because the protected product escapes biohydrogenation.

Before entering the rumen, most of the unsaturated fatty acids have the double bond in a *cis* formation, after biohydrogenation the remaining double bonds are in the *trans* configuration. Trans-FA that escape rumen biohydrogenation are easily desaturated in

presence of an enzyme called stearoyl Co-A desaturase, resulting in oleic acid (C18:1) formed from stearic acid (C18:0) and CLA (C18:2) arising from oleic acid (C18:1; Wood, 2007).

Conjugated linoleic acid (CLA) has been studied because this FA grouping and its isomers are related with a decrease in body fat. Ostrowska (1999) found that carcass lean tissue increased with increasing CLA supplementation. Lactating dairy cows also reduced milk fat arising from de novo synthesis (Chouinard, 1999) and results have shown that the inhibitory effects of CLA are only related to milk fat, whereas milk yield and other milk components are not affected (Bauman, 2003a). It is well known that FA can alter the profile of the membrane phospholipids (Scott, 1993; Maddock, 2007; Calder 2002), such as liver and adipocytes and also alter composition of the meat and milk. However, it is not known if the supplementation of lipids can alter the lipid profile of the rumen epithelium.

2.7.1 Phospholipids membrane structure

The cell membrane is a complex structure, and its complexity is due to lipids and proteins that are designed to enable specialized functions (Lingwood and Simons, 2010). The membrane has a bilayer organization characterized by a polar (hydrophilic) and a non-polar group of lipid compounds (hydrophobic; Figure 3; Cullis and Hope, 1991). The fluidity of the membrane depends on the nature of the acyl chain regions. Most of the lipids are present individually as a viscous gel or as a fluid, depending on the temperature they are found (Cullis and Hope, 1991). Lipids have the ability to self-modulate into fluid bilayer structures influencing the permeability barrier and generating the matrix that proteins will bind to or associate with (Cullis and Hope, 1991). The permeability to small ions, such as Na^+, K^+, and H^+ are of importance in order to establish an electrical gradient and to create a membrane potential. The membrane potential is necessary to facilitate transport processes (Cullis and Hope, 1991).

Membrane lipids are predominantly composed of glycerol-based phospholipids (glycerophospholipids). Sphingolipids and glycosphingolipids also constitute a major fraction, with sphingomyelin being an important lipid (Meer et al., 2008). Cholesterol is one of the main components of the cellular membrane, particularly in mammalian plasma

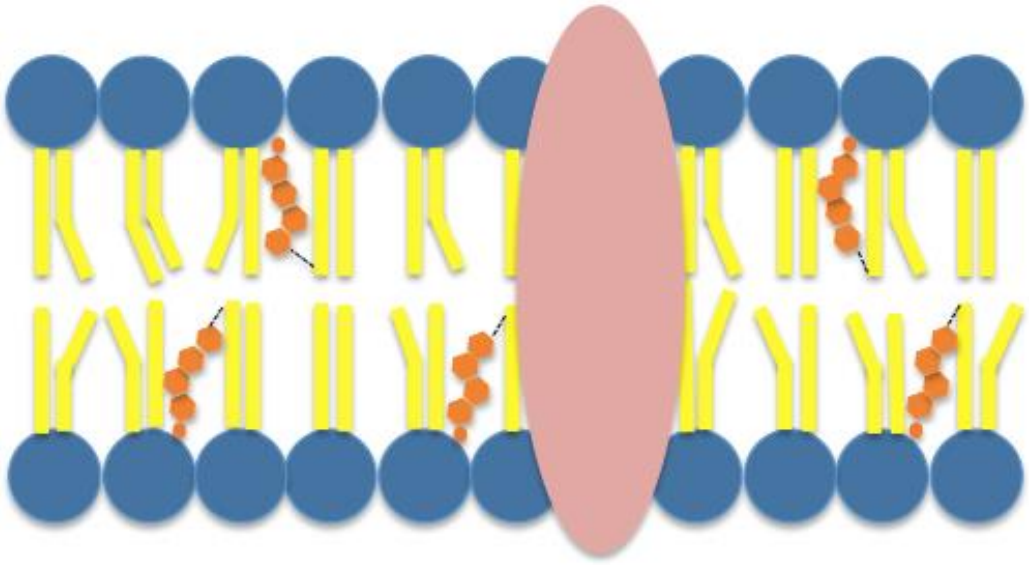

Figure 1.3 Static diagram of phospholipid membrane. The membrane is composed by a hydrophilic head with a hydrophilic tail, where fatty acid binding protein in embedded. The head contains a phosphate group and glycerol and the tail could be saturated or unsaturated fatty acids. Cholesterol interacts with the fatty acids in the hydrophobic tail, with the interaction favoring saturated fatty acids.

membranes. Cholesterol is often present in association with phospholipids (Cullis and Hope, 1991). The cholesterol content of mammalian cell membranes is greater when compared to other species (Cullis and Hope, 1991). Cholesterol may increase membrane fluidity because it preferentially interacts with sphingomyelin, which is mostly saturated (Cullis and Hope, 1991). Some important integral membrane proteins are preferentially located between sphingomyelin and cholesterol (Samsonov et al, 2001). The lipid bilayer separation is cholesterol-dependent (Ipsen et al., 1987). The segregation favors the interaction with hydrocarbon chain of saturated lipids over interactions with unsaturated lipids (Simons and Vaz, 2004). The interaction of the phospholipids with cholesterol also changes the conformation of the hydrocarbon chain, increasing membrane thickness (García-Sáez et al., 2007). The increase in membrane thickness occurs as acyl chains and cholesterol become more tightly packed as they are required to share a limited space between the phospholipid head groups (Simons and Vaz, 2004). Cholesterol also interacts with sphingolipids. The sphingosine-based lipids have a hydroxyl group and amido nitrogen acting as hydrogen-bond donors as well as acceptors (Simons and Vaz, 2004). Together with fatty acid carbonyl group, they can bind to hydrogen in water and in other lipids (Simons and Vaz, 2004). Thus, decreasing the supply of saturated FA as an important source of precursors for cholesterol biosynthesis should also decrease cholesterol in the plasma membrane and decrease epithelial permeability (Pizzo et al., 2002).

2.8 Conclusion

In ruminants, dietary fatty acids are saturated by ruminal microbes. However, feeding unsaturated fatty acids can increase duodenal flow of unsaturated fatty acids. Increasing the supply of unsaturated fatty acids modulates the fatty acid composition of numerous tissues and fatty acid composition of epithelial membranes is related to membrane fluidity and permeability. However, there is a paucity of data regarding whether dietary lipid can alter the fatty acid composition of the ruminal epithelium and whether fatty acid composition of the ruminal epithelium affects SCFA transport across the rumen.

2.9 Hypothesis

The hypothesis of this study was that feeding a greater proportion of rumen-protected unsaturated FA will result in a greater concentration of unsaturated FA in the ruminal epithelium and contribute to increased passive diffusion of SCFA across the ruminal epithelium.

2.10 Objective

The objective of this study was to evaluate the effect of FA supplementation and the type of FA on the FA composition of the ruminal epithelium and the passive uptake and flux of SCFA across the ruminal epithelium.

3.0 Effect of lipid supplementation on ruminal epithelial membrane composition and short-chain fatty acid absorption

3.1 Introduction

Membrane permeability of the gastrointestinal epithelium is critical to ensure selective permeability and to maintain gradients in pH, osmolality, and solutes between the luminal contents and portal circulation (Lande et al., 1995). It is clear that fatty acids are rapidly integrated within cellular membranes and interact with phospholipids where long-chain saturated fatty acids have been reported to generally decrease fluidity and apparent permeability to solutes relative to mono and polyunsaturated fatty acids (Ibarguren et al., 2014). In a study using unilaminar vesicles (Lande et al., 1995), increasing the proportion of cholesterol decreased the permeability coefficients for urea and ammonia, but not protons. Similar results for decreased permeability with greater cholesterol inclusion have been confirmed by Jedlovszky and Mezei (2003). In young piglets, perfusion of oleic acid into the intestine increased the permeability of the tissue measured using ^{51}Cr-EDTA appearance (Velasquez et al., 1993). Hence, it appears that dietary fatty acid supply may modulate the composition of the cellular membrane and ionic transport.

Short-chain fatty acids (SCFA) are lipophilic molecules that contribute to the metabolizable energy supply in both ruminants (Bugaut, 1987) and monogastric species (Engelhardt, 1995). Permeability coefficients for SCFA depend on chain length with the permeability ranking being butyrate>propionate>acetate (Gutknecht and Walter,1981). Differences between permeability coefficients and absorption rates indicate that SCFA absorption does not occur solely through passive permeation (diffusion) across the cellular membrane. Indeed, Aschenbach et al. (2009) demonstrated that SCFA are absorbed via a bicarbonate-dependent pathway, bicarbonate-independent pathway that is sensitive to nitrate, and via passive diffusion. However, it should be noted that the relative importance of individual pathways differs among SCFA where acetate relies to a greater extent than butyrate on bicarbonate-dependent and nitrate-sensitive pathways (Penner et al., 2009; Aschenbach et al., 2010; Schurmann et al., 2014). Although

pathways for SCFA transport have been partially elucidated, it is not clear if manipulating the fatty acid composition of the ruminal epithelium will modulate the pathway for SCFA absorption. Thus, I hypothesized that increasing the proportion of long-chain unsaturated FA in ruminal epithelial cells would increase passive diffusion of SCFA.

3.2 Materials and Methods

3.2.1 Experimental Design

Twenty-one Holstein steers (mean ± SD for body weight of 194.12 ± 26.77 kg) were used for this study. The use of steers and all procedures involving steers were pre-approved by the University of Saskatchewan Animal Research Ethics Board (protocol number 20100021) and followed the guidelines presented by the Canadian Council on Animal Care (2009). Prior to the start of the study the steers were group-housed for a minimum of 2 wk and fed a common diet consisting of (% DM basis) corn silage (50), rolled barley grain (12), and a vitamin and mineral pellet (38) containing ground barley, canola meal, wheat bran, beet pulp and mineral/vitamin supplement.

Subsequently, steers were blocked by age and body weight and, within block, were randomly assigned to 1 of 3 treatments differing in lipid supply and the type of lipid. Treatments imposed included the control (**CON**) diet, a diet enriched with saturated lipid sources (**SAT**), and a diet enriched in unsaturated lipid sources (**UNSAT**; Table 3.1). The SAT and UNSAT diets had a similar total dietary lipid concentration but for the SAT diet, porcine tallow and palmitic acid (Jefo Dairy Fat 99%, Jefo, Saint-Hyacinthe, Quebec, CA) were used as saturated lipid supplements. For the UNSAT diet, whole flaxseed and Megalac (Church and Dwight Co., Inc., Princeton, NJ) were used to increase the supply of unsaturated fatty acids to the small intestine. All diets were formulated to meet or exceed the requirements for a growing steer with a 1 kg/d body weight gain. The whole flax and Megalac combination was chosen to increase the flow of oleic and polyunsaturated fatty acids to the duodenum. In addition, monensin (Elanco Animal Health, Greenfield, USA) was included in all diets to achieve a concentration of 33 mg/kg and to increase the probability for partial inhibition of biohydrogenation

(McGuffey et al., 2001). Dietary FA composition of the ingredients is reported in Table 3.2.

At the start of the study, each steer was placed in an individual pen and fed at 0700 and 1700 h. Pens were cleaned once daily and steers had ad libitum access to water. The steers were transitioned to their treatment diet by feeding the common diet, that was the same as CON diet, and their experimental diet in a 1:1 ratio for 1 d prior to being exposed to their final diet. Subsequently, each steer was exposed to a 30-d feeding period with the start of the feeding period staggered (but balanced across treatments) to facilitate a staggered slaughter schedule for the Ussing chamber measurements (described below).

3.2.2 Feed intake and growth performance

Steers were weighted on 2 consecutive d at the start and end of the study (0630 h on d 1 and 2 and d 28 and 29). The average BW was calculated for the start and end of study weights and the change in weight between the start and end was used to determine average daily gain (kg/d). The weight of the feed offered was 3.0% of starting BW to limit potential confounding effects of DMI among treatments and the amount of feed offered was recorded daily. If refusals were present, the weight of the refusals were measured, recorded, and the DM concentration determined. Dry matter intake was calculated based on the provision of DM, and when necessary, the DM of the refusals were subtracted. Refusals accounted for an average of 11% (as fed basis) of the total diet fed. Twice weekly, samples of the feed ingredients were collected for DM analysis in order to adjust the diets to maintain the specified ingredient inclusion rates, and these feed samples were used for chemical analysis. Prior to analysis, feed samples were placed in a forced air oven at 55°C until achieving a constant weight. These weekly samples were then ground to pass through a 1-mm sieve and stored until being analyzed for chemical and fatty acid composition.

Table 3.1. Composition of the control diet (CON), saturated lipid diet (SAT) and unsaturated lipid diet (UNSAT) fed to growing Holstein steers.

	Dietary treatment		
Ingredients, %DM	CON	SAT	UNSAT
Corn silage	50.0	50.0	50.0
Barley grain	12.0	12.0	12.0
Megalac[1]	-	-	2.76
Palmitic acid[2]	-	1.09	-
Pellet			
Barley grain	15.0	15.0	15.0
Wheat grain	5.7	4.17	4.73.
Canola meal	9.87	11.44	9.73
Mineral and vitamin supplement[3]	2.24	2.24	2.24
Monensin[4]	0.02	0.02	0.02
Limestone	2.13	2.13	1.4
Beet pulp	3.07	-	-
Porcine tallow	-	1.93	-
Ground flaxseed	-	-	2.13
Chemical composition, % DM			
Crude Protein	12.42	12.34	12.43
Soluble protein	2.78	2.71	3.03
NDF	35.13	33.96	35.78
Sugar	2.15	1.97	1.89
Starch	32.80	31.55	31.41
Ether extract	2.2	4.9	5.1
Ca	1.26	1.28	1.34
P	0.60	0.61	0.61
NEm, Mcal/kg	1.55	1.60	1.59
NEg, Mcal/kg	0.95	0.99	0.99

[1]Church and Dwight Co., Inc., Princeton, NJ

[2]Jefo Dairy Fat 99%, Jefo, Saint-Hyacinthe, Quebec, CA

[3]Dairy Premix, Masterfeeds, Saskatoon, SK. Mineral and vitamin supplement contained: Crude protein (min), 0.8%; Crude Fat, 1.0-1.5%; Calcium, 16%; Phosphorus, 6.5%; Salt, 15.5%; Sodium, 6.3%; Magnesium, 7.0%; Potassium, 2.0%; Sulfur, 0.1%; Cobalt, 30 mg/kg; Copper, 675 mg/kg; Iodine, 80 mg/kg; Iron, 3,085 mg/kg; Manganese, 1,500 mg/kg; Zinc, 2,500 mg/kg; Fluorine, 700 mg/kg; Vitamin A, 250,000 IU/kg; Vitamin D3, 80,00 IU/kg; Vitamin E, 2,000 IU/kg, monensin 33 mg/kg

[4]Elanco Animal Health, Greenfield, Massachusetts, USA

Table 3.2. Fatty acids composition of feed ingredients used for the control diet (CON) saturated fatty acid diet (SAT) and unsaturated fatty acid diet (UNSAT) fed to growing Holstein steers.

Variable	Corn Silage	Barley grain	CONT pellet	SAT pellet	UNSAT pellet	Palmitic acid[1]	Megalac[2]
Total fatty acids g/100g	1.37	1.75	2.16	6.00	4.09	96.37	84.00
Fatty acids, %							
C14:0	0.05	0.41	0.34	0.88	0.28	1.79	0.57
C14:1	0.01	0.03	0.02	0.02	0.01	0.00	0.00
C15:0	0.05	0.16	0.11	0.09	0.07	0.12	0.02
C16:0	22.52	22.88	21.90	20.79	12.72	89.78	11.65
C16:1	0.10	0.08	0.54	1.60	0.16	0.04	0.30
C18:0	4.70	3.47	3.04	10.03	3.88	1.28	1.39
C18:1	23.51	15.66	26.57	37.25	27.05	5.99	65.11
C18:2N6	42.76	51.14	42.41	22.85	29.73	0.91	19.29
C18:3N6	0.00	0.01	0.00	0.00	0.01	0.00	0.00
C18:3N3	4.55	4.28	3.84	2.72	24.62	0.00	0.79
C18:4N3	0.02	0.01	0.01	0.00	0.00	0.00	0.00
C20:0	1.13	0.19	0.27	0.35	0.30	0.05	0.17
C20:1	0.14	0.76	0.41	1.19	0.46	0.03	0.23
C20:2N6	0.01	0.00	0.02	0.30	0.04	0.00	0.01
C20:3N6	0.01	0.00	0.00	0.05	0.00	0.00	0.00
C20:4N6	0.01	0.00	0.00	0.22	0.00	0.00	0.00
C20:3N3	0.01	0.03	0.00	0.07	0.01	0.00	0.00
C20:4N3	0.03	0.00	0.00	0.00	0.00	0.00	0.00
C20:5N3 (EPA)	0.01	0.04	0.00	0.00	0.00	0.00	0.03
C22:0	0.08	0.86	0.33	0.19	0.25	0.00	0.10
C22:1	0.14	0.00	0.00	1.14	0.27	0.00	0.04
C22:2N6	0.01	0.00	0.00	0.00	0.00	0.00	0.00
C22:4N6	0.00	0.00	0.00	0.05	0.00	0.00	0.00
C22:5N6	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C22:5N3	0.01	0.00	0.00	0.03	0.00	0.00	0.00
C22:6N3 (DHA)	0.02	0.00	0.00	0.00	0.00	0.00	0.25
C24:0	0.11	0.00	0.19	0.10	0.13	0.00	0.02
C24:1	0.02	0.00	0.00	0.06	0.00	0.00	0.02
Saturated	28.64	27.97	26.17	32.43	17.62	93.02	13.92
Monounsaturated	23.90	16.53	27.54	41.26	27.96	6.06	65.71
Polyunsaturated	47.45	55.50	46.29	26.31	54.42	0.92	20.37
Omega-3	4.64	4.36	3.85	2.83	24.63	0.00	1.07
Omega-6	42.81	51.14	42.44	23.48	29.79	0.91	19.29

[1]Jefo Dairy Fat 99%, Jefo, Saint-Hyacinthe, Quebec, CA

[2]Church and Dwight Co., Inc., Princeton, NJ

3.2.3 Blood, digesta, and tissue sample collection and analysis

On d 30 at 1000 h (3 h post-feeding), steers were killed via captive bolt stunning, pithing, and exsanguination. To facilitate the Ussing chamber measurements, only 1 calf was killed each day. Thus, the starting date of the study was staggered so that all calves were exposed to the same treatment duration with the start of the feeding period balanced across treatments.

Blood was collected at the time of killing on d 30 (1000 h) into one container containing Li-heparin (148 IU; coated Vacutainer tube, Becton Dickinson, Franklin Lakes, NJ) and one without an anticoagulant (Vacutainer tube; Becton Dickinson, Franklin Lakes, NJ). The vial for plasma was immediately placed on ice and centrifuged at 1,500 × *g* for 20 min at 4°C. After centrifugation, the plasma was transferred into four 2-mL microcentrifuge tubes and stored at -20°C until being analyzed for glucose, insulin, and total fatty acids. Samples for serum were allowed to clot for 4 h at room temperature before being centrifuged as described for plasma. Serum was then transferred into microcentrifuge tubes and stored at -20°C until analyzed for beta-hydroxybutyric acid (BHBA). Plasma insulin was determined using a bovine-specific insulin ELISA kit (Mercodia, Uppsala, Sweden) with all analysis completed on a single plate. The coefficient of variation was on average 5.2%. Plasma glucose (Sigma-Aldrich, St. Louis, MO) and serum BHBA (Roche Diagnostics, Laval, QC, Canada) were quantified using commercial kits (Penner et al., 2009). Glucose analysis was completed on 1 plate and the average coefficient of variation was 1.57%. For BHBA, 3 plates were used with inter-assay and intra-assay coefficients of variation of 1.32% and 1.10%, respectively.

Immediately after killing, the abdominal cavity was opened and the reticulo-rumen was removed. The weight of the reticulo-ruminal digesta was determined and the entire reticulo-ruminal digesta was mixed and pH was measured using a portable pH meter (AP110, Fischer Scientific, Ottawa, ON). A representative sample of rumen digesta (1 L) was collected, and strained through 2 layers cheesecloth. Subsequently, two 35-mL aliquots were collected into 7-mL of metaphosphoric acid (25% wt/vol) to prevent microbial fermentation. The sample was then stored at −20°C until being analyzed for SCFA concentration using gas chromatography (Agilent Technologies, Santa Clara, CA) as described by Khorasani et al. (1996).

A section of the ruminal epithelium (approximately 30 cm^2) including the ventral and caudal ventral blind sacs were collected. Immediately after collection, two biopsies from the ventral sac were collected using sterile forceps and scissors. The tissue was rinsed in ice-cold PBS buffer (pH 7.4), placed in RNAlater (Sigma-Aldrich, St. Louis, Missouri, United States) and stored at -80°C until analyzed for gene expression using quantitative real time PCR.

Frozen ruminal tissue was ground using a mortar and pestle with liquid nitrogen to keep the tissue frozen during grinding. Total RNA was extracted using a Trizol-reagent based assay (adapted from Thermo Fisher, Waltham, MA, USA). After the tissue was ground, 50 to 100 mg of tissue was placed into a 2 mL micro centrifuge tube. Samples were kept on dry ice until 1 mL of Trizol was added. Samples were manually mixed for 5 min, and 200 μL of chloroform was added to each tube and samples were mixed again for 2 min. Samples were placed in pre-cooled centrifuged at 10,000 × g for 10 min. This step separated the phases: the bottom contained the phenol, the middle contained protein and DNA, and the clear layer contained the RNA. A total of 600 μL of the supernatant was placed in fresh tubes and same volume (600 μL) of isopropanol was added and mixed well. Samples were left to sit on ice for a minimum of 45 min, but no more than 1.5 h. Samples were centrifuged again at 14,000 × g for 15 min. Supernatant was removed and 1 mL of cold ethanol was added to the samples. Samples were centrifuged at 14,000 × g for 15 min. The supernatant was removed and 200 μL of nuclease-free water was placed into tubes. After 5 min a pipet was used to mix and to re-suspend the pellet. A total of 10 μL of 3 *M* sodium acetate and 200 μL of isopropanol were added to each tube, then mixed and placed in the -20°C freezer overnight. The next morning, samples were centrifuged at 14,000 × g for 15 min and the supernatant was removed. One milliliter of cold ethanol was added to the pellet and samples were centrifuged one more time at 14,000 × g for 15 min. The supernatant was removed and 50 μL of nuclease free water was added to the pellet. A pipet was used to mix and re-suspend the pellet.

The concentration of RNA was analyzed using a Nanodrop 2000c Spectrophotometer (Thermo Scientific Waltham, MA). Samples were deemed acceptable when the nucleic acid concentration was greater than 600 ηg/μl and the ratio of absorbance at 260:280 wavelengths (nm) was between 1.8 and 2.0. Subsequently, RNA

samples were treated to minimize DNA contamination (TURBO DNA-free Kit, Thermo-Fisher Scientific) and RNA integrity was assessed using a 1.2% agarose gel with a denaturing gradient. The bands arising from individual samples were visually inspected to confirm the 18S and 28S ribosomal RNA band separation (Thermo Fisher Scientific). The RNA was then used for cDNA synthesis (GoScript Reverse Transcription System, Promega, Madison, WI). the resulting cDNA was subjected to quantitative real-time PCR to measure specific transcript abundance.

The gene specific forward and reverse primers used in this study were designed using sequence data from NCBI (National Center for Biotechnology Information). Primers were designed to yield a PCR product size of 100 to 200 base pairs with a theoretical melting temperature 58 to 63ºC. Selected primers also spanned an exon-exon junction. Target genes of interest and their corresponding NCBI accession number, forward and reverse primer sequences, general function, and source are shown in Table 3.3.

Quantitative real-time PCR (qPCR) was performed in triplicates using a CFX96 Real-Time PCR system and SsoFast EvaGreen Supermix (Bio-Rad Laboratories, Hercules, CA). Prior to the start of PCR, a 30s enzyme activation period (95°C) was initiated followed by 40 cycles consisting of denaturation (5 s at 95°C), annealing and extension phases (5 s at 60°C), and one cycle consisting of melt curve (10 s at 95°C). The temperature decrease and increase proceeded at an average of 3.3°C/s. A serial dilution series of known template concentrations, previously assigned, were used to establish a standard curve for determining primer efficiency. A PCR efficiency of 100% corresponds to a slope of -3.32, as determined by the following equation (Ramakers et al., 2003):

$$\text{Efficiency} = 10^{(-1/\text{slope})} - 1$$

The log of each known concentration in the dilution series was then plotted against the Ct value for that concentration (Ct; number of cycles required for fluorescent signals to cross the threshold). From this standard curve, the slope, y-intercept, and correlation coefficient were derived. The mean slope was -3.32 ± 0.12. The range in efficiency for the primers was between 94.6 to 108.2%, with a mean of 99.23%. Gene expression fold change was calculated using the ΔΔCt approach with the assumption of 100% primer efficiency (Litvak and Schmittgen, 2001). Average Ct was used to calculate

ΔΔCt. Prior to calculating fold change, the house-keeping genes were tested for stability, and were considered to be stable when the Ct was not different among treatments ($P > 0.10$). Housekeeping genes included 60S acidic ribosomal protein P0 (RPLP0), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and beta-actin (ACTB). The *P*-values for RPLP0, GAPDH, and ACTB were 0.47, 0.22, and 0.84 respectively. Each plate was organized by steer such that all target genes for an individual steer were included in one 96-well plate. The three housekeeping genes were also analyzed for each steer on each 96-well plate.

The remaining ruminal tissue from the ventral and caudal ventral blind sacs were cleaned thoroughly using a pre-heated (38.5°C) incubation buffer saturated with oxygen during transportation to the laboratory (Table 3.4). The transportation buffer did not include antibiotics. Epithelia were then prepared for mounting in Ussing chambers by gently removing the submucosal layers using hand stripping. The prepared epithelia were then placed in buffer for transport back to the laboratory. In the laboratory (within 40 min of killing), the tissue was cut into strips and then mounted between 2 halves of an Ussing chamber (exposed surface area of 3.14 cm^2; Free University of Berlin, Germany). Another piece of the ruminal epithelium was collected for analysis of fatty acid composition and stored at -20°C.

3.2.4 Ussing Chamber Experiment

3.2.4.1 Buffer Solutions. Buffer solutions were prepared for incubation of the mucosal (i.e. luminal; pH of 6.2) and serosal (i.e. blood; pH of 7.4) sides. The use of mucosal and serosal buffer solutions differing in pH was designed to be representative of the pH conditions exposed in vivo and this approach has been used previously (Penner et al., 2009; Schurmann et al., 2014). To achieve the desired pH, the serosal and mucosal buffers were adjusted using either 1 *M* NaOH or 3 *M* gluconic acid. Buffers were contained in glass columns with water jackets and were kept at 38.5ºC using a circulating water bath. Buffer in the mucosal and serosal sides were mixed by gas lift. The composition of the buffer solutions used is reported in Table 4. Buffers containing bicarbonate were gassed with carbogen (5% CO_2 and 95% O_2), and buffers not containing bicarbonate were gassed with 100% O_2.

Table 3.3. Target gene name, National Center for Biotechnology Information (NCBI) accession number, forward and reverse sequences and gene function.

Target gene name (Abbreviation)	NCBI accession number	Forward and reverse sequence	Gene function
ACTB	NM_173979.3	F: GCGGCATTCACGAAACTACC R: GCCAGGGCAGTGATCTCTTT	House-keeping
GAPDH	NM_001034034.2	F: TCTGGCAAAGTGGACATCGT R: ATGACGAGCTTCCCGTTCTC	House-keeping
RPLP0	NM_001012682.1	F: TTGTGGGAGCAGACAACGTG R: GCCGGGTTGTTTTCCAGATG	House-keeping
NHE1	NM_174833.2	F: CTGGTGGAAAGTGGAGGCAT R: TGTGTCTGTTGTAGGACCGC	High affinity isoform Na/H exchanger
NHE3	NM_001192154.1	F: CTTCAAATGGCACCACGTCC R: GAAGAAGAACACCGTTGGCG	Low affinity isoform Na/H exchanger
MCT4	XM_005221026.3	F: GTTGGACCTGAGCGTCTTCA R: GGTGGGCCTAGCAAAGATGT	Transport of short-chain fatty acids

The annealing temperature for all genes was 60°C, except for NHE3 that was 63°C
References genes include ACTB, GAPDH and RPLP0 and target genes were NHE1, NHE2m, and MCT4
ACT = beta-actin; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; RPLP0 = 60S acidic ribosomal protein P0; NHE1, sodium hydrogen exchanger 1; NHE3 = sodium hydrogen exchanger 3; MCT4 = monocarboxylic acid transporter 4; F = forward sequence; R = reverse sequence.

The use of buffer containing bicarbonate was to enable measurement of uninhibited acetate, propionate, and butyrate uptake and flux while the buffer that did not contain bicarbonate and included nitrate was designed to maximally inhibit acetate, propionate, and butyrate uptake and flux based on known pathways of transport (Bilk et al., 2005; Aschenbach et al., 2009; Schurmann et al., 2014). The flux in the buffer designed for maximal inhibition was interpreted to equate to passive diffusion. Subsequently, the uptake and flux of SCFA that was mediated by transporters was calculated by difference (transporter-mediated uptake and flux = non inhibited uptake and flux – maximally inhibited uptake and flux). All Ussing chamber buffers contained broad spectrum antibiotics [penicillin G Na salt (60 mg/L), kanamycin sulfate (100 mg/L), and flurocytosine (50 mg/L)] to inhibit microbial activity. Buffer, antibiotics, and chemicals were purchased from Sigma Aldrich, 1-^{14}C-butyrate was purchased from Moravek Biochemicals (Moravek Biochemicals, Brea, CA), and all other radiolabeled chemicals were purchased from Perkin Elmer (Woodbridge, ON, Canada).

3.2.4.2 Electrophysiology. All epithelia were incubated under short-circuit conditions as previously described (Aschenbach et al., 2000; Penner et al., 2009) and the potential difference was measured using Argenthal reference electrodes (Mettler Toledo, Urdorf, Switzerland) that were connected to each half (serosal and mucosal) of a Ussing chamber using agar bridges (3% agar in 3 mol/L of KCl). Current was applied using a voltage clamp device (Ing.-Büro für Mess- und Datentechnik, Aachen, Germany) such that the amount of current applied was sufficient to clamp the transepithelial potential difference to 0 mV. In addition, bipolar pulses of current were applied every 6 s for determination of transepithelial conductance (**Gt**). Tissue conductance was determined according to Ohm's law by measuring the impulse-induced change in the transepithelial potential difference following the application of short bipolar current impulses. Data for Gt are reported from tissues used for flux measurements.

3.2.4.3 Uptake and flux measurements. A total of 12 Ussing chambers were used for uptake measurements and an additional 12 chambers for flux measurements. Within the uptake and flux measurements, the transport of acetate, propionate, and butyrate were measured in separate Ussing chambers. All measurements were conducted in duplicate

Table 3.4. Chemical composition of the transport buffer, and the mucosal and serosal buffers used to determine the total and bicarbonate-independent nitrate-insensitive uptake and flux of acetate, propionate, and butyrate in Ussing chambers.

	Buffer				
	Transport	Bicarbonate		Bicarbonate-free	
Substance, m*M*		Serosal	Mucosal	Serosal	Mucosal
Na-gluconate	60.0	60.0	60.0	69.6	34.6
K-glusonate	5.5	5.5	5.5	5.5	5.5
Ca-gluconate	1.0	1.0	1.0	1.0	1.0
Mg-glusonate	1.3	1.3	1.3	1.3	1.3
Na-phosphate	0.6	0.6	0.6	0.6	0.6
Disodium hydrogen phosphate	2.4	2.4	2.4	2.4	2.4
Acetic acid	10.0	0	0	0	0
L-glutamine	1.0	1.0	1.0	1.0	1.0
HEPES-free acid	10.0	10.0	10.0	10.0	10.0
Na-propionate	10.0	0	0	0	0
Na-butyrate	10.0	0	0	0	0
Na-bicarbonate	25.0	25.0	25.0	0	0
Glucose	10.0	10.0	10.0	10.0	10.0
Na-nitrate	0	0	0	0	40.0
Acetazolamide	0	0	0	0.1	0.1
Mannitol	115.0	115.0	115.0	135.0	135.0
Gluconic acid	0	0	0	10.0	1
NaOH	0	0	0	5	0
Antibiotics, mg/L					
Penicillin G	0	60	60	60	60
Kanamycin sulfate	0	100	100	100	100
Flurocytosine					
Buffer characteristics					
pH	7.4	7.4	6.2	7.4	6.2
Temperature, ^{o}C	38.5	38.5	38.5	38.5	38.5
Osmolality, mOsmol/kg	314.4 ± 21.0	283.7 ± 6.8	277.1 ± 4.6	306.4 ± 7.4	313.7 ± 4.8

with the duplicates considered to be technical replicates. As described above, the uptake and flux were measured without inhibition (bicarbonate containing buffer) and under maximal inhibition conditions (buffer not containing bicarbonate but containing nitrate). After mounting tissues, 20 min was provided for stabilization of electrophysiology. Tissues were then ranked based on tissue conductance and randomized to either flux or uptake measurements based on the incubations buffers. Within the flux and uptake measurements, tissues were further assigned to measure acetate, propionate, or butyrate transport. For all measurements, a final concentration of 25 m*M* of acetate, propionate, or butyrate was applied to the mucosal side. The isotopes added to an individual column included [^{3}H]- acetate (150 kBq/15 μL), [1-^{14}C]-propionate (75 kBq/15 μL), or [1-^{14}C]-butyrate (75 kBq/ μL).

3.2.4.4 Acetate, propionate, and butyrate uptake. For uptake measurements, the protocol previously described by Aschenbach et al. (2009) was used. Briefly, a radio-labelled solution of acetate, propionate, or butyrate was added to the mucosal side and allowed to incubate for 1 min. For acetate, propionate, and butyrate, the volume added was 302, 302, and 308 mL, respectively in order to achieve a final concentration of 25 mL in the mucosal buffer with 150 kBq for ^{3}H-acetate or 75 kBq for ^{14}C-propionate and ^{14}C-butyrate. After 20 sec of mixing via gas lift, duplicate samples (100 μL) of the mucosal buffer were collected. Following 1 min of incubation, the mucosal and serosal columns were drained and rinsed 3 times (20 sec/wash) over a 1-min duration using ice-cold buffer solution. The tissue was then dismounted and placed in a pre-cooled lysing device with the mucosal side facing up. A total of 4 mL of Solvable (Perkin Elmer, Waltham, MA) was added to the lysing device and the Solvable was gently agitated over the tissue for 3 min. Subsequently, two 500-μL samples of lysate were transferred into scintillation vials and 5 mL of scintillation cocktail was added. Samples were then counted on a scintillation counter (Tri-Carb 2910TR, Perkin Elmer) and the average of the 2 vials were used for calculations. Additionally, a 500 μL sample of lysate was transferred into a microcentrifuge vial for analysis of protein content using bicinchoninic acid disodium salt hydrate (Smith et al., 1985). Uptakes were calculated as described by Aschenbach et al. (2002).

3.2.4.5 Acetate, propionate, and butyrate flux. After assigning tissues to treatments, radio-labelled acetate, propionate, or butyrate was added to the mucosal side as described for uptakes. A total of 45 min was allocated to allow for isotope equilibration and a 100-μL sample was collected from the mucosal side at the start and end of the incubations. For the 100-μL samples, an additional 400 μL of fresh buffer was placed in each scintillation vial and 5-mL of scintillation cocktail was added. Subsequently, three 500-μL samples spaced 60-min apart were collected from the serosal side. After each sample, an equal volume of fresh buffer was replaced to equalize hydrostatic pressure and the dilution was accounted for. The 500-μL samples were each placed in a scintillation vial and 5-mL of scintillation cocktail was added. All samples were placed on a scintillation counter (Tri-Carb2910TR, Perkin Elmer) and the decays per minute were measured. The mucosal-to-serosal flux rates for acetate ($J_{ms\text{-}acetate}$), propionate ($J_{ms\text{-}propionate}$), and butyrate ($J_{ms\text{-}butyrate}$) were calculated as described by Gäbel et al. (1991)

3.3 Statistical analysis

Data were analyzed as a randomized complete block design using the MIXED model in SAS. The model included treatment as a fixed effect and block as a random effect. Treatment differences were considered to be significant when $P < 0.05$ and means were compared using the Tukey-Kramer test.

3.4 Results

3.4.1 Feed intake and growth performance

Diets were formulated to be different in the total FA concentration when comparing the CON and treatments, and to differ in the FA composition for the SAT and UNSAT treatments. Supporting the formulation strategy, both the SAT (4.34 g/100 g) and UNSAT (4.47 g/100 g) treatments had a greater concentration of total dietary FA than CON (1.72 g/100 g; $P < 0.001$; Table 3.5) with no differences between SAT and UNSAT.

With respect to the proportions of individual FA, the most abundant FA present (those representing > 1% of the total FA concentration) in the diets included C16:0, C18:0, 18:1, C18:2N6, and C18:3N3. These FA contributed to over 95% of the dietary FA for each treatment. For C16:0, the concentration in the UNSAT was less (634.4 mg/100 g, $P < 0.001$) than SAT (1769.6 mg/100 g) and CON (381.4 mg/100 g). The SAT diet had a greater ($P < 0.001$) concentration of C18:0 (276.8 mg/100 g), intermediate for UNSAT (125.2 mg/100 g) and for CON (64.4 mg/100 g) The concentration of C18:1 differed among all treatments and was greatest ($P < 0.001$) for UNSAT, intermediate for SAT, and least for CON. In contrast to C18:1, the concentration of C18:2N6 was greatest ($P < 0.001$) for UNSAT, intermediate for SAT, and least for CON. As the UNSAT treatment contained flax and Megalac, the concentration of C18:3N3 was greatest ($P < 0.001$) at 412.1 mg/100 g, with no difference between CON (71.8 mg/100 g) and SAT (99.9 mg/100 g). Thus when considering the most abundant dietary FA, the formulation strategy achieved differences in the total fatty acid concentration between the CON and the SAT and UNSAT treatments, and substantial differences in the FA profile for the SAT and UNSAT treatments. The previous statement is further supported by the greater concentration ($P < 0.001$) of saturated and mono-unsaturated FA observed for SAT than CON and UNSAT, with the concentration of saturated and monounsaturated FA being least for the CON treatment. Likewise, the proportion of polyunsaturated FA were greatest ($P < 0.001$) for UNSAT, intermediate for SAT, and least for CON. The omega-3 FA concentration and the omega-6 FA concentration was greatest for UNSAT relative to CON and SAT, intermediate for SAT, and least for CON.

With respect to FA of lower abundance (< 1% of the total FA), the UNSAT diet had the greatest ($P < 0.001$) concentration of C14:0, with an intermediate concentration for SAT, and least for CON. No differences were found for C14:1 among treatments. The diet for SAT calves had a greater ($P < 0.001$) concentration of C15:0, without any differences between CON and UNSAT. The diet for SAT calves had a greater ($P < 0.001$) concentration of C16:1, without any differences between CON and UNSAT. The proportion of C18:3N6 was very low (< 0.04 to 0.15 mg/100 g) in all diets and did not differ among treatments. Likewise, C18:4N3, C20:4N3 and C22:2N6 did not differ among treatments. That said, SAT had greater concentrations of C20:2N6 ($P < 0.001$),

Table 3.5. Fatty acid composition of the control diet (CON; negative control; n = 3), saturated fatty acid diet (SAT; n = 3) and unsaturated fatty acid diet (UNSAT; n = 3) fed to growing steers.

	Treatment				
	CON	SAT	UNSAT[1]	SEM	*P* value
Total g/100 g diet	1.72[b]	4.34[a]	4.47[a]	0.053	< 0.001
FA composition, mg/100 g diet					
C14:0	3.95[c]	17.30[b]	42.89[a]	0.803	< 0.001
C14:1	0.32	0.62	0.26	0.173	0.37
C15:0	1.63[b]	4.14[a]	2.08[b]	0.203	<0.001
C16:0	381.39[c]	1769.61[a]	634.41[b]	14.923	< 0.001
C16:1	5.26[b]	36.59[a]	9.70[b]	1.660	< 0.001
C18:0	64.36[c]	276.79[a]	125.18[b]	7.67	< 0.001
C18:1	411.19[c]	1090.48[b]	1973.27[a]	16.82	< 0.001
C18:2N6	747.22[c]	917.38[b]	1241.11[a]	12.725	< 0.001
C18:3N6	0.04	0.07	0.16	0.055	0.35
C18:3N3	71.83[b]	99.90[b]	412.10[a]	6.741	< 0.001
C18:4N3	0. 20	0.18	0.15	0.100	0.93
C20:0	10.34[b]	16.53[a]	16.01[a]	0.263	< 0.001
C20:1	5.74[c]	29.23[a]	14.05[b]	1.86	0.003
C20:2N6	0.23[b]	6.69[a]	0.85[b]	0.294	< 0.001
C20:3N6	0.06[b]	1.20[a]	0.06[b]	0.177	0.006
C20:4N6	0.09[b]	5.00[a]	0.13[b]	0.220	< 0.001
C20:3N3	0.16[b]	1.72[a]	0.25[b]	0.221	0.004
C20:4N3	18.23	18.23	18.23	0.127	1.00
C20:5N3	0.20[b]	0.10[b]	0.70[a]	0.089	0.006
C22:0	4.95[b]	6.77[a]	8.08[a]	0.323	0.014
C22:1	0.94[b]	26.05[a]	5.85[b]	2.117	< 0.001
C22:2N6	0.06	0.12	0.06	0.030	0.35
C22:4N6	0.02[b]	1.15[a]	0.02[b]	0.62	< 0.001
C22:5N3	0.09[b]	0.80[a]	0.09[b]	0.061	0.003
C22:6N3	0.12[b]	0.12[b]	5.53[a]	0.093	< 0.001
C24:0	2.29	2.93	3.10	0.247	0.12
C24:1	0.16[b]	1.54[a]	0.58[b]	0.161	0.002
Saturated	468.92[c]	2119.56[a]	806.17[b]	22.720	< 0.001
Monounsaturated	423.61[c]	1184.17[a]	2003.71[b]	18.965	< 0.001
Polyunsaturated	820.56[c]	1034.64[b]	1661.43[a]	16.449	< 0.001
Omega-3	72.80[c]	103.01[b]	419.01[a]	6.670	< 0.001
Omega-6	747.76[c]	931.63[b]	1242.42[a]	12.848	< 0.001

[abc]Means with uncommon superscripts differ ($P < 0.05$).

[1]A theoretical value of 84 g/100 g of FA was used for Megalac.

C20:3N6 (P = 0.003), C20:4N6 (P < 0.001), C20:3N3 (P = 0.004), C22:1 (P < 0.001), C22:4N6 (P < 0.001), C22:5N3 (P = 0.003), C24:1 (P = 0.002) than CON and UNSAT, without any differences between CON and UNSAT. The concentration of C20:1 was also greater for SAT (P = 0.003), intermediate for UNSAT, and least for CON. The UNSAT diet had a greater concentration of C20:5N3 (P = 0.006) and C22:6N3 (P < 0.001), than SAT, without any differences between UNSAT and CON.

There were no differences for initial or final BW between CON, SAT, and UNSAT steers (P = 0.95 and 0.65, respectively; Table 3.6). Moreover, there were nodifferences between treatments for DMI, ADG, reticulo-ruminal digesta weight, and ruminal pH immediately after killing (P > 0.10).

3.4.2 Fatty acid composition of rumen fluid, blood and ruminal tissue

In support of the lack of differences for DMI and ruminal digesta mass among treatments, the concentration of SCFA in ruminal fluid did not differ among steers fed CON, SAT, or UNSAT (P = 0.11; Table 3.7). There were no differences in the proportion of individual SCFA in ruminal fluid among treatments.

Total FA concentration in ruminal fluid was less (P < 0.001) for CON (0.30 g/100 g) compared to SAT (0.52 g/100 g) and UNSAT (0.56 g/100 g) without differences between SAT and UNSAT (Table 3.8). The most abundant FA in ruminal fluid (>1%), included C14:0, C15:0, C16:0, C16:1, C18:0, C18:1, C18:2N6, C183:N3, C20:0, C20:1, and C22:6N3. The concentration of C14:0 was less (P = 0.021) in ruminal fluid from calves fed CON than UNSAT, but there was no difference between SAT and UNSAT. For C15:0 (P = 0.24) and C16:1 (P = 0.21), there were no differences among treatments with average concentrations of 3.14 and 27.03 mg/100 g of ruminal fluid. Ruminal fluid from CON calves had less C16:0 (P < 0.001) and C18:0 (P = 0.004) than SAT and UNSAT, but SAT and UNSAT did not differ. The concentration of C18:1 (P < 0.001) and C18:3N3 (P < 0.001) was greater for UNSAT than SAT and CON, without any differences between SAT and CON. Calves fed UNSAT also had a greater concentration of C18:2N6 (P = 0.040) than SAT, but CON was not different than SAT or UNSAT. Calves fed SAT had a greater (P = 0.029) concentration of C20:0 than CON, with UNSAT being intermediate but not different than either CON or SAT. The concentration

Table 3.6. Body weight, average daily gain, dry matter intake, reticulo rumen digesta weight, and ruminal pH for steers receiving the control diet (CON; n = 7), saturated lipid diet (SAT; n = 7), and unsaturated lipid diet (UNSAT; n = 7).

	Treatment				
Variable	CON	SAT	UNSAT	SEM	*P* value
Initial body weight, kg	194.83	192.96	194.58	10.662	0.95
Final body weight, kg	234.83	229.77	237.44	12.323	0.65
Dry matter intake, % BW	2.64	2.79	2.75	0.072	0.36
Dry matter intake, kg/d	6.38	6.49	6.64	0.295	0.65
Average daily gain, kg/d	1.33	1.23	1.43	0.131	0.29
Reticulo-rumen digesta, kg	34.31	33.10	32.37	1.931	0.49
Ruminal pH	5.99	5.81	5.77	0.142	0.45

[abc]Means with uncommon superscripts differ ($P < 0.05$).

Table 3.7. Ruminal short-chain fatty acid (SCFA) concentration from growing Holstein steers fed the control diet (CON; n=7), saturated lipid diet (SAT; n=7), and unsaturated lipid diet (UNSAT; n=7).

	Treatment				
Variable	CON	SAT	UNSAT	SEM	*P* value
Total SCFA, m*M*	143.08	128.67	152.02	7.384	0.11
Molar proportion, %					
Acetic acid	58.71	56.45	60.19	1.772	0.34
Propionic acid	25.60	28.84	25.60	1.709	0.33
Isobutiric acid	0.09	0.27	0.39	0.104	0.058
Butyric acid	10.42	11.40	10.81	1.259	0.88
Isovaleric acid	1.28	1.61	1.77	0.156	0.10
Valeric acid	1.16	1.43	1.24	0.092	0.12

[abc]Means with uncommon superscripts differ ($P < 0.05$).

Table 3.8. Fatty acid composition (mg/100 g) of ruminal fluid for steers receiving the control diet (CON; negative control; n = 7) saturated fatty acid diet (SAT; n = 7), and unsaturated fatty acid diet (UNSAT; n = 7).

	Treatment				
Variable	CON	SAT	UNSAT	SEM	*P* value
Total, g/100 g of ruminal fluid	0.30[b]	0.52[a]	0.56[a]	0.034	<0.001
FA composition, mg/100g					
C14:0	3.88[b]	6.03[a]	6.21[a]	0.583	0.021
C14:1	0.74	0.41	1.05	0.241	0.196
C15:0	2.71	3.33	3.39	0.305	0.242
C16:0	87.67[b]	200.96[a]	174.21[a]	11.639	< 0.001
C16:1	17.01	30.87	33.21	12.599	0.213
C18:0	125.02[b]	191.21[a]	211.28[a]	16.275	0.004
C18:1	44.17[b]	64.02[b]	98.58[a]	6.298	< 0.001
C18:2N6	15.68[ab]	14.08[b]	19.59[a]	1.428	0.040
C18:3N6	0.02	0.00	0.00	0.011	0.397
C18:3N3	0.89[b]	0.61[b]	3.89[a]	0.259	< 0.001
C18:4N3	0.02	0.00	0.00	0.009	0.278
C20:0	1.08[b]	2.62[a]	2.49[ab]	0.409	0.029
C20:1	1.27	1.67	1.21	0.622	0.854
C20:2N6	0.02	0.02	0.00	0.019	0.546
C20:3N6	1.68	0.00	0.00	0.949	0.382
C20:4N6	0.00	0.00	0.00	0.002	0.397
C20:3N3	0.02	0.00	0.00	0.009	0.213
C20:4N3	0.04	0.00	0.00	0.014	0.141
C20:5N3	0.03	0.00	0.00	0.012	0.164
C22:0	0.17	0.06	0.00	0.093	0.414
C22:1	0.05	0.26	0.00	0.089	0.114
C22:2N6	0.01	0.01	0.01	0.009	0.760
C22:4N6	0.05	0.02	0.03	0.031	0.825
C22:5N3	0.01	0.01	0.00	0.007	0.372
C22:6N3	0.50[b]	2.39[ab]	4.98[a]	0.857	0.006
C24:0	0.08	0.01	0.00	0.026	0.107
C24:1	0.01	0.04	0.07	0.046	0.590
Saturated	220.52[b]	404.29[a]	397.58[a]	27.982	< 0.001
Monounsaturated	63.24[b]	97.21[b]	143.13[a]	12.412	< 0.001
Polyunsaturated	19.01[b]	17.16[b]	28.58[a]	1.955	0.002
Omega-3	1.51[b]	3.01[b]	8.88[a]	0.876	< 0.001
Omega-6	17.50	14.15	19.70	1.642	0.081

[abc]Means with uncommon superscripts differ ($P < 0.05$).

of C22:6N3 was greater ($P = 0.006$) for UNSAT than CON, with SAT being intermediate but not different that the other treatments. Calves fed SAT and UNSAT did not differ but had a greater concentration of saturated FA than CON ($P < 0.001$). The UNSAT calves also had greater concentration of monounsaturated FA ($P < 0.001$), polyunsaturated FA ($P = 0.002$), and omega-3 ($P < 0.001$) in ruminal fluid than SAT and CON, without any differences between SAT and CON.

There was no difference for plasma glucose, serum BHBA, and plasma insulin concentrations among treatments ($P > 0.10$; Table 3.9). The total FA concentration in plasma was greater for steers receiving UNSAT and SAT ($P < 0.001$) than CON, but did not differ between SAT and UNSAT.

The most abundant FA (>1%) in plasma included C14:0, C15:0, C16:0, C16:1, C18:0, C18:1, C18:2N6, C18:3N3, C20:3N6, C20:3N3 and C22:4N6. Steers fed CON had less C14:0 ($P = 0.003$), C16:0 ($P < 0.001$), C18:0 ($P < 0.001$), C18:1 ($P < 0.001$), and C18:2N6 ($P < 0.001$) in plasma than SAT and UNSAT. The concentrations of C16:1 ($P < 0.001$), C18:3N6 ($P < 0.001$) and C20:3N6 ($P < 0.001$) were greater for SAT than CON and UNSAT ($P < 0.001$) with the latter not different. The concentration of C18:3N3 was greatest for UNSAT, intermediate for SAT, and least for CON ($P < 0.001$). For FA of low abundance in plasma (<1 mg/100 g plasma), steers fed CON had lower concentrations of C20:5N3 ($P < 0.001$), C22:0 ($P < 0.001$), C22:2N6 ($P = 0.002$), C24:0 ($P < 0.001$), and C24:1 ($P < 0.001$) than SAT and UNSAT. The concentration of saturated, monounsaturated FA, polyunsaturated FA and omega-6 were lower ($P < 0.001$) for CON than SAT and UNSAT, without any differences between SAT and UNSAT. The concentration of omega-3 in plasma was greater ($P < 0.001$) for UNSAT diets, intermediate for SAT, and least for CON.

Total FA concentration in the ruminal epithelium tended ($P = 0.069$; Table 3.10) to be greater for SAT than UNSAT. The most abundant FA (>1%) in ruminal tissue among all treatments included C14:0, C16:0, C16:1, C18:0, C18:1, C18:2N6, C20:3N6, C20:3N3, and C22:5N3. When reported in percentage of the total C18:2N6 was greater

Table 3.9. Plasma fatty acids from growing Holstein steers fed the control diet (CON; negative control; n = 7), saturated lipid diet (SAT; n = 7), and unsaturated lipid diet (UNSAT; n = 7).

	Treatment				
Variable	CON	SAT	UNSAT	SEM	*P* value
Glucose, mg/dL	67.15	75.74	66.30	5.550	0.43
Insulin, µg/L	0.41	0.31	0.37	0.067	0.54
BHBA, mmol/L	1.52	1.37	1.44	0.149	0.70
FA composition ml/100 g plasma					
Total	129.72^{b}	225.97^{a}	215.65^{a}	8.850	< 0.001
C14:0	0.72^{b}	1.06^{a}	0.96^{a}	0.053	0.003
C14:1	0.28	0.46	0.44	0.166	0.69
C15:0	1.32	1.36	1.49	0.188	0.79
C16:0	18.33^{b}	36.64^{a}	33.05^{a}	1.341	< 0.001
C16:1	1.27^{b}	4.48^{a}	2.02^{b}	0.364	< 0.001
C18:0	26.00^{b}	39.16^{a}	38.41^{a}	1.874	< 0.001
C18:1	18.29^{b}	38.40^{a}	33.51^{a}	1.759	< 0.001
C18:2N6	45.02^{b}	74.95^{a}	78.00^{a}	4.119	< 0.001
C18:3N6	1.72^{b}	2.76^{a}	1.71^{b}	0.120	< 0.001
C18:3N3	1.68^{c}	4.04^{b}	6.87^{a}	0.546	< 0.001
C18:4N3	0.02^{b}	0.19^{ab}	0.25^{a}	0.020	0.020
C20:0	0.03^{b}	0.22^{ab}	0.25^{a}	0.052	0.023
C20:1	0.05	0.05	0.05	0.033	1.00
C20:2N6	0.15^{ab}	0.24^{a}	0.13^{b}	0.027	0.030
C20:3N6	3.54^{b}	5.26^{a}	3.90^{b}	0.306	< 0.001
C20:3N3	6.01^{b}	9.25^{a}	6.77^{b}	0.469	< 0.001
C20:4N6	0.00	0.00	0.03	0.010	0.14
C20:4N3	0.05c	0.31^{b}	0.42^{a}	0.035	< 0.001
C20:5N3	0.36^{b}	0.95^{a}	1.02^{a}	0.056	< 0.001
C22:0	0.23^{b}	0.42^{a}	0.41^{a}	0.028	< 0.001
C22:1	0.26	0.00	0.46	0.306	0.57
C22:2N6	0.35b	0.62^{a}	0.57^{a}	0.052	0.002
C22:4N6	1.03^{ab}	1.10^{a}	0.78^{b}	0.094	0.015
C22:5N3	1.92	2.44	2.58	0.280	0.13
C22:6N3	0.18^{b}	0.31^{a}	0.29^{ab}	0.034	0.022
C24:0	0.31^{b}	0.55^{a}	0.61^{a}	0.040	< 0.001
C24:1	0.23^{b}	0.51^{a}	0.45^{a}	0.025	< 0.001
Saturated	46.99^{b}	79.38^{a}	75.18^{a}	3.288	< 0.001
Monounsaturated	20.37^{b}	43.89^{a}	36.93^{a}	2.180	< 0.001
Polyunsaturated	62.36^{b}	102.70^{a}	103.53^{a}	5.279	< 0.001
Omega-3	4.35^{c}	8.24^{b}	11.46^{a}	0.713	< 0.001
Omega-6	58.01b	94.45a	92.07a	4.656	< 0.001

^{abc}Means with uncommon superscripts differ ($P < 0.05$).

Table 3.10. Fatty acid composition of the ruminal epithelia from growing Holstein steers fed the control diet (CON; n = 7), saturated lipid diet (SAT; n = 7), and unsaturated lipid diet (UNSAT; n = 7).

	Treatment				
Variables	CON	SAT	UNSAT	SEM	*P* value
Total g/100 g of wet tissue	1.98	2.17	1.39	0.476	0.069
FA composition, mg/100g					
C14:0	29.18	38.78	17.36	12.020	0.084
C14:1	5.49	8.12	3.29	2.376	0.094
C15:0	13.86	15.52	8.78	3.587	0.89
C16:0	476.43^{ab}	545.87^{a}	336.30^{b}	119.090	0.050
C16:1	33.42	42.37	17.09	13.126	0.060
C18:0	349.01	379.74	251.42	81.985	0.059
C18:1	821.10	760.28	445.68	233.206	0.094
C18:2N6	152.41	167.50	167.82	11.421	0.51
C18:3N6	2.26^{ab}	2.42^{a}	1.13^{b}	0.495	0.023
C18:3N3	8.78^{b}	10.84^{ab}	14.87^{a}	1.996	0.009
C18:4N3	0.06	0.00	0.00	0.038	0.43
C20:0	6.70	6.98	6.02	0.931	0.59
C20:1	6.42	8.46	3.04	2.535	0.075
C20:2N6	3.46^{ab}	3.73^{a}	2.60^{b}	0.366	0.021
C20:3N6	21.14	20.02	17.62	1.538	0.28
C20:4N6	0.00	0.00	0.50	0.147	0.052
C20:3N3	66.55^{a}	58.91^{ab}	54.59^{b}	3.215	0.048
C20:4N3	0.26	0.33	0.77	0.228	0.12
C20:5N3	3.32^{ab}	2.63^{b}	4.64^{a}	0.486	0.025
C22:0	3.74	3.94	3.50	0.262	0.50
C22:1	0.00	0.45	0.01	0.181	0.16
C22:2N6	0.00	0.03	0.00	0.020	0.43
C22:4N6	10.53^{a}	9.79^{a}	6.93^{b}	0.549	< 0.001
C22:5N3	14.21	13.14	14.14	0.733	0.51
C22:6N3	1.39	1.55	1.35	0.324	0.74
C24:0	9.60	9.59	7.88	9.020	0.44
C24:1	1.24	1.38	0.28	0.354	0.077
Saturated	887.30	1000.42	631.94	215.760	0.053
Monounsaturated	806.63	881.88	469.38	250.896	0.088
Polyunsaturated	285.52	291.78	288.21	16.772	0.95
Omega-3	28.02^{b}	28.56^{b}	36.26^{a}	2.684	0.006
Omega-6	257.61	263.23	251.95	14.486	0.80

^{abc}Means with uncommon superscripts differ ($P < 0.05$).

for UNSAT than SAT, with no differences between UNSAT and CON or SAT and CON (Appendices, Table 5.4). Calves fed SAT tended to have greater concentrations of C14:0 than UNSAT, and had greater C16:0 $P = 0.050$) than UNSAT. Ruminal epithelium from CON calves had concentrations of C16:0 that were not different from either SAT or UNSAT. In addition, the concentration of C18:0 and C18:1 in the ruminal epithelium tended to be greater for SAT than UNSAT, while C18:2N6 did not differ among treatments. The concentration of C18:3N6 ($P = 0.023$) was greater for calves fed SAT than UNSAT but the CON was not different than the other treatments. Calves fed UNSAT had greater concentration of C18:3N3 ($P = 0.009$) than CON, but UNSAT and SAT did not differ.

Feeding SAT also increased ($P = 0.021$), the concentration of C20:2N6 relative to UNSAT. Calves fed UNSAT had less C22:4N6 ($P < 0.001$) than SAT and CON calves. The ruminal epithelium from UNSAT had also greater ($P = 0.025$) C20:5N3 than SAT, without any differences between UNSAT and CON or CON and SAT. The net result was that SAT tended to have a greater concentration of saturated FA than UNSAT, and UNSAT had greater ($P = 0.006$) omega-3 FA concentration than CON and SAT.

3.4.3 Short-chain fatty acid uptake and flux

Acetate uptake was not affected by treatment ($P \geq 0.18$; Table 3.11), but SAT increased total propionate uptake ($P = 0.038$) relative to CON, but differences were not detected between UNSAT and any other treatment. The increased propionate uptake was caused by greater uptake via passive diffusion ($P = 0.015$) for steers fed SAT relative to CON and UNSAT. Moreover, steers provided SAT diets had greater ($P = 0.008$) total butyrate uptake than UNSAT and CON, and tended to have greater uptake of butyrate via passive diffusion ($P = 0.056$) than CON. Tissue Isc and Gt were not affected by treatments. There was a tendency for increased propionate flux across the ruminal epithelium for steers fed SAT diets relative to CON and UNSAT ($P = 0.072$; Table 3.12). However, there was no other differences in SCFA flux across the ruminal epithelium.

3.4.4 Quantitative Real-Time PCR

There were no differences in the expression of genes MCT4, NHE1, and NHE3 in CON, SAT and, UNSAT ($P > 0.05$; Table 3.13). However, there was a tendency in NHE3 to have greater expression in UNSAT steers than SAT ($P = 0.080$).

Table 3.11: Apical uptake of acetate, propionate, and butyrate across the isolated bovine ruminal epithelia harvested from growing steers fed the control diet (CON; n = 7), saturated lipid diet (SAT; n = 7), and unsaturated lipid diet (UNSAT; n = 7).

Uptake, ηmol/(mg protein × min)	Treatment				
	CON	SAT	UNSAT	SEM	*P* value
Acetate					
Total	0.38	0.52	0.41	0.064	0.27
Passive	0.26	0.27	0.33	0.063	0.50
Transporter mediated	0.12	0.25	0.08	0.077	0.18
Propionate					
Total	0.37^{b}	0.73^{a}	0.49^{ab}	0.091	0.038
Passive	0.37^{b}	0.48^{a}	0.38^{b}	0.069	0.015
Transporter mediated	0.00	0.24	0.10	0.107	0.30
Butyrate					
Total	0.45^{b}	1.06^{a}	0.59^{b}	0.119	0.008
Passive	0.56	1.00	0.84	0.136	0.056
Transporter mediated	-0.11	0.06	-0.25	0.137	0.22

^{ab}Means with uncommon superscripts differ ($P < 0.05$).

Table 3.12. Mucosal-to-serosal flux of acetate, propionate, and butyrate across the isolated ruminal epithelia from growing steers fed the control diet (CON; n = 7), saturated lipid diet (SAT, n = 7), and unsaturated lipid diet (UNSAT; n = 7).

	Treatment				
	CON	SAT	UNSAT	SEM	*P* value
Isc, μEq/(cm^2 × h)	0.42	0.26	0.50	0.245	0.77
Gt, mS/cm^2	4.24	4.60	6.35	0.841	0.19
Acetate flux, μmol/(cm² × h)					
Total	0.56	0.65	0.55	0.069	0.57
Passive	0.37	0.39	0.36	0.044	0.81
Transporter mediated	0.19	0.25	0.19	0.080	0.79
Propionate flux, μmol/(cm² × h)					
Total	0.56	0.70	0.61	0.039	0.072
Passive	0.41	0.43	0.36	0.027	0.22
Transporter mediated	0.15	0.26	0.25	0.046	0.16
Butyrate flux, μmol/(cm² × h)					
Total	1.52	1.45	1.61	0.204	0.85
Passive	0.88	1.05	1.02	0.138	0.63
Transporter mediated	0.64	0.40	0.59	0.231	0.72

[ab]Means with uncommon superscripts differ ($P < 0.05$).

Isc and Gt were calculated as average of acetate, propionate and butyrate for each treatment.Total flux represents the uninhibited flux of ^{3}H-acetate, ^{14}C-Propionate, and ^{14}C-Butyrate. Passive flux that represents the flux that was not inhibited with the absence of bicarbonate and inclusion of nitrate in the buffer. Transporter mediated flux was calculated by difference using the uninhibited flux and maximally inhibited flux.

Table 3.13. Relative expression (fold change) of genes for the rumen, standard error of mean for control (CON; n=7), SAT (RA; n=7), and UNSAT (LFI; n=7) steers

	Treatment				
	CON	SAT	UNSAT	SEM	*P* value
NHE1	1.01	0.99	1.02	0.084	0.93
NHE3	1.03	1.05	1.30	0.108	0.080
MCT4	1.14	1.31	1.52	0.398	0.76

MCT4 = monocarboxylic acid transporter 4; NHE1, sodium-hydrogen exchanger 1; NHE3 = sodium-hydrogen exchanger 3.

3.5 DISCUSSION

The hypothesis of this study was that feeding a greater proportion of unsaturated lipid would result in a greater concentration of unsaturated FA in the ruminal epithelium. I further hypothesized that the increase in unsaturated fatty acid concentration in the ruminal epithelium would increase permeability of the rumen epithelium thereby increasing the uptake and flux of acetate, propionate, and butyrate via passive diffusion. The results support the hypothesis that feeding a greater proportion of unsaturated lipid can increase the proportion of unsaturated fatty acids in the ruminal epithelium when reported as a percentage of the total FA in the ruminal epithelium (data not reported) but not when reported as concentration (mg/100 g). In addition, we noted increased omega-3 FA concentration in the ruminal epithelium for UNSAT relative to CON and SAT. This is congruent with the FA profile supplied by flaxseed and Megalac in the diet, and the increased concentrations of monounsaturated and polyunsaturated FA in ruminal fluid and omega-3 FA in plasma. Masur et al. (2016) recently reported that ruminal epithelial cells in vitro are responsive to the supply of FA and in particular the supply of conjugated linoleic acid. They further observed that the increased exposure to the previously mentioned FA altered cellular metabolism of FA and the expression of MCT4. On the other hand, increasing the supply of dietary saturated lipid increased the proportion of C16:0, C18:3N6, C20:2N6 and C22:4N6 in ruminal tissue relative to UNSAT lipid supply. The increase in unsaturated FA may be a result of stearyl-CoA desaturase (**SCD**) activity. Our finding supports that of Mazur et al. (2016) where the exposure of the ruminal epithelia to trans-vaccenic acid increased the expression of stearyl-CoA desaturase (**STD**) thereby, allowing for greater net synthesis of C18:1. Furthermore, in the present study, feeding SAT tended to increased total FA concentration in the ruminal epithelia. Thus, it is clear that the ruminal epithelium is responsive to dietary lipid supply when approaches are used to limit the extent of biohydrogenation.

A clear response in the present study was that providing lipid supplements increased the rate of total propionate and butyrate uptake. To my knowledge, this is the first study reporting an affect of lipid supplementation on the uptake of SCFA. That said, Masur et al. (2016) did report increases in MCT1 and MCT4 expression, in vitro, when

ruminal epithelial cells were exposed to cis-9 trans-11 C18:2, and MCT1 expression was increased when exposed to trans-10 cis-12 C18:2 fatty acids. For propionate, but not butyrate, there was also a tendency for increased flux. The detectable response for propionate but not butyrate may be related to the greater extent of intraepithelial metabolism for butyrate (Weigand et al., 1972; Gäbel et al., 2002; Graham et al., 2007) and that as a result of oxidation, a portion of the ^{14}C-label may have been released apically and thus not detected on the serosal side. This concept is supported by Sehested et al. (1999) where they reported considerable metabolism of SCFA to CO_2 and further suggested that the arising CO_2 was preferentially released on the mucosal side.

Interestingly, uptake of acetate was not affected by dietary lipid content or lipid source. The lack of response for acetate is likely due to a greater reliance on anion exchange pathways relative to propionate and butyrate (Aschenbach et al., 2009). Nevertheless, the results support the concept that dietary lipid supplementation modulates the permeability of the ruminal epithelium and improves uptake of SCFA that rely on passive diffusion. Our results partially support that of Schurmann et al. (2014) where they found that passive diffusion of acetate and butyrate was the most responsive pathway when cattle were exposed to a dietary change. Collectively, this data suggests that membrane permeability may be a key factor regulating SCFA uptake.

In addition to the changes observed with lipid supplementation, this study demonstrated that the profile of the lipid supplement affects the response of the ruminal epithelium and modulates SCFA uptake. For example, it was observed that total uptake of propionate was greater for steers supplemented with SAT than CON, without any differences between SAT and UNSAT or UNSAT and CON. Total butyrate uptake was greater for steers supplemented with SAT than CON and UNSAT. Moreover, the uptake via passive diffusion for propionate was greater for steers supplemented with SAT than CON and UNSAT. However, it should be noted that lipid supplementation and the type of lipid did not affect the expression of NHE1 and MCT4. However, a tendency for increased expression of NHE3 was observed when calves were fed UNSAT relative to CON and SAT. The tendency for increased expression of NHE3 does not support our results as it would be expected that the greater passive uptake observed for propionate and butyrate for calves fed SAT would have stimulated mechanisms to help regulate

intracellular pH. Thus, it is unclear why NHE3 tended to increase for UNSAT, even though uptake values were greater for SAT.

Although contrary to my hypothesis, a main finding from this study was that SAT increased passive diffusion of propionate and butyrate. Previous studies have generally reported that provision of saturated FA decrease membrane fluidity and apparent permeability to solutes relative to mono and polyunsaturated FA (Ibarguren et al., 2014). Similar results for decreased permeability with greater cholesterol inclusion have been confirmed by Jedlovszky and Mezei (2003). Thus, it is not clear why we observed greater permeability with SAT in the present study. Although the concentration of specific FA in the ruminal epithelium were altered in response to the dietary FA, total PUFA and MUFA were not altered in the ruminal epithelium when evaluating supply (mg/100 g). That said, when values were reported as a proportion of the total FA supply, the proportion of C18:2N6 and omega-3 FA were greater for UNSAT than SAT (appendices, Table 5.4). Moreover, we did observe that SAT steers tended to have a greater concentration of total FA in the ruminal epithelium than CON and UNSAT. The increased concentration of FA in the ruminal epithelium may suggest an obligatory requirement for, or at least beneficial effects of, saturated FA for ruminants. The beneficial response may be related to increased supply of cholesterol precursors, such as acetoacetyl-CoA. Cholesterol is abundant in cellular membranes and is integrated with phospholipids (Cullis and Hope, 1991). Cholesterol is known to affect membrane permeability, and its interaction with the hydrocarbon chain of saturated fatty acids may promote passive diffusion (Simons and Vaz, 2004).

Another possible explanation is that some FA isomers may have a negative impact on the epithelial tissue function and permeability. For example, the composition of C18:2N6 did not differ between treatments; however, the proportion of C18:2N6 was greater (P = 0.049; not reported in the tables) for UNSAT (13.2%) than CON (9.5%) and SAT (10.3%). Past studies have demonstrated that certain isomers of C18:2, specifically *trans-10, cis-12* and to a lesser extent cis-10 trans-11, decrease the percentage of milk fat in lactating dairy cows (Chouirnad, 1999; Bauman, 2003) by depressing de novo synthesis in the mammary gland (Loor and Herbein, 1998). In caco-2 cells, exposure to *trans-10, cis-12* C18:2 disrupted the distribution of occludin and ZO-1, and decreased

transepithelial resistance. The net result was increased paracellular permeability. The FA analysis used in the present study did not allow for separation of the C18:2N6 isomers. Future research is needed to confirm the effect of individual FA isomers on the ruminal epithelium.

While increases in total propionate uptake were observed, it is challenging to interpret the uptake data for the CON treatment as we were not able to isolate any meaningful transporter-mediated uptake or flux. This differs from that reported by Aschenbach et al., (2009) where propionate uptake was partially dependent on HCO_3^- /$SCFA^-$ exchange. That said, most studies evaluating mechanisms involved in SCFA uptake and flux use acetate and butyrate in the evaluation due to their markedly differing transport pathways (Gäbel et al., 2002; Aschenbach et al., 2009; Schurmann et al., 2009). Thus, further work is needed to evaluate mechanisms of propionate uptake and flux.

3.6 CONCLUSION

Increasing the concentration of dietary lipid alters ruminal epithelial FA composition and enhanced the uptake of propionate and butyrate relative to non-supplemented steers. In addition, provision of saturated FA alters the fatty acid composition of the ruminal epithelium, tending to increase the total FA concentration in the ruminal epithelium and further improved propionate uptake via passive diffusion and butyrate uptake relative to steers fed unsaturated lipid sources.

4.0 GENERAL DISCUSSION

The first objective of this project was to determine whether supplemental lipid could alter the FA profile of the ruminal epithelium. Studies have shown that supplemental lipids might modulate cell membrane lipids (Scott, 1993; Maddock at al., 2007; Calder, 2012). However there is no evidence for whether lipid supplementation could change the ruminal epithelium cell composition. That said, past studies have clearly shown that lipid supplementation can modulate the composition of meat and milk (Wood et al., 2008; Chilliard and Ferlay, 2004). In this study I reported that lipid supplementation can modulate the FA profile of the ruminal epithelium. This study also demonstrated that the type of lipid in the supplement also affects ruminal epithelial tissue composition. For example, steers that were exposed to a diet with greater amount of unsaturated FA had more omega-3 FA in their ruminal tissue than steers fed a saturated diet. Total FA concentration in the tissue tended to be greater for steers fed saturated FA.

The second objective was to evaluate if the lipid supplementation affects SCFA absorption by the ruminal epithelium. Past studies have demonstrated that the composition of membrane lipids regulate the movement of nutrients across the membrane due to changes in membrane permeability (Scott, 1993; Maddock at al., 2007; Calder, 2012). To my knowledge, this is the first study that measured the FA composition of the rumen epithelium and how this composition can alter SCFA absorption. This study also supports the results from Masur et al. (2016), who reported each supplemented FA resulted in an increase in the amount of the same FA in the rumen epithelial cells. To accomplish this objective, the Ussing chamber model was used to measure SCFA uptake and flux. I observed that tissue permeability was increased with lipid supplementation, based on propionate and butyrate uptake, when steers where fed saturated FA. What was not expected was that the SAT treatment exerted a greater response than the UNSAT treatment. In fact, I hypothesized that feeding more UNSAT FA in the diet would increase total FA in the ruminal tissue and also would increase SCFA uptake and flux by the rumen epithelium. Polyunsaturated FA such as omega-3 have been shown to have positive effects on human health (Su et al., 2008) and animal health (Simopoulos, 1991). They can be incorporated into cell membranes (Lazzarin et al., 2009) and they are an

important agent for suppression of inflammation (Smith et al., 2011). Omega-3 FA such as EPA and DHA are also related to fetal development (Dunstan et al., 2007), brain and retina health (Krauss-Etschmann et al., 2007), and prevention and treatment of several diseases (Serhan et al., 2008) such as cardiovascular disease. The present study showed that additional lipid supplementation can alter the FA profile of the membrane lipid in the ruminal epithelium but feeding the saturated FA diet tended to increase the total FA concentration. Ruminants might have an obligate requirement for saturated FA, especially because the diet consumed is hydrolyzed to a great extent by ruminal lipases. Free FA that are released then undergo biohydrogenation thereby increasing the proportion of saturated FA in the ruminal epithelium (Beam, 2000). Future studies are needed to expand on the current work to further evaluate effects of FA supply on other regions of the gastrointestinal tract.

In the current study the FA profile of the ruminal tissue did not allow for the presentation of specific FA isomers. Further classification may be needed in future studies as different isomers exert differential biological effects or have differing potencies. For example, *trans*-10, cis-12 C18:2 that was the first intermediate to be identified as a potent inhibitor of milk fat synthesis (Bauman, 2008). Bauman (2008) also demonstrated that the *trans*-10, cis-12 isomer is more potent than the cis-9 trans-11 isomer for reducing milk fat synthesis. It is possible that these C18:2 isomers may have contributed to tendency for lower concentration of FA in the ruminal epithelium for the UNSAT compared to SAT, but this is only speculation as there is no evidence that this isomer is the one present. Moreover, the effect of *trans*-10, cis-12 on other tissues has not been elucidated. Finally, when considering C18:2N6 supply (mg/100 g), SAT and UNSAT were not different. That said, there is evidence to suggest that the isomers are recognized differently, thereby affecting lipid metabolism (Metges et al., 2003). Metges et al. (2003) looked at the effect of trans-10 cis-12 C18:2 and cis- 9 tran-11 C18:2 on white and brown adipocytes cells of hamsters. They reported that trans-10 cis-12 isomer decreased neutral lipid in both cells when added to a concentration of 35.7 and 71.4 μmol/L. They further noted a greater reduction in neutral lipid concentration in brown cells than white cells (60% and 30%, respectively). In contrast, incubating cells with 71.4 μmol/L of the cis-9 trans-11 isomer, increased lipid content in brown and white cells

occurred. An explanation to the effect of trans-10 cis-12 C18:2 may include an increase in lipolysis, a decrease of FA and triacyl-glycerol synthesis, an increase in FA oxidation, and stimulated apoptosis (Metges et al., 2003). The previously stated outcomes do not occur with cis-9 trans-11 CLA.

Furthermore, while this study demonstrated effects of lipid supplementation, the model included dietary lipid extremes (2.2 vs. 4.9% and 5.2%). As such, the amount of supplemental lipid required induce changes in tissue FA composition is not known. Moreover, a 30-d feeding period was used to ensure sufficient exposure was utilized to detect changes. Again, understanding the duration of time required to induce such responses is important. Adaptation may occur rapidly and changes have been observed for SCFA absorption within 7 days of dietary change (Schurmann et al., 2014).

Producers already use lipid supplements with the goal to increase the energetic density of the diet; however, attention to the type and composition of the supplements and how it is beneficial may not be receiving adequate attention. In this study, lipid supplementation had a positive effect for SCFA uptake. The increased uptake may translate to improved energetic efficiency but larger and longer studies would be needed to confirm this suggestion. This suggests that it is necessary to know what type of lipid should be fed. Protected lipids that escape biohydrogenation, such as saturated lipid sources, Ca-soaps, and encapsulated FA are expensive. This study suggests that a more favourable response may be observed for more saturated FA relative to unsaturated FA.

4.1 Future research

This study was the first to show that lipid supplementation can modulate the composition of the ruminal epithelium and affect its permeability. However, more research is needed to explain some of the results of this study. For example, a question that remains is whether the ex vivo responses observed in the present study will translate to similar positive outcomes for SAT when measured in vivo? Further research is also needed to determine the effect of individual FA isomers in terms of affecting membrane permeability. An important question to confirm is whether the C18:2 isomer, cis-10 trans-12, affects tissue permeability and tissue FA concentration. This could be accomplished

using arterial infusions of specific isomers to help establish whether the type FA affects membrane permeability and nutrient absorption.

Subsequent studies should determine the ideal percentage of lipid in the diet required to induce a response. In the present study, the lipid supplemented treatments incorporated 5% the optimal concentration of dietary lipid to induce positive effects. With the current study, we cannot evaluate whether lower inclusion rates of lipid would be effective. Following the same thought, research evaluating the timeline for a response is needed. Going on even further, more research would be interesting in how lipid supplementation modulates composition of other tissues, such as the intestine and if it changes in composition are related to post-ruminal digestion and nutrient absorption.

5.0 APPENDICES

Table 5.1 Fatty acid composition (%) of the control diet (CON; negative control; n = 3), saturated fatty acid diet (SAT; n = 3) and unsaturated fatty acid diet (UNSAT; n = 3) fed to growing steers.

	Treatment				
	CON	SAT	UNSAT	SEM	*P* value
FA composition of diet, %					
C14:0	0.42[a]	0.20[b]	0.19[b]	0.019	<0.001
C14:1	0.02	0.01	0.01	0.005	0.7023
C15:0	0.09	0.07	0.07	0.010	0.2963
C16:0	22.79[a]	22.32[a]	18.78[b]	0.255	<0.001
C16:1	0.65[a]	0.26[b]	0.12[c]	0.316	<0.001
C18:0	0.83[b]	6.46[a]	4.17[b]	0.832	<0.001
C18:1	23.71[c]	27.41[a]	24.81[b]	0.129	<0.001
C18:2N6	43.65[a]	35.91[c]	38.48[b]	0.265	<0.001
C18:3N6	0.00	0.00	0.00	0.002	0.4219
C18:3N3	4.26[b]	3.77[b]	11.48[a]	0.126	<0.001
C18:4N3	0.01	0.01	0.01	0.009	0.8638
C20:0	0.69	0.72	0.70	0.052	0.927
C20:1	0.31[b]	0.60[a]	0.33[b]	0.035	0.0018
C20:2N6	0.01[b]	0.12[a]	0.02[b]	0.009	<0.001
C20:3N6	0.00[b]	0.02[a]	0.00[b]	0.005	0.037
C20:4N6	0.00[b]	0.09[a]	0.00[b]	0.003	<0.001
C20:3N3	0.01	0.03	0.01	0.007	0.1715
C20:4N3	0.01	0.01	0.01	0.010	1
C20:5N3	0.01	0.01	0.01	0.004	0.5787
C22:0	0.26	0.22	0.23	0.010	0.0618
C22:1	0.07[b]	0.49[a]	0.17[b]	0.039	<0.001
C22:2N6	0.01	0.01	0.01	0.003	1
C22:4N6	0.02[a]	0.00[b]	0.00[b]	0.003	0.0073
C22:5N3	0.01	0.02	0.01	0.004	0.1133
C22:6N3	0.02	0.01	0.01	0.007	0.512
C24:0	0.13	0.09	0.10	0.012	0.2322
C24:1	0.03	0.01	0.01	0.006	0.0787
Saturated	27.62[b]	30.79[a]	24.24[c]	0.311	<0.001
Monounsaturated	24.3733[c]	29.19[a]	25.46[b]	0.132	<0.001
Polyunsaturated	48.01[b]	40.03[c]	50.07[a]	0.235	<0.001
Omega-3	4.33[b]	3.86[b]	11.55[a]	0.125	<0.001
Omega-6	43.68[a]	36.17[c]	38.52[b]	0.253	<0.001

[abc]Means with uncommon superscripts differ ($P < 0.05$).

Table 5.2 Fatty acid composition (%) of ruminal fluid for steers receiving the control diet (CON; negative control; n = 7) saturated fatty acid diet (SAT; n = 7), and unsaturated fatty acid diet (UNSAT; n = 7).

	Treatment				
	CON	SAT	UNSAT	SEM	*P* value
FA composition of ruminal fluid , %					
C14:0	1.29	1.16	1.10	0.109	0.35
C14:1	0.24	0.07	0.18	0.063	0.18
C15:0	0.89[a]	0.3[b]	0.61[b]	0.044	<0.001
C16:0	28.89[b]	39.01[a]	31.11[b]	1.119	<0.001
C16:1	5.44	5.89	6.11	2.837	0.96
C18:0	41.46	36.53	37.51	1.691	0.075
C18:1	14.68[ab]	1.05[b]	17.55[a]	1.055	0.004
C18:2N6	5.13[a]	2.75[b]	3.57[b]	0.329	<0.001
C18:3N6	0.01	0.00	0.00	0.003	0.40
C18:3N3	0.30[b]	0.11[b]	0.72[a]	0.069	<0.001
C18:4N3	0.01	0.00	0.00	0.003	0.30
C20:0	0.36	0.49	0.43	0.084	0.52
C20:1	0.37	0.34	0.22	0.168	0.80
C20:2N6	0.01	0.00	0.00	0.006	0.44
C20:3N6	0.64	0.00	0.00	0.361	0.38
C20:4N6	0.00	0.00	0.00	0.001	0.40
C20:3N3	0.01	0.00	0.00	0.003	0.20
C20:4N3	0.01	0.00	0.00	0.005	0.15
C20:5N3	0.01	0.00	0.00	0.005	0.18
C22:0	0.06	0.01	0.00	0.030	0.39
C22:1	0.02	0.05	0.00	0.018	0.20
C22:2N6	0.00	0.00	0.00	0.002	0.83
C22:4N6	0.02	0.00	0.01	0.010	0.57
C22:5N3	0.00	0.00	0.00	0.002	0.32
C22:6N3	0.15	0.43	0.85	0.157	0.026
C24:0	0.01	0.00	0.00	0.005	0.12
C24:1	0.00	0.01	0.02	0.009	0.60
Saturated	72.43[b]	77.84[a]	70.76[b]	2.255	0.005
Monounsaturated	20.75	18.85	24.08	2.342	0.065
Polyunsaturated	6.31[a]	3.31[b]	5.16[ab]	0.528	0.002
Omega-3	0.49[b]	0.55[b]	1.56[a]	0.160	<0.001
Omega-6	5.82[a]	2.76[b]	3.59[b]	0.516	0.001

[abc]Means with uncommon superscripts differ ($P < 0.05$).

Table 5.3 Plasma fatty acids (%) from growing Holstein steers fed the control diet (CON; negative control; n = 7), saturated lipid diet (SAT; n = 7), and unsaturated lipid diet (UNSAT; n = 7).

	Treatment				
	CON	SAT	UNSAT	SEM	*P* value
FA composition of Plasma, %					
C14:0	0.56[a]	0.47[b]	0.45[b]	0.020	0.003
C14:1	0.21	0.19	0.21	0.081	0.98
C15:0	1.04[a]	0.58[b]	0.71[ab]	0.096	0.009
C16:0	14.08[b]	16.19[a]	15.42[a]	0.330	0.001
C16:1	0.98[b]	1.95[a]	0.94[b]	0.146	<0.001
C18:0	19.10[a]	17.32[b]	17.81[b]	0.481	0.002
C18:1	14.08[b]	16.91[a]	15.70[ab]	0.693	0.032
C18:2N6	34.81	33.35	35.94	1.303	0.39
C18:3N6	1.33[a]	1.22[a]	0.80[b]	0.057	<0.001
C18:3N3	1.29[c]	1.78[b]	3.13[a]	0.157	<0.001
C18:4N3	0.12	0.09	0.12	0.012	0.088
C20:0	0.03	0.10	0.11	0.024	0.047
C20:1	0.04	0.02	0.03	0.020	0.85
C20:2N6	0.12	0.11	0.06	0.018	0.087
C20:3N6	2.72[a]	2.33[a]	1.79[b]	0.107	<0.001
C20:3N3	0.00	0.00	0.01	0.005	0.14
C20:4N6	4.65[a]	4.08[a]	3.13[b]	0.169	<0.001
C20:4N3	0.04[c]	0.14[b]	0.20[a]	0.017	<0.001
C20:5N3	0.28[b]	0.42[a]	0.47[a]	0.022	<0.001
C22:0	0.18	0.19	0.19	0.014	0.92
C22:1	0.19	0.00	0.23	0.169	0.61
C22:2N6	0.28	0.27	0.26	0.024	0.92
C22:4N6	0.78[a]	0.49[b]	0.36[c]	0.037	<0.001
C22:5N3	1.50	1.08	1.20	0.152	0.13
C22:6N3	0.14	0.14	0.13	0.018	0.98
C24:0	0.24	0.24	0.29	0.020	0.17
C24:1	0.18[b]	0.23[a]	0.21[ab]	0.013	0.026
Saturated	36.13	35.08	34.98	0.766	0.52
Monounsaturated	15.67[b]	19.31[a]	17.32[ab]	0.860	0.029
Polyunsaturated	48.20	45.61	47.71	1.344	0.37
Omega-3	3.37[b]	3.65[b]	5.26[a]	0.210	<0.001
Omega-6	44.84	41.96	42.45	1.241	0.25

[abc]Means with uncommon superscripts differ ($P < 0.05$).

Table 5.4 Fatty acid composition (%) of the ruminal epithelia from growing Holstein steers fed the control diet (CON; n = 7), saturated lipid diet (SAT; n = 7), and unsaturated lipid diet (UNSAT; n = 7).

	Treatment				
	CON	SAT	UNSAT	SEM	*P* value
FA composition of wet tissue, %					
C14:0	1.21	1.49	1.04	0.217	0.15
C14:1	0.24	0.31	0.21	0.046	0.26
C15:0	0.72	0.69	0.64	0.044	0.49
C16:0	24.05[b]	25.17[a]	24.16[b]	0.271	0.018
C16:1	1.38	1.65	1.03	0.222	0.061
C18:0	18.18	17.42	17.99	0.270	0.15
C18:1	33.28	35.06	30.28	2.431	0.23
C18:2N6	10.28[ab]	9.50[b]	13.23[a]	1.428	0.049
C18:3N6	0.13	0.11	0.09	0.152	0.18
C18:3N3	0.00	0.00	0.03	0.010	0.23
C18:4N3	0.00	0.00	0.00	0.003	0.43
C20:0	0.36	0.36	0.51	0.043	0.025
C20:1	0.25	0.35	0.18	0.055	0.10
C20:2N6	0.21	0.21	0.20	0.025	0.94
C20:3N6	1.43	1.19	1.46	0.245	0.63
C20:4N6	4.63	3.45	4.48	0.694	0.29
C20:3N3	0.00	0.00	0.03	0.011	0.069
C20:4N3	0.01[b]	0.02[ab]	0.06[a]	0.013	0.022
C20:5N3	0.22[b]	0.17[b]	0.38[a]	0.048	0.006
C22:0	0.25	0.23	0.29	0.046	0.52
C22:1	0.00	0.02	0.00	0.007	0.21
C22:2N6	0.00	0.00	0.00	0.000	0.43
C22:4N6	0.70	0.55	0.56	0.086	0.15
C22:5N3	0.97[ab]	0.77[b]	1.16[a]	0.147	0.059
C22:6N3	0.08	0.09	0.11	0.022	0.45
C24:0	0.61	0.54	0.66	0.122	0.74
C24:1	0.08	0.08	0.03	0.032	0.37
Saturated	45.42	45.91	45.30	0.457	0.61
Monounsaturated	35.46	37.46	31.73	2.704	0.18
Polyunsaturated	19.25	16.63	22.97	2.636	0.091
Omega-3	1.77[b]	1.57[b]	2.87[a]	0.237	<0.001
Omega-6	17.47	15.05	20.10	2.412	0.16

[abc]Means with uncommon superscripts differ ($P < 0.05$).

6.0 LITERATURE CITED

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