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Kevin J. Harvatine

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**EFFECTS OF RUMEN-PROTECTED FATTY ACID SATURATION ON MILK
YIELD, INTAKE, CHEWING BEHAVIOR AND RUMINAL FERMENTATION
IN LACTATING DAIRY COWS**

By

Kevin J. Harvatine

A THESIS

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ABSTRACT

Effects of rumen-protected fatty acid saturation on milk yield, intake, chewing behavior and ruminal fermentation in lactating dairy cows

By

Kevin J. Harvatine

Two experiments were conducted to investigate the effects of rumen-protected unsaturated (UNS) and saturated (SAT) fatty acids (FA) on milk yield, intake, chewing behavior, and ruminal fermentation of lactating dairy cows. In the first experiment, UNS (2% calcium salts of palm FA) decreased dry matter intake (DMI, 0.8 kg/d), rumination time (25 min/d), plasma insulin and milk protein concentrations compared to SAT (2% prilled FA) in a 32 cow crossover design. In the second experiment, UNS (2.5 % calcium salts of blended FA) decreased milk fat percent and increased body weight gain compared to SAT (2.5% prilled FA) in 8 duodenally and ruminally cannulated cows. Calcium salts only provided partial rumen-protection as UNS FA were highly biohydrogenated. A simplified model of rumen biohydrogenation was developed and used to determine that UNS decreased fractional biohydrogenation rate of trans-C18:1. Saturated FA decreased rumen organic matter digestibility possibly because of modification of particle passage rate related to increased rumination. UNS also decreased dry matter intake and meal size compared to SAT (1.6 kg), and SAT increased rumination over 50 min per day compared to control and UNS. Duodenal FA profile is important for prediction and manipulation of animal response because of physiological and metabolic effects of individual FA.

**Dedicated to the Harvatine and Foster Families of
Northeastern Pennsylvania**

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LIST OF ABBREVIATIONS

BCS	Body Condition Score
βHBA	β-hydroxybuturate
BW	Body Weight
CCK	Cholecystokinin
CLA	Conjugated Linoleic Acid
CON	Control
CP	Crude Protein
DE	Digestible Energy
DIM	Days in Milk
DM	Dry Matter
DMI	Dry Matter Intake
FA	Fatty Acid
FCM	Fat Corrected Milk
GE	Gross Energy
GI	Gastro-intestinal
GLP-1	Glucagon-like-peptide-1
INDF	Indigestible Neutral Detergent Fiber
INT	Intermediate
L	Linear
MUN	Milk Urea Nitrogen
NDF	Neutral Detergent Fiber
NEFA	Non-esterified Fatty Acid

NE_L	Net Energy of Lactation
NE_m	Net Energy of Maintenance
OM	Organic Matter
pdNDF	Potentially Digestible NDF
pFCMY	Pretrial Fat Corrected Milk Yield
PUFA	Poly unsaturated FA
Q	Quadratic
RPF	Rumen Protected Fatty acids
SAT	Saturated
TRT	Treatment
UNS	Unsaturated
VFA	Volatile Fatty Acid

INTRODUCTION

Early lactation, high producing dairy cows experience negative energy balance due to limitation of energy intake, while late lactation, low producing cows gain excessive body weight due to failure to control energy intake. Although, lactation and maintenance of body weight are under homeorhetic control, milk production and tissue gain are not perfectly coordinated with intake causing asynchrony of nutrient intake and energy expenditure. Negative energy balance places the cow under metabolic stress, decreasing milk yield and persistency and increasing susceptibility to metabolic disorders. Excessive body weight gain wastes feed resources and decreases whole farm efficiency, and increases the incidence of dystocia, ketosis, and fatty liver during the next lactation.

Dairy nutritionists attempt to regulate energy balance through dietary intervention. Concentrates are commonly fed to increase the energy density of a diet, which increases fermentation acid production, and decreases dietary fiber and daily rumination time (Allen, 1997). A reduction in ruminal pH with increased concentrate feeding can decrease rumen fiber digestion, decrease microbial protein production, and increase systemic acidosis. In contrast, fat supplementation increases dietary energy density without increasing diet fermentability. Fat sources can be protected in the rumen; decreasing FA biohydrogenation and inhibition of fermentation (Wu et al., 1991). Research in other animal models demonstrates the bioactivity of FA, including modulation of intake and body weight gain. Although increased dietary energy density is not required in late lactation, FA supplementation may be used to decrease intake and body weight gain, or decrease digesta passage rate and increase diet digestibility. In late

lactation, decreasing intake and partitioning nutrients towards milk synthesis and away from fat storage would allow continued feeding of a high concentrate diet that provides more propionate to help maintain higher levels of milk yield (Leaver, 1988; and Hansen et al., 1991).

Intake and diet nutrient density and digestibility determines total nutrients absorbed. Fat supplementation increases the energy density of the diet, but intake and digestibility must be maintained to increase daily energy intake. The intake response to dietary fat depends on the FA profile reaching the duodenum; with unsaturated FA more hypophagic than saturated FA (Drackley et al., 1992; Christensen et al., 1994; Bremmer et al., 1998; and Allen, 2000). Saturated FA digestibility is lower than unsaturated FA fed in triglyceride form, but saturation may not affect digestibility when fed as unesterified FA (Pantoja et al., 1995; Pantoja et al., 1996; and Elliot et al., 1999).

Fatty acid protection methods and effects of FA supplements on rumen fermentation have not been tested with high producing cows fed fermentable diets and high concentrations of rumen available FA. Few studies in lactating cows have reported digestibility of unesterified FA differing in saturation directly in the same experiment (Eastridge and Firkins, 1991; Palmquist, 1991; and Schauff and Clark, 1992), and there are no reports of FA digestibility in high producing cows with high passage rates.

Cow response to energy supplementation may depend on metabolic state. High and low producing cows are considerably different in energy metabolism and intake regulation. Two experiments were conducted to evaluate cow response to rumen-protected FA saturation. A crossover experiment using 2% calcium salts of palm oil and prilled, hydrogenated FA was first used to observe difference in response to FA

saturation by low and high milk yield cows. A second intensive experiment used 2.5% of a more unsaturated FA calcium soap than palm oil and prilled, hydrogenated FA to observe effects on ruminal digestion and feeding behavior. An additional block of non-cannulated cows was used in the second experiment to increase the number of intake and milk production observations.

It may be possible to select FA supplements to modify metabolism based on production goals such as increased energy intake and efficiency in early lactation and decreased body weight gain and intake in late lactation. The objective of this research was to evaluate the effects of supplemental FA saturation on milk yield, energy balance, ruminal fermentation, feed intake, and chewing behavior. The hypothesis of the first experiment was that more highly unsaturated FA would decrease intake relative to saturated FA at equal FA concentrations, and individual cow response would depend on production level. The hypothesis of the second experiment was that more unsaturated FA would decrease intake by decreasing meal size, and increase ruminal digestion by decreasing passage rate, but would not affect FA digestibility.

CHAPTER 1

A REVIEW OF LITERATURE

Dietary Fat

Dietary fatty acids (FA) serve a number of functions in lactating dairy cows. Traditionally, fat has been considered an energy source, providing energy required for maintenance and production of tissue and product. Dietary FA also serve as integral structural components of cellular membranes, and regulatory molecules. More recently, FA are appreciated as biological modifiers of physiology and metabolism, making them bioactive compounds (Drackley, 2000). Dairy cows experience vastly different metabolic states during a lactation cycle and dietary FA serve different roles during these states. It is reasonable to speculate that cow response will depend on FA profile, metabolic state, and their interaction.

Fat supplementation in ruminants is not a new area of investigation. Palmquist and Jenkins (1980) provided a short history of fat research, discussing a 1907 review of data from 10 European experiment stations showing little benefit of fat on milk and milk fat yield (Kellner, 1907). However, research in the late 1920's to early 1940's consistently observed a 2 to 10% milk production response to increased dietary lipids. In a 1960 review, Warner (1960) discussed reduced fiber digestion and milk production with fat supplementation, leading to the conclusion that fat was rarely superior to cereal grain. Palmquist and Jenkins (1980) focused their review on the renewed interest in using fat supplementation to increase dietary energy density, without increasing dietary

starch content, to support energy requirements of high producing cows. Recently, dietary fat has also gained interest for increasing reproductive efficiency (Staples et al., 1998), and changing the FA profile of animal products (Grummer, 1991; Palmquist and Beaulieu, 1993; and Mansbridge and Blake, 1997). Consumers are increasingly concerned about the intake of saturated FA with their link to health problems including heart disease and diabetes (Mansbridge and Blake, 1997). Increasing CLA intake may decrease the incidence of cancer and obesity (Kelly, 2001). Dietary manipulation may allow designing FA profiles of meat and milk products to meet consumer demands for low saturated FA concentration (Grummer, 1991). Lastly, FA type has a profound effect on animal physiology including metabolic signaling and gene transcription that may have application to increase production and efficiency (Drackley, 2000).

Fat and the Rumen

Dietary FA must first pass through the rumen before absorption in the intestine, making rumen lipid metabolism an important starting point for discussion of fat supplementation. Rumen activity and fate of FA is well studied and the subject of many reviews including those by Harfoot (1981), Harfoot and Hazlewood (1988), and Jenkins (1993). Extensive lipolysis of triglycerides and hydrogenation of unsaturated FA occur in the rumen by bacteria and protozoa. Theoretically, digestion of long-chain FA in the rumen should be low, with minimal absorption across the ruminal epithelium and minimal catabolism to VFA and CO₂ (Jenkins, 1993). Microbial *de novo* FA synthesis for incorporation into phospholipids should produce a net positive flow of lipids to the

duodenum (Drackley, 2000). In contrast to the expected positive rumen FA flow, digestion studies commonly observe a net loss of FA in the rumen (Jenkins, 1993).

Lipid complexes (triglycerides, galactolipids and phospholipids) are hydrolyzed to their individual components in the rumen, releasing long-chain FA. The rapid hydrolysis of esterified FA, especially diglycerides, was recognized with identification of the lipolytic capacity of many bacteria (Harfoot, 1981). Although considered a rapid process, lipolysis may be rate limiting, as different biohydrogenation end products are observed in esterified and non-esterified treatments (Palmquist and Jenkins, 1980). In addition, the rate of lipolysis may be affected by the saturation of FA in lipid complexes (Elliot et al., 1997).

Free unsaturated FA are biohydrogenated by rumen bacteria and protozoa. The importance of protozoal biohydrogenation was disregarded after observing that defaunation had little effect on rumen FA biohydrogenation (Harfoot, 1981). Protozoa may play a secondary role to bacteria, or bacterial hydrogenation capacity may be very high. Hydrogenation of unsaturated FA may be a protective mechanism, as unsaturated FA are toxic to rumen bacteria (Harfoot, 1981). However, in the rumen, unsaturated FA are normally present as a triglyceride or are associated with feed particles that would have little interaction with bacteria (Harfoot, 1981). Harfoot (1981) proposed that because bacteria require more highly saturated FA for formation of phospholipid membranes they may hydrolyze and biohydrogenate FA for incorporation into their membranes, eliminating the metabolic burden of synthesizing saturated FA.

Microbial biohydrogenation is a multi-step process of which the rate and control are not well understood. A free carboxyl group is required for hydrogenation, limiting

the availability of FA in triglycerides or associated with metal cations. The requirement for a free carboxyl group was first concluded after observing a lower plasma appearance rate with ruminal infusion of linoleic acid in free compared to esterified form, and was later demonstrated *in vitro* (Harfoot, 1981). Biohydrogenation of linoleic and linolenic acid are multistep pathways that include trans-diene intermediates (Harfoot and Hazelwood, 1988). The first step of hydrogenation of linoleic acid is isomerization of the cis-12 bond to trans-11, forming cis-9, trans-11 C18:2, known as conjugated linoleic acid (CLA) (Drackley, 2000). A hydrogenation reaction then removes the cis-9 double bond forming vaccenic acid. In the final step, the trans-11 bond is removed producing stearic acid. Biohydrogenation of oleic acid also includes formation of a number of trans-FA intermediates (Mosley et al., 2002). The competition between FA biohydrogenation and passage rates determines duodenal FA flow. Considerable levels of vaccenic acid and other trans-C18:1 isomers reach the duodenum, but very little CLA escapes from the rumen (Piperova et al., 2002).

Allen (2000) proposed that the extent of biohydrogenation is a result of the characteristics of the fat source, retention time in the rumen, and characteristics of the microbial population. Using simple enzyme kinetic theory, total biohydrogenation is determined by the pool size of available FA, rumen retention time, and bacterial hydrogenation capacity that is a function of bacteria concentration, microbial population, and rumen environment. Beam et al. (2000) observed decreased rates of lipolysis and biohydrogenation with increased concentrations of polyunsaturated triglycerides. Even at high concentrations of FA the rate of lipolysis was over three times the rate of biohydrogenation, thus lipolysis was not rate limiting (Beam et al., 2000). However,

Van Nevel and Demeyer (1996) observed decreased lipolysis with lower pH, making lipolysis the rate-limiting step. *In vitro* experiments showed severe inhibition of fermentation with addition of free unsaturated FA, but no effect of esterified unsaturated FA or free saturated FA (Chalupa et al., 1984). Traditionally, hydrolysis of triglycerides is considered rate limiting, but it appears that numerous factors affect hydrolysis. In some situations unsaturated FA availability is limited or slowed by esterification, but not in all. Factors determining the rate of hydrolysis are not well understood, limiting nutritionist's ability to predict the level of rumen FA protection provided by esterification.

Loss of dietary FA from the rumen through absorption across the rumen wall and oxidative metabolism is often considered minimal, and bacterial synthesis of FA is commonly expected to produce a net positive flow of FA through the rumen. Low absorption and metabolism of FA from the rumen was first concluded with minimal plasma recovery of radioisotope labeled linoleic acid infused into the rumen while diverting nutrients with a reentrant cannula (Jenkins, 1993). Ruminant bacteria contain 10-15% lipid on a DM basis. These fats originate from preformed FA uptake and *de novo* synthesis (Jenkins, 1993). Rumen bacteria and protozoa readily incorporate dietary FA into their cellular membranes, and increased availability of exogenous FA decreases endogenous synthesis (Palmquist and Jenkins, 1980). Wu and Palmquist (1991) reported synthesis of 6.6 mg of FA per g of non-lipid diet during 24 h *in vitro* incubations; lipid synthesis was not affected by source of fat (calcium salts and TG) or addition of acetate or isoacids. Duodenal FA flow cannot be partitioned into dietary and microbial synthesized origins in simple digestion studies, and bacterial synthesis may hide possible

oxidation and absorption of FA from the rumen. In contrast to the net positive FA flow expected, Jenkins (1993) observed that 15 out of 47 published treatment means reported a loss of FA from the rumen. Regression analysis predicted an 8 percent loss of lipid intake and showed up to 30 percent lipid loss in the dataset (Jenkins, 1993). Ferlay et al. (1993) reported a 14% increase in FA flow with control diet and 36.7 and 21.3% rumen FA loss with rapeseed FA fed as calcium salts and triglycerides, respectively. Loss of FA in the rumen may be due to flow marker error causing under-prediction of duodenal flow. However, Doreau and Chilliard (1997) proposed that negative FA flux observed through the rumen is not because of flow marker bias, but caused by absorption and oxidation of FA especially with higher fat diets. Fatty acid oxidation was observed by rumen epithelium *in vitro*, and bacteria adhering to the rumen wall can absorb oxygen from the epithelial cells and are capable of oxidative metabolism (Doreau and Chilliard, 1997). The authors proposed that higher fat diets experience greater loss of FA and hypothesized that FA are less adsorbed to feed particles in high fat diets leading to increased contact with the rumen wall and increased opportunity for absorption and oxidation. The increasing occurrence of rumen FA loss reported in digestion studies merits investigation of rumen FA metabolism that has been ignored as technical bias.

Dietary fat can alter microbial growth and have profound associative effects on ruminal nutrient digestibility. Chalupa et al. (1984) observed that unsaturated FA inhibited fermentation, but saturated FA had no effect. Fat supplementation has variable effects on ruminal digestion, but normally fiber digestion is decreased, and nonstructural carbohydrate digestion is not changed (Jenkins, 1993). Total tract digestibility is

normally not affected by fat supplementation because of compensatory digestion in the lower tract (Merchen et al., 1997).

Devendra and Lewis (1974) reviewed four theories for fat mediated depression of fiber digestion including: 1. physical coating of fiber preventing microbial attachment, 2. modification of rumen microbial population due to toxic effects, 3. inhibition of microbial activity due to coating of bacterial cell surface, and 4. reduction in cation availability for microbes from formation of salts with long-chain FA. The authors preferred the physical coating of fiber theory. In contrast, Palmquist and Jenkins (1980) concluded that most data support inhibitory effects on microbial activity, which changes bacterial competitiveness and shifts the microbial population, especially causing a decrease in protozoa and cellulytic bacteria. Jenkins (1993) attributed the variable effects of fat on fiber digestion to the structure of the lipid including degree of saturation and presence of a free carboxyl group. Free FA can directly inhibit microbial growth through disruption of membrane function (Jenkins, 1993). Unsaturated FA are more toxic than saturated FA, possibly because of increased FA absorption, detrimental effects of biohydrogenation, or disruption of cellular membrane function (Jenkins, 1993).

Inhibitory effects of FA on fiber digestion can be partially alleviated. Addition of metal cations (ex. Ca) increases formation of insoluble salts, blocking FA absorption and inhibition of microbial growth (Palmquist and Jenkins, 1980). Increasing saturation and chain length of the FA increases the amount and strength of salt formed (Jenkins and Palmquist, 1982). The formation of the metal salts is determined by the binding affinity of the cation and the dissociation constant of the FA. Fatty acid binding to metal cations is partially dependent on pH of the rumen and the pK_a of the FA. Sukhija and Palmquist

(1990) determined the pK_a for calcium salts of stearate, tallow, palm FA and soy oil to be 4.5, 4.5, 4.6 and 5.6 respectively. These pK_a values are misleading because they are determined for a FA mixture. Soy oil contains a much higher concentration of unsaturated FA than the other treatments and demonstrates the high pK_a of unsaturated FA. Finally, addition of other feed particles, particularly fiber, decreases detrimental effects presumably through increased competition in FA absorption (Doreau and Chilliard, 1997).

Ruminal lipid metabolism changes FA profile reaching the duodenum. The profile of long-chain FA absorbed in the small intestine is the combined result of the FA fed and ruminal biohydrogenation. Duodenal FA are more saturated than dietary FA, and include many FA isomers from incomplete biohydrogenation, and odd-carbon and branch-chain FA from microbial synthesis.

Sources of Dietary Fat

Diets of dairy cows contain four appreciable sources of FA including forages, grains, oilseeds, and fat supplements. The sources vary in FA form and type and have different effects in the rumen (Allen, 2000).

The large dietary proportion of forage fed makes it a significant source of lipid, even though forage contains a small concentration of FA (1-4%). This is especially true in low total fat diets. Forage lipids are found predominantly in the plant leaf, mostly in the form of glycolipids and some as phospholipids (Harfoot, 1981). The high concentration of glycolipids may cause an overestimation of the energy value because glycolipids have less energy than estimated by the 2.25 factor used in calculation of total

digestible nutrients (TDN; Van Soest, 1996). Vegetative FA composition is highly unsaturated, normally containing over 70% linoleic and linolenic acid. Doreau and Chilliard (1997) found it interesting that plant FA are only slightly less hydrogenated in the rumen than free oils. High efficiency of forage lipid hydrogenation may not be surprising since forage lipids are mostly associated with the leaf which is rapidly broken down in the rumen, especially with legumes. The FA are in complex lipids but are in close association with bacteria digesting the leaves. Furthermore, high concentrations of FA are present in the leaf chloroplast (Harfoot, 1981) that would be spilled when plant cells are ruptured.

Grain supplements vary in their FA concentration, profile and availability. Corn grain FA content varies with variety including specially bred high-oil corn. Although corn FA content is low, it can contribute considerably to FA intake when fed at high inclusion rates. Grain byproducts can also provide a considerable amount of lipid. Soybean meal retains residual FA after extraction and many corn grain byproducts contain considerable concentrations of FA including corn distillers grain (10% EE, NRC, 2001). Fat availability is expected to depend on association of FA with grain components, particle size and processing methods. The saturation, esterification, and rate of rumen availability of grain FA is expected to vary considerably.

Oilseeds contain a high concentration of lipid in the form of triglycerides and can increase the dietary lipid concentration even at low inclusion rates (2-12% of DM). Cottonseed, soybean, canola, and rapeseed are common oilseeds fed to dairy cows depending on location, availability, and price. Oilseed lipid contains high concentrations of unsaturated fat (50-90% of FA) mostly as oleic and linoleic acid with low

concentrations of linolenic, although there are large differences in the oleic to linoleic acid ratios between oilseeds (Van Soest, 1996). Most oilseed FA are in the form of triglycerides and must be released by lipolysis before biohydrogenation, as previously discussed. The triglycerides are contained within the seed coat and are adsorbed to the seed components. The seed must be mechanically broken down by chewing and microbial digestion to release the triglycerides. Triglyceride release is predicted from the digestion rate (inherent feed characteristics and fermentation) and passage rate from the rumen.

Although oilseeds contain very high concentrations of unsaturated FA, processing method determines the extent of FA biohydrogenation and interference with fermentation. Grinding, extruding, roasting, flaking, formaldehyde coating, and hydroxide treatment change FA availability in the rumen. The processing challenge is to slow the rate of unsaturated FA availability in the rumen while not impeding FA digestion in the small intestine. *In vitro* fermentation observed decreased lipolysis and increased fiber digestion with whole and roasted oilseed compared to free oil (Reddy et al., 1994). Roasting decreased FA availability linearly with increasing roasting temperature and extrusion had little protective effect presumably caused by the release of oil from cells during processing (Reddy et al., 1994). In agreement, *in vivo* comparison of extruded, ground, and roasted soybeans concluded that FA were protected the least with extrusion and the greatest with roasting (Chouinard et al., 1997). Tice et al. (1994) observed no difference in reducing particle size of roasted soybeans on rumen biohydrogenation; biohydrogenation of roasted soybean C18 unsaturated FA ranged from 52.1 to 60.1% and was not different from calcium salts of palm oil control (57.6%).

Whole rapeseed and canola seed provide excellent rumen protection of FA but are also indigestible in the small intestine. Grinding rapeseed and canola seed increases intestinal digestibility but dramatically increases rumen FA availability, while chemical treatment to weaken the seed coat increases intestinal digestibility while maintaining rumen protection (Hussein et al., 1996). Whole cottonseed was moderately biohydrogenated (56.6-67.1% of unsaturated C18 FA), maintained total tract FA digestibility (62.5-70.9%), and had no detrimental effect on fiber digestion and microbial fermentation when fed at different concentrations in diets differing in fermentability (Harvatine et al., 2002).

Animal and vegetable fat by-products provide an economical source of FA for dairy rations. These fat sources vary in their rumen activity and can be processed to increase saturation or decrease esterification. Fatty acids that do not interfere with rumen fermentation are considered rumen inert, protected, or bypass. Protected fat sources do not interfere with rumen fermentation as their characteristics make FA unavailable to microorganisms and prevent coating of feed particles. Highly saturated FA are naturally protected in the rumen considering their low interference with fermentation (Chalupa et al., 1984), presumably because of their high melting point, hydrophobia, and limited disturbance of cellular membranes. Esterification may partially decrease FA availability and protect microbial fermentation when lipolysis limits FA availability. *In vitro*, fermentation is not inhibited by esterified FA (Chalupa et al., 1984), but results may differ depending on hydrolysis rate, which may be affected by bacterial population, rumen environment and triglyceride saturation. Vegetable oil and animal fat are mostly esterified FA with varying FA profile depending on source. The degree of saturation of esterified fat has variable effects on rumen fermentation and digestibility. Saturation of

tallow normally does not change rumen fermentation in dairy cows (Pantoja et al., 1995; Pantoja et al., 1996), although decreased DM and fiber digestibility have been observed in steers (Elliott et al., 1997)

Dietary rumen available FA concentrations above 5% DM are generally considered detrimental to rumen fermentation. Protected products have been developed including encapsulated, calcium soaps, prilled and hydrogenated, and amide bonded FA (Jenkins, 1993). Rumen protected fat serves the dual purpose of protecting rumen fermentation against the detrimental effects of FA, and protecting unsaturated FA from biohydrogenation.

The first protection method was developed over 30 years ago and included encapsulation of oil by formaldehyde-treated protein that was indigestible in the rumen, but was digestible in the abomasum (Doreau and Chilliard, 1997). The method was only partially effective with challenges in manufacturing and breakdown during mastication (Doreau and Chilliard, 1997).

Calcium soaps of long-chain FA are a common, commercially available product used as a source of protected unsaturated FA. Calcium ions are bound to the free carboxyl groups of FA rendering them unavailable for biohydrogenation. Calcium salts of palm oil seem to have no effect on rumen fiber digestion although biohydrogenation of the FA may be extensive (Doreau and Chilliard, 1997). Wu et al. (1991) and Wu and Palmquist (1991) reported *in vivo* and *in vitro* hydrogenation of calcium salts of palm oil at 57 and 47% of unsaturated FA respectively. As previously presented, pH has a large effect on the dissociation of the metal salts, and increasing FA saturation increases cation binding (Sukhija and Palmquist, 1990). The large postprandial drop in pH is expected to result in

dissociation of the calcium complex, especially from highly unsaturated FA as demonstrated *in vitro* (Doreau and Chilliard, 1997). Sakhija and Palmquist (1990) determined 10% dissociation of calcium salts of palm oil and tallow at pH 5.5. They also determined that soy oil, which contains a high concentration of unsaturated fat, had a pK_a of 5.5, much higher than the 4.5 pK_a of the more highly saturated palm FA (Sukhija and Palmquist, 1990). In a mixed blend, unsaturated FA will dissociate more than saturated FA as pH decreases because unsaturated FA have a higher pK_a . Fatty acids may reattach to calcium when rumen pH increases after the postprandial drop, but can be biohydrogenated while dissociated.

Saturated FA are considered inert in the rumen as their chemical characteristics limit their dispersion in the rumen and decrease their direct contact with bacteria. Saturated FA also have limited physiological influence on bacteria because they do not interfere with membrane integrity and are not easily absorbed because of their hydrophobic nature. Prilling and hydrogenating fat takes advantage of this characteristic by simply saturating fat sources. The process results in very saturated free FA that do not interfere with rumen fermentation and digestion of DM and fiber (Elliott et al., 1994; Elliott et al., 1997).

The most recently developed technique to create rumen protected fat binds FA to primary amines producing fatty acyl amides (Fotouhi and Jenkins, 1992; and Jenkins et al, 1996). This process protects FA by blocking the carboxyl group much the same as metal cations. Fatty acyl amides provide exceptional rumen protection with a 0.4%/h rate of biohydrogenation compared to 4.7%/h for free FA (Fotouhi and Jenkins, 1992), and either do not affect or increase total tract digestion of DM and fiber (Jenkins, 1999; and

DeLuca and Jenkins, 2000). The goal of manufactured fat products is to protect the FA in the rumen to prevent interference with fermentation, but still deliver highly digestible FA to the small intestine.

Metabolic Utilization of Absorbed Fatty Acids

Dietary FA are used as a concentrated source of energy. Early nutritionists recognized the increased energy value of fat, assigning it a physiologic fuel value 2.25 times that of protein and carbohydrates (Stipanuk, 2000). This is the result of increased efficiency during digestion, oxidation, and tissue deposition. Although some rumen loss of FA has been reported, FA are not extensively destroyed by fermentation in the rumen. Whereas, fermentation of dietary carbohydrates results in a large loss of energy from maintenance and growth of bacteria and methane production. Fatty acid digestion in the small intestine results in roughly 80% absorption of available FA (Drackley, 2000). Biochemically, FA contain a large amount of free energy stored in carbon bonds. The metabolism of FA yields energy for maintenance and production through complete oxidation or partial oxidation and ketogenesis. Finally, transferring dietary fat to product is very efficient as preformed FA can be directly deposited in adipose or milk and do not have to enter synthesis pathways that result in energy loss.

Biological systems are engineered to use fat for insulation, cushioning, cellular structure, long-term storage of energy, and production of second messengers. Animals can synthesize FA *de novo* from nutrients such as protein and glucose. However, the ability to produce unsaturated FA are limited. Mammals can desaturate FA but cannot place a double bond within nine carbons from the methyl end of the FA. This prohibits

the synthesis of FA with double bonds in the ω -6 and ω -3 positions that are required for normal formation of cellular membranes and synthesis of key regulatory molecules such as prostaglandins (Sardesai, 1992). Many of the required FA can be synthesized by elongation and desaturation of linoleic and linolenic acid. In addition, unsaturated fat and more specifically poly-unsaturated FA (PUFA) help maintain the fluidity of cellular membranes (Sardesai, 1992). Increasing unsaturated FA increases membrane fluidity because of their lower melting point. Changes in fluidity can affect membrane integration and movement of proteins such as receptors and transporters (Drackley, 2000). These changes affect the activity and efficiency of membrane transporters, enzymes and receptors.

Fatty Acid Requirements

The many roles of FA and their bioactivity complicate the determination of dietary FA requirements. This highlights the ambiguous nature of defining nutrient and animal requirements. The terms dispensable and indispensable are used to categorize amino acids. Reeds (2000) discussed application of these categorizes in protein metabolism and highlighted dependence on their definition that may change from a nutritional, metabolic or functional perspectives. Likewise, the same concept has been applied to FA, categorizing each as essential or nonessential based on the animals capacity to synthesize or conserve the required amounts (Cunnane, 2000). Cunnane (2000) proposed that the essential FA should be renamed “conditionally dispensable” due to adequate capacity to synthesize or conserve the essential long-chain polyunsaturates and their parent molecules.

Animal requirements are difficult to quantify as they may be defined as the substrate required for maintenance and sustained production, or nutrient concentrations that stimulate maximum production through changing physiology and metabolism. The first definition employs simple accounting and a factorial approach to first calculate expenditure in maintenance and production activities, and then determines required intake based on biochemical assumptions of efficiency and metabolic conversion. A FA requirement is thus the amount of the FA secreted in milk plus that retained in tissue and oxidized for energy. The second definition recognizes that absorbed nutrients change physiology and metabolism that determine animal response. Through this definition, FA requirement depends on the amount and profile of FA that directs nutrients to lactation and increases efficiency through gene regulation and endocrine stimulation. Recognizing the second level of complexity demands research into not only the energy value of dietary nutrients consumed, but also the physiological and metabolic effect of individual FA.

Linoleic and linolenic acid are required as sources of ω -6 and ω -3 FA that the cow cannot synthesize. These FA are normally considered essential and must be supplied in the diet (Drackley, 2000). Although it is easy to label these FA as essential, a requirement is much harder to define. The metabolic requirement for the essential FA has not been determined in the ruminant, but would be expected to be a function of FA oxidation and turnover, growth and production of message.

Essential FA have been a subject of conversation in ruminant nutrition for many years. The discussion has centered on the ruminant's ability to absorb PUFA when their diet is low in fat and the rumen actively biohydrogenates PUFA. Although essential FA flow to the duodenum is severely limited, there are no reports of FA deficiency in adult

ruminants. Mattos and Palmquist (1977) measured linoleic acid biohydrogenation and transfer to milk fat in cows fed a high grain diet, and observed linoleic acid available at twice the requirement for female weanling rats on a metabolic body weight basis. In addition, ruminants may be adapted to sparing PUFA, preserving them for required purposes. Essential FA are less available for oxidation since they are highly incorporated into phospholipids and cholesteryl esters (Drackley, 2000). The slow turnover of phospholipids and cholesteryl esters pools ensure retention of the essential FA. Dietary FA are also incorporated into milk and tissue, however the efficiency of conversion of dietary unsaturated FA to milk is lower than saturated FA (Chilliard, 1993). A final conservation method for PUFA is lower oxidation. Reid and Husbands (1985) observed lower linoleic acid oxidation in cultured hepatocytes, and Linsay and Valerio (1975) showed a 25-40% lower oxidation rate for linoleic acid than stearic and palmitic acid. The essential FA may be more correctly labeled conditionally dispensable since the cow appears to have adapted adequate conservation methods and does not experience clinical deficiency.

Using the factorial approach it appears that essential FA are normally available in adequate concentrations, especially since there are no reported cases of deficiency. Adult ruminants have adapted their metabolism to work with limited PUFA, although there may be benefits to FA supplementation including improving reproductive efficiency, increasing energy balance, and modulating physiology.

Some nutritionists have proposed that essential FA limit maximum reproductive efficiency. Supplemental FA may increase reproductive efficiency through increased energy balance, increased progesterone synthesis, and increased or decreased

prostaglandin $F_{2\alpha}$ secretion, although results are inconsistent as reviewed by Grummer and Carroll (1991), Staples et al. (1998), and Williams and Stanko (1999). Dietary FA supplementation to increase reproductive efficiency is an example of an attempt to maximize production by increasing nutrient intake above the pure substrate requirement.

Energy balance has long been recognized as a major cause of anestrus and infertility as the cow directs nutrients to lactation. Anestrus and infertility in low energy balance may be mediated through low plasma leptin, insulin, or IGF-I concentration. Insulin and IGF-I are associated with increased follicular growth (Williams and Stanko, 2000), and leptin may mediate GnRH secretion through neuropeptide Y in the hypothalamus (Harris, 2000; and Spicer, 2001). The effect of dietary FA on energy intake and balance will be discussed in detail later. Plasma cholesterol is consistently increased with dietary fat supplementation, being linked to increased demand for lipid transport. Cholesterol is a precursor for progesterone synthesis in the corpus luteum and fat supplementation has been shown to increase blood plasma progesterone concentration (Staples et al., 1998). Progesterone synthesis may not be controlled by substrate availability as maximum synthesis of progesterone was reached *in vitro* at a much lower cholesterol concentration than normally observed in blood (Carroll et al., 1992). In addition, Rabiee et al. (1999) observed no relationship between ovarian uptake of cholesterol and progesterone release; failing to implicate cholesterol availability as a limitation of the steroids production. Increased plasma progesterone concentration is expected to increase maintenance of pregnancy especially during early gestation. Increasing unsaturated FA is proposed to increase prostaglandin- $F_{2\alpha}$ secretion through substrate availability, or decrease secretion through direct synthesis inhibition. As

previously mentioned, the essential FA are used to produce the precursors of PGF_{2α} and the availability of the FA may limit synthesis during early lactation when PGF_{2α} is required for termination of anestrous and maintaining normal estrus cycles. Linoleic acid has also been shown to inhibit PGF_{2α} secretion both *in vitro* and *in vivo* possibly through competitive inhibition of arachidonic acid at a key synthesis enzyme (Staples et al., 1998). Inhibition of PGF_{2α} is expected to protect implanted embryos that are not recognized by the dam and decrease fetal mortality in the first 28 d of gestation. It has been proposed that the concentration of the FA reaching the target tissues dictates the stimulatory or inhibitory response of PGF_{2α} (Staples et al., 1998).

The reproductive benefits of FA supplementation are normally associated with unsaturated FA, although reproductive benefits are observed across all fat sources (Staples et al., 1998). Biohydrogenation is normally not measured in reproductive trials, and the oilseed, tallow, and palm oil treatments used are expected to provide limited polyunsaturated FA to the duodenum. The metabolic hormone, energy balance and cholesterol mechanisms proposed to increase reproduction are not specific for PUFA. Energy balance is not consistently increased with fat supplementation because of decreased dry matter intake, especially with higher unsaturated FA concentrations (Allen, 2000), and increased milk yield in some experiments (Chilliard, 1993). Cholesterol was increased with duodenal FA infusion with no effect of FA type (Drackley et al., 1992; Christensen et al., 1994; and Bremmer et al., 1998). Increased reproductive efficiency through stimulating or attenuating PGF_{2α} has a number of practical limitations. First PUFA are highly biohydrogenated in the rumen, even when initially complexed as calcium soaps. Secondly, if unsaturated FA was capable of both increasing and

decreasing $\text{PGF}_{2\alpha}$ secretion dependent on plasma concentration we would expect to observe variable effects (increased or decreased) on reproductive efficiency with essential FA supplementation. Logically, additional essential FA supplementation cannot be utilized to both increase $\text{PGF}_{2\alpha}$ secretion to minimize anestrus and maintain normal estrus cycles, and decrease $\text{PGF}_{2\alpha}$ secretion to protect newly implanted embryos. All animals have an indispensable requirement for energy to maintain homeostasis, repair tissue, and grow, lactate and reproduce. The energy requirement of the high producing dairy cow is extreme with the large demand of lactation. In many cases cows cannot consume enough dietary carbohydrate to meet their energy requirement, and increasing diet energy density through increasing fermentability leads to rumen acidosis (Allen, 1997). Dietary FA are over twice as energy dense as other dietary components. Substitution of dietary fat for starch may make room in the diet for other nutrients such as protein and fiber. Decreasing starch and increasing fiber is expected to increase rumination and stabilize rumen fermentation (Allen, 1997). In this situation, dietary fat might allow greater production if DMI is maintained

Fatty acids can regulate physiology and metabolism through modification of metabolic hormones, tissue sensitivity, and gene expression (Drackley, 2000). Dietary FA stimulate secretion of a number of gut peptides including gastric inhibitory peptide (GIP), glucagon-like-peptide-1 (GLP-1), and cholecystokinin [CCK (Dawson et al., 1999; Meier et al., 2002; and Reidelberger, 1994)]. These hormones have large impacts on insulin secretion and glucose disposal after a meal (van der Burg et al., 1995). Fatty acids also directly stimulate pancreatic insulin secretion, with longer chain and more saturated FA having greater effects (Gravena et al., 2002). At the tissue level,

unsaturated FA increases insulin sensitivity in rats (Clarke, 2000). Palmquist and Moser (1981) observed decreased insulin sensitivity in cows fed calcium salts of palm oil compared to no fat control. Fatty acid saturation may have differential effects on insulin signaling between cows and rodents, or palm oil may not contain a high enough concentration of unsaturated FA to increase insulin sensitivity. Polyunsaturated FA have a clear impact on glycolytic, lipolytic, and lipid oxidation enzyme gene regulation in rodent models (Sessler and Ntambi, 1998; and Raclot and Oudart, 1999). A high PUFA diet results in preferential partitioning of ingested energy towards oxidation at the expense of storage (Raclot and Oudart, 1999). The interaction of metabolic regulation and FA metabolism is demonstrated in leptin repression of stearoyl-CoA desaturase-1 in the liver that is important in leptin mediated weight loss (Cohen et al., 2002). Effect of fat type on endocrine response and gene expression in cattle has not been investigated.

Dietary FA concentration and profile are quite different from the rat to the ruminant, but FA appear to have an important role in mammalian metabolic regulation. Drackley (2000) identified FA regulation of physiology as “one of the most exciting current areas of research in lipid metabolism.” In the future we may select FA supplementation to manage metabolic states and increase product yield or efficiency.

Fatty Acid Digestibility

Fat supplements must be efficiently digested and absorbed to benefit the cow. Fatty acid digestibility and the associative effects of FA on ruminal nutrient digestion are very important considerations in energy intake. Lipid complexes are hydrolyzed in the rumen delivering nonesterified FA to the small intestine, in contrast to the esterified FA

flow in non-ruminants. Abomasal acid secretion decreases digesta pH to less than 2.0, and greatly reduces the concentration of FA associated with metal cations. Pancreatic juice and bile entering in the duodenum are essential for digestion of any remaining triglycerides, formation of micelles, and absorption of FA (Drackley, 2000). Bile salts and lysolecithins aid in absorption of FA from feed particles and the formation of micelles that diffuse across the intestinal cell membrane in the jejunum (Doreau and Chilliard, 1997). Lipases are present in pancreatic secretion but have an optimal pH of 7.5 with little activity below 5.0 (Noble, 1981). The low pH of the duodenum prohibits lipase function and conditions for appreciable lipase activity are not expected until well into the jejunum, delaying digestion of triglycerides and decreasing the opportunity for FA absorption (Noble, 1981). Total tract digestibility of esterified FA is lower than unesterified FA (Elloitt et al., 1994; and Elloitt et al., 1999), and triglyceride digestibility decreases with increasing saturation (Pantoja et al., 1996; and Pantoja et al. 1995). Elliot et al. (1999) observed that highly saturated TG are more resistant to ruminal and intestinal lipolysis than unsaturated TG, leading to low digestibility of highly saturated TG. Finally, low intestinal pH is also expected to decrease the solubility of FA and bile salts, but may solubilize calcium soaps allowing their absorption.

Lower tract and total tract digestibility can be determined for total FA, but such measures for individual FA are meaningless due to the hydrogenation of unsaturated FA, and bacterial synthesis of FA (Merchen et al., 1997). Biohydrogenation and microbial FA synthesis, as discussed in reference to rumen digestion, also occur in the large intestine, but to a lesser extent (Bock et al., 1991; Ferlay et al., 1993; and Elliot et al., 1999). Individual FA digestibility can be measured through experimental treatments

differing in FA profile and with duodenally and ileally cannulated cows. Nutritionists have attempted to assign digestibility values to individual FA in a mixed FA diet (Moate et al., 2000). This is a misrepresentation of the data as the recovered saturated FA pool is inflated and unsaturated FA pool is decreased by hydrogenation in the rumen and hindgut and oxidation in sample preparation and handling (Palmquist, personal communication). There are very few direct comparisons of rumen protected saturated and unsaturated FA digestibility, because of the cost and complexity of multiple cannulation experiments necessary to make such comparisons. Christensen et al. (1994) and Bremmer et al. (1998) measured digestibility of abomasally infused free FA and observed no difference between saturated and unsaturated fat treatments. Also, Schauff and Clark (1989), Grummer et al. (1988) and Palmquist (1991) directly compared calcium salts of palm oil and prilled FA, finding no difference in total tract digestibility of energy, lipid and FA. Doreau and Chilliard (1997) summarized 64 treatment groups reporting FA digestibility in the small intestine or the lower tract, finding significant differences across chain lengths (although C16 and C18 did not differ), and observed only slight differences between lower tract saturated and unsaturated C18 FA digestibility (77, 85, 83 and 76% for 0, 1, 2 and 3 double bonds respectively). The small differences in C18 FA digestion are at least partially attributed to biohydrogenation of unsaturated FA in the large intestine because the majority of the data set measured FA disappearance across both the small and large intestine.

Dietary Fatty Acid Effects on Intake

Fatty acid supplements increase the energy density of the diet, but daily energy intake depends on energy concentration and dry matter intake. Intake is highly regulated by animal nutrient requirements and metabolic state, and also by the type and temporal pattern of fuels absorbed (Allen, 2000). Fatty acid supplementation can cause hypophagia, and fat source, form and type are significant predictors of intake response (Allen, 2000). Within commonly fed rumen protected FA sources, calcium salts of palm oil linearly decreased intake with increasing dietary concentration while saturated FA had no effect on intake (Allen, 2000). Benson et al. (2001) summarized 11 infusion studies representing 26 treatment groups showing intake depression with all but two treatments; regression analysis revealed a negative relationship between infused C18:1 and C18:2 FA concentration and intake, with C18:2 creating greater intake depression. Abomasal infusions of unsaturated FA with a lower C16:C18 FA ratio decreased DMI and digestible energy intake (Drackley et al., 1992), and DMI and gross energy intake (Christensen et al. 1994). Bremmer et al. (1998) demonstrated a negative relationship between intake and unsaturated FA with the same C16:C18 FA ratio. Experiments feeding free oil versus protected unsaturated FA provide comparison of saturated and unsaturated FA treatments because free oil is highly biohydrogenated in the rumen. Oleamide consistently decreased intake compared to free oil and linearly decreased intake with increasing inclusion rate (Jenkins, 2000; Jenkins et al., 2000; and DeLuca and Jenkins 2000). Finally, four-day continuous intravenous infusion of both palmitic and oleic acid significantly decreased intake, while stearic acid only numerically decreased intake (Vandermeersch-Doize and Paquay, 1984).

Regulation of Intake

Fatty acid profile reaching the duodenum determines hypophagia, and FA saturation may affect intake through a number of modes including: metabolic fate of nutrients, metabolic hormones, and gut peptides. The goal of intake regulation is balancing short-term and long-term nutrient supply and demand. Numerous tissues are proposed to be involved in intake regulation including splanchnic, hepatic, and adipose tissue. The gut and liver are well positioned to first detect absorbed nutrients, and adipose tissue is well positioned to detect long-term energy balance.

Koopmans (1995) proposed that intake is regulated by internal signals arising from the small intestine and the metabolic effect of absorbed nutrients, with differences between nutrients and metabolic states. In the rodent model, intake compensation for intravenously infused energy depends on the nutrient, with a 55% compensatory reduction in energy intake for infused glucose energy, 103% for amino acid energy, and 41% for lipid (Walls and Koopmans, 1992). In addition, intake recovered rapidly following glucose and amino acid treatments, returning to baseline within 2 d after infusion, while DMI recovery following lipid treatment and incomplete (Walls and Koopmans, 1992). Intravenous and intragastric infusions were used to explore the interaction of the gut in intake regulation. Intragastric infusion decreased energy intake 15% more than intravenous nutrient infusion (Burggraf et al., 1997). The interaction of absorbed nutrients and gut signals was shown with intravenous nutrient infusion during fed and fasted states. Energy intake decreased to compensate for intravenous nutrient infusion during the dark cycle while rats actively ate (99% energy intake compensation),

but daily intake failed to decrease to compensate for energy infused during the light period when rats were fasted [56% energy intake compensation (Walls et al., 1991)]. The authors concluded that intake response to absorbed nutrients depends on a threshold level of gut hormones (Walls et al., 1991). Nutrient infusion during a fed state may decrease intake because endogenous gut peptide secretion has primed the regulatory system to respond to absorbed nutrients. Without gut peptide secretion, intake regulatory mechanisms might fail to respond to absorbed nutrients.

Parabiotic cross-intestinal rats are created by sewing together the skin and muscle along the flank, creating one peritoneal cavity (Koopmans et al., 1997). Connection of skin and muscle creates a slow exchange of blood (1%/min), with full blood exchange expected in 3-4 h (Koopmans et al., 1997). Intestinal crossing was achieved by splicing a 30 cm segment of the lower duodenum and upper jejunum of one rat's intestine into the intestine of its partner without severing nerves or blood supply. The rat losing nutrients through absorption in its partner's intestine increased intake by 50% and the rat gaining nutrients decreased intake by 50% (Koopmans, 1991). Intake adjustment was quick and repeatable, and body weight of rats was not different at the end of the experiments (Koopmans, 1991). Plasma glucose, insulin, and glucagon did not vary with food intake across rats, but plasma lactate was increased in the low intake rat and decreased in the high intake rat (Koopmans, 1997). With a slow turnover of blood, transfer of metabolites is expected to create long-term energy balance in the pair, but temporal signals of nutrient absorption and short half-life hormones such as the gut peptides (CCK, GIP, and GLP-1) would be blocked. The large difference in intake between the paired rats with the same energy balance emphasizes the importance of temporal variation in nutrient absorption.

Further research using different dietary treatments for each rat in the pair may provide more insight into interaction of the gut and intake.

Satiety and the Gastrointestinal Tract

The gastrointestinal tract serves as an endocrine gland to change gut motility, stimulate digestive secretions, and regulate intake. The gastrointestinal tract has the first opportunity to sense the energy density and amount of food ingested. Four important hormones released in response to a meal are CCK, GLP-1, glucagon-like-peptide-2 (GLP-2), and gastric inhibitory peptide (GIP). CCK is a well-researched peptide that decreases gut motility and stimulates satiety in other species (Reidelberger, 1994). GLP-1, GLP-2, GIP are also involved in prandial endocrine response and stimulation of satiety (Dawson et al., 1999; Meier et al., 2002; and Burrin et al., 2003)

In ruminants, dry matter intake was decreased and postprandial CCK was increased when diets were supplemented with calcium salts of palm oil (Choi and Palmquist, 1996). Direct intravenous infusions of CCK depressed reticular-rumen motility and intake in sheep (Grovum, 1981). Finally, Nicholson and Omer (1983) showed that intestinal infusion of unsaturated FA decreased rumen motility of sheep. These observations are consistent with CCK decreasing gut motility and centrally signaling satiety (Reidelberger, 1994). Recently, Benson and Reynolds (2001) tested the effect of unsaturated fat (rapeseed oil) infusion on gut peptide secretion, observing increased plasma GLP-1 without an increase in CCK, although CCK was pooled over time eliminating observation of temporal variation. Rapeseed infusion had no effect on feeding or chewing behavior, although a large reduction in time spent ruminating was

noted for some cows (Benson and Reynolds, 2001). Experiments testing gut peptide response to FA infusion have only tested unsaturated FA and low FA controls, little data is available reporting the effect of saturated FA on endocrine signaling and rumen motility. Gut peptides may act centrally to stimulate satiety or may change rumen motility and passage rate, increasing gut fill and stimulating tension receptors.

Satiety and Absorbed Nutrients

Metabolic fate of nutrients can regulate intake through the site, rate and timing of oxidation. Hepatic oxidation of FA has been demonstrated to decrease intake in the rat (Langhans and Scharrer, 1987; Scharrer and Langans, 1989; and Friedman et al., 1999). The location and function of the liver as a central site of fuel storage and metabolic conversion makes it an ideal site to assess fuel utilization, and communicate this signal to the brain via afferent nerves (Langhans, 1995). The hepatic oxidation theory of satiety proposes that oxidation of nutrients increases hepatocyte intracellular ATP that affects the firing rate of the hepatic vagus; the exact mechanism by which hepatocytes interact with the hepatic vagus has not been elucidated (Langhans, 1995). Emery et al. (1992) proposed that uptake and oxidation of FA by the liver might depress intake particularly in early lactation. Palmquist (1994) and Allen (2000) found hepatic oxidation regulation of intake appealing and defensible, however it has not been proven in the ruminant.

The rate of FA oxidation would depend on FA uptake by the liver, and enzyme concentration and activity. Emery et al. (1992) reported hepatic NEFA uptake as a constant proportion to blood concentration, and reviewed malonyl CoA and propionic acid inhibition of oxidation and ketogenesis. In nonruminants, PUFA are preferentially

oxidized and saturated FA appear to be directed towards fat storage (Storlein, 2000). The essential PUFA are conserved in the ruminant since they are highly associated with phospholipid pools and have low reported oxidation rates. Substantial intakes of PUFA are not expected even with protected sources, but high levels of mono-unsaturated FA including trans- FA are absorbed. The oxidation rates of these FA have not been studied. Dietary unsaturated FA may change liver oxidation and phosphorylation potential by changing rate of gluconeogenesis, PUFA affect gene regulation of metabolic enzymes, and FA have a large effect on insulin signaling through gut peptides and directly in the pancreas.

Satiety and Energy Balance

Fatty acids may change energy balance through increased digestibility and metabolic efficiency, or decreased milk fat production. Chemostatic regulation of intake may feedback to decrease intake through leptin stimulation of satiety in the paraventricular nucleus (Ingvarsen and Bosclair, 2001). Energy balance is expected to be an important factor in intake response to fat supplementation, as long-term intake of fat should increase energy balance and thus decrease intake. Fatty acid type may change partitioning of FA by preferential oxidation or storage of FA causing changes in leptin secretion. Increased oxidation of FA in the liver could also increase function of the somatotrophic axis, increasing IGF-1 production, and changing nutrient partitioning (Renaville et al., 2002). Recent research has identified trans-FA intermediates of rumen PUFA biohydrogenation as key regulators of milk fat synthesis (Bauman and Griinari, 2003). Infusion of trans-10,cis-12 CLA reduces milk fat by decreasing lipogenic enzyme

gene expression (Baumgard et al., 2002a). Tyrell and Moe (1972) observed decreased efficiency of ME utilization for milk synthesis during milk fat depression. Milk fat depression normally occurs with little effect on dry matter intake leading to increased body weight gain in proportion to milk fat depression (Baumgard et al., 2002b). Increased body weight gain in response to trans-10,cis12 CLA in lactating cows is contrary to decreased body fat gain observed in growing animals (Mersmann, 2001). Milk fat depression of 25 to 50% resulted in no change in plasma glucose, insulin and leptin concentration or insulin stimulated glucose clearance, but did result in 24 to 33% reduced lipolytic response to an epinephrine challenge consistent with slightly increased energy balance (Baumgard et al., 2002a). Gaynor et al. (1996) observed no effect of abomasal infusion of cis or trans-C18:1 on disappearance rates of glucose, secretion of insulin after glucose challenge, and appearance rates of NEFA and triglycerides after norepinephrine challenge. Romo et al. (1996) measured energy metabolism during cis and trans-C18:1 FA infusion, observing increased production of milk energy for cis compared to trans-FA but failed to find differences in energy expenditure or tissue retention. Incomplete compensatory adjustment in energy intake resulting in increased body weight gain during milk fat depression represents a failure or shift in regulation of energy balance that cannot be attributed to homeostatic signaling or regulation of lipid and glucose metabolism.

Other Factors Stimulating Satiety

The rate of feed intake and rate of digesta passage out of the rumen create an important balance to maximize rumen digestion but not limit intake by physical fill.

Dietary lipids can have profound effects on rumen fermentation as previously presented. Changing microbial activity may decrease digestion rate of fiber and other feed components. Decreased digestion rate results in slower passage since feed particles must be reduced in size and buoyancy before they may pass out of the rumen (Allen, 1996). If dietary fat has a large impact on digestion and passage rate of fiber, ruminal fill will increase and stimulate tension receptors (Allen, 2000).

Isomers of unsaturated FA have profound biological activity as observed with CLA and milk fat depression (Bauman and Griinari, 2003). Feeding dietary fat yields a number of isomers from incomplete rumen biohydrogenation. The type and amount of isomer is related to the type of fat fed, ruminal fermentation and residence time. The effect of unsaturated FA type on animal physiology and metabolism may be mediated through very small quantities of certain FA isomers. Which may explain the variation observed in feeding experiments. Abomasal infusion of trans-C18:1 isomers numerically decreased intake compared to cis-C18:1 FA although there are a large number of candidate isomers that could depress intake including the trans-C18:1 isomers (Romo et al., 1996; and Romo et al., 2000). Trans-FA isomers may be involved in intake regulation, but satiety is consistently demonstrated with duodenal infusion of vegetable oils low in trans-FA isomers (Benson and Reynolds, 2001).

Forbes proposed that intake is not regulated by one mechanism, but is determined by the central integration of many signals (Forbes, 1996). The animal likely integrates these signals and adjusts intake to minimize physical and metabolic discomfort (Forbes, 2000). The possible mechanisms discussed included both short and long-term regulators, both of which probably play a role in FA induced intake depression.

CHAPTER 2

The effect of production level on feed intake, milk yield and plasma metabolite response to rumen protected fatty acid saturation in lactating cows.

ABSTRACT

Animal response to dietary treatment may interact with metabolic state, which differs for cows across a wide range of milk yield. Response to dietary saturated versus unsaturated rumen-protected fatty acids (FA) was evaluated using 31 multiparous Holstein cows arranged in a crossover design with 14 d periods. Cows averaged 43.7 kg milk (range 34.0-57.5 kg) for the 4 d immediately prior to initiation of the experiment when fed a diet intermediate in composition to the treatment diets. Treatments were 2.5% FA rumen-protected fat sources from unsaturated FA (UNS, calcium soaps of palm FA) or saturated FA (SAT, prilled hydrogenated free FA). UNS had decreased DMI (0.8 kg/d) and time spent ruminating (25 min/d) with no effect on time spent eating compared to SAT. No difference was observed between treatments for milk or 3.5% fat corrected milk (FCM) yield. Intake and milk yield responses were not related to milk yield across cows. SAT significantly increased milk protein and lactose concentration, but treatment did not affect yield of milk components. SAT increased insulin over 25% and decreased non-esterified FA nearly 20% with no effect on plasma bST, leptin, glucose or β -hydroxybutyrate. Milk protein concentration and yield responses to treatment were positively correlated with pretrial fat-corrected milk yield. Milk protein response was

negatively correlated to insulin response, signifying the importance of insulin sensitivity in control of milk protein synthesis. UNS decreased DM intake and rumination time compared to SAT, consistent with reports of unsaturated fat increasing satiety and decreasing gut motility. Depression of milk protein synthesis by fat supplementation may be related to FA saturation and milk yield of cows.

INTRODUCTION

Energy required for milk yield is often greater than the cow's ability to consume dietary energy, resulting in a negative or low energy balance. Addition of fat to the diet increases energy density without increasing rumen acid production, thus stabilizing rumen function relative to addition of grain. Prilled, hydrogenated free fatty acids (FA) and calcium salts of FA are two commercially available fat sources that have been designed to reduce adverse effects of rumen active FA on rumen microbial fermentation.

The ability of the cow to increase daily energy intake depends on the digestible energy density of the diet and daily dry matter intake (DMI). Intake is highly regulated by animal nutrient requirements and metabolic states, and also by the type and temporal absorption of fuels. Allen (2000) observed in a meta-analysis that fat supplements differing in FA source, form, and type have different hypophagic effects. Within commonly fed rumen protected FA sources, calcium salts of palm oil linearly decreased intake with increasing dietary concentration while hydrogenated FA had no effect on intake (Allen, 2000). The concept that FA of varying saturation reaching the duodenum have different hypophagic effects was demonstrated through a series of abomasal

infusion studies that showed decreasing FA saturation decreased feed intake (Drackley et al., 1992; Christensen et al., 1994; and Bremmer et al., 1998).

Dietary fat also has demonstrable effects on milk protein production (DePeters and Cant, 1992). Decreased milk protein yield and concentration by increased dietary fat might be caused by changes in rumen fermentation, endocrine signaling, milk yield or mammary nutrient metabolism (DePeters and Cant, 1992). Drackley et al. (1992) observed a linear decrease in milk crude protein yield with abomasal unsaturated FA infusion, and Christensen et al. (1994) observed decreased milk true protein and casein yield with C18 unsaturated FA infusion compared to saturated FA, indicating that FA profile has an important role in fat-stimulated milk protein depression.

Dietary energy density is often increased by addition of fat in an attempt to improve the energy balance of high producing cows that are unable to consume the required amount of forage and grain to meet energy requirements. It is expected that cows response to energy supplementation will depend on cow metabolic state or milk yield. Crossover design experiments with a pretrial covariant period have been used to study cow responses across production levels to evaluate low lignin corn silage (Oba and Allen, 1999) and forage to concentrate ratio (Voelker et al., 2002). Similarly, response to fat supplementation was expected to differ for cows varying in milk yield.

The objective of this experiment was to determine the relationship between production level and responses for feed intake, milk yield, and plasma hormones and metabolites to diets supplemented with saturated or unsaturated rumen-protected FA. We hypothesized that more highly unsaturated FA would decrease intake relative to saturated

FA at equal FA concentrations, and individual cow response would depend on milk yield.

MATERIALS AND METHODS

Cows and Treatments

Thirty-two multiparous Holstein cows (Table 1) in mid- to late lactation (144 ± 70 ; Mean $\pm$ SD) at the Michigan State University Dairy Cattle Teaching and Research Center were randomly assigned to sequence in a crossover design with a pretrial covariant period. Treatments were 2.5% added dietary FA from saturated FA (SAT) or unsaturated FA (UNS) sources (SAT- prilled hydrogenated free FA, Energy Booster 100[®], Milk Specialties Company Inc., Dundee, IL; UNS- calcium soaps of palm FA, Megalac[®], Church and Dwight Company, Inc., Princeton, NJ). Treatments were fed as a mix using ground corn as a carrier, and were balanced for calcium and FA concentration using limestone and rice hulls (Table 2). Covariant and treatment periods were 14 d in length with the first 10 d for diet adaptation followed by 4 d of sample collection. Diets contained alfalfa silage (~50% of forage DM), corn silage (~50% of forage DM), dry ground corn, whole linted cottonseed (12.5% of ration DM), protein mix (soybean meal, corn gluten meal, and blood meal), and mineral and vitamin premix (Table 3). The base diet contained ~5.0% FA with 2.0% FA from cottonseed. Milk yield averaged 43.7 kg/d and ranged from 34.0 to 57.5 kg/d during the 4 d immediately prior to the experiment, when cows were fed a diet intermediate to both treatments. Cows were housed in tie stalls throughout the experiment, except for a 1.5-hour exercise period twice daily prior to milking in a parlor. Samples and data were collected during the last 4 d of each period.

Experimental procedures were approved by the All University Committee on Animal Use and Care at Michigan State University.

Data and Sample Collection

Throughout the experiment, cows were fed once daily (1100 h) at 110% of expected intake. The amount of feed offered and orts were weighed for each cow daily during the collection period. Samples of all dietary ingredients (0.5 kg) and treatment diets were collected daily during the collection period and composited into one sample per period. Samples of orts (12.5%) were collected daily during the collection period and composited into one sample per cow period. Blood was collected from the coccygeal vessel into a tube containing sodium heparin. Six samples were collected over two days (d 10 and 11) represent 4-h intervals of a 24-h period to account for diurnal variation. Blood was centrifuged at 2,000 x g for 15 min immediately after sample collection, and plasma was harvested and frozen at -20°C until analysis. Cows were milked twice daily in the milking parlor throughout the experiment. Milk yield was measured and sampled at each milking from d 11 to d 14 and averaged over the period. Feeding behavior was recorded manually every five minutes for 24 h on d 14. Activity was classified as eating, ruminating, drinking, or idle. Cows were fed and milked as normal during feeding behavior observation. Body weight was recorded on the day prior to the start of the first period and on d 14 of each period to determine body weight change. On the same days, three trained investigators determined body condition score using a five-point scale (1 = thin, 5 = fat; Wildman et al., 1982).

Sample Analyses

Milk samples were analyzed for fat, true protein, and lactose by infrared spectroscopy at Michigan DHIA (East Lansing). Diet ingredients and orts were dried in a 55°C forced-air oven for 72 h. All samples were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Diet ingredients were analyzed for DM, NDF, ADF, lignin, starch, crude protein, ash, and FA concentration and profile. NDF concentrations were determined with the addition of heat-stable amylase (Van Soest et al., 1991; method A). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide. Crude protein was analyzed according to Hach et al. (1987). Ash content was determined after 6 h oxidation at 500 degree C in a muffle furnace. Total FA concentration and FA profile were analyzed by GLC (Sukhija and Palmquist, 1988). Concentrations of all nutrients except DM were expressed as percentages of DM determined from drying at 105 degree C in a forced-air oven.

Blood samples were analyzed for insulin, glucagon, somatotropin (**bST**), cholecystokinin (**CCK**), leptin, non-esterified fatty acids (**NEFA**), glucose and beta-hydroxybutyrate (**βHBA**). Commercial radioimmunoassay kits were used to determine plasma concentration of insulin (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA), glucagon (Glucagon kit GL-32K; Linco Research, St. Charles, MO), and CCK (Eurica-CCK kit RB30; ALPCO, Windham, NH). Plasma somatotropin and leptin concentration was determined by radioimmunoassays (Gaynor et al. 1995; and Ehrhardt et al. 2000). Enzymatic kits were used for determination of glucose (Glucose kit #510; Sigma Chemical Co., St. Louis, MO), NEFA (NEFA C-kit; Wako Chemicals USA,

Richmond, VA), and β -hydroxybutyrate (β HBA kit #310-A; Sigma Chemical Co., St. Louis, MO).

Net energy of body weight change and milk production was calculated according to NRC 2001.

Milk NE_L (Mcal/d) = MY (kg) x [0.0929 x (Fat %) + 0.0563 x (True Protein%) + 0.0395 x (Lactose%)]

Statistical Analyses

For treatment effects, all data were analyzed by the fit model procedure of JMP Version 5.0 (JMP, 2000) according to the following model:

$$Y_{ijkl} = \mu + S_i + C_j(S_i) + P_k + T_l + e_{ijkl}$$

where

μ = overall mean,

S_i = effect of sequence (I = 1 to 2),

$C_j(S_i)$ = effect of cow nested in sequence (j = 1 to 16),

P_k = effect of period (k = 1 to 2),

T_l = effect of treatment (l = 1 to 2),

e_{ijkl} = residual error.

Pretrial fat-corrected milk yield (**pFCMY**) was calculated as the average daily production over eight milkings during the 4 d immediately prior to the initiation of the experiment. Relationships between response to treatment and pFCMY were analyzed according to the following model:

$$Y_i = \mu + S_i + pM + pM^2 + e_i$$

Where

$$Y_i = y_{SAT} - y_{UNS}$$

y_{SAT} = response for the saturated FA treatment

y_{UNS} = response for the unsaturated FA treatment

μ = overall mean,

S_i = effect of sequence ($i = 1$ to 2),

pM = pFCMY

pM^2 = pFCMY²

e_i = residual error

Data points with Studentized Residuals greater than 3 were considered outliers and excluded from the data set. One cow was diagnosed with clinical mastitis in the first treatment period and was excluded from statistical analysis.

RESULTS AND DISCUSSION

Thirty-one cows completed the experiment and are profiled in Table 1. Cows ranged from 34.0 to 57.5 kg milk during the covariant period. Fat treatments differed in FA profile with UNS containing nearly 2.5 times more unsaturated FA than SAT, primarily as C18:1 and C18:2 (Table 2). Treatments also differed in C16:C18 FA ratio

because the UNS treatment contained high levels of palmitic acid. Diets contained nearly equal concentrations of starch, CP, and FA, but differed slightly in NDF concentration because of the addition of rice hulls in the UNS mix to equalize calcium concentrations across treatments (Table 3).

Intake and Chewing Behavior

UNS decreased DMI 0.8 kg/d relative to SAT ($P<0.01$). UNS decreased intake of starch, crude protein and total FA ($P<0.01$). In previous direct comparisons of saturated and unsaturated rumen protected FA, no differences were observed in intake when supplemented at 0.68 kg of FA (Grummer, 1988; and Schauff and Clark, 1989), and at 2 and 5% of the diet (Eastridge and Firkins, 1991). However, both of these studies had fewer observations, lower producing cows and lower basal dietary FA concentration compared to the present study. Allen (2000) reported that in 11 out of 24 studies calcium salts of palm oil caused a linear decrease in feed intake, while 22 of the 24 trials resulted in a numerical decrease in feed intake. In contrast, hydrogenated TG or FA resulted in decreased feed intake in only one study and increased feed intake in two out of 21 studies reported.

Heinrichs et al. (1982) observed that supplemented fat decreased the length and size of the initial meal but tended to increase the number of spontaneous meals. De Visser et al. (1982) reported smaller meals and a slower eating rate with concentrates containing added fat. Manual observation of feeding behavior, as used in this study, lacks sensitivity for accurate determination of meal length and time between meals compared to automated methods (Dado and Allen, 1993). Meals were identified and data was

analyzed for meal length and intermeal interval; no response variables were significantly different and the data is not presented in this paper.

Abomasal infusion of unsaturated fat consistently decreases feed intake relative to no fat and saturated fat infusions (Benson and Reynolds, 2001). Drackley et al. (1992) and Christensen et al. (1994) both showed abomasal infusions of 450g of unsaturated FA with a lower C16:C18 FA ratio decreased DM intake compared to an equal amount of more saturated FA, while Drackley et al. (1992) also reported decreased digestible energy intake. The experimental treatments in the current study differed in both unsaturated fat concentration and C16:C18 FA ratio. Bremmer et al. (1998) demonstrated the negative relationship between intake and diet unsaturated FA concentration at the same C16:C18 FA ratio in abomasal infusions. In addition, protected oleamide FA consistently linearly decreases intake compared to free oil that is readily biohydrogenated in the rumen (Jenkins, 1998; Jenkins, 2000; and DeLuca and Jenkins, 2000). The above experiments provide strong evidence that FA-mediated intake depression is a function of FA saturation, not chain length.

Rumen biohydrogenation of unsaturated FA can be extensive and may explain differences in the magnitude and consistency of response when unsaturated fat is fed versus directly infused into the abomasum. Klusemeyer and Clark (1990 and 1991) and Wu et al. (1991) reported rumen biohydrogenation of unsaturated C18 FA fed as calcium salts of palm oil at 34, 33 and 48% respectively. Unsaturated fat in the form of calcium salts must first dissociate from the calcium ion and then be absorbed by rumen bacteria and biohydrogenated. The rate of this process is expected to be a function of the pK_a for the calcium salt, rumen pH, and the microbial population, which affects absorption of FA

and enzyme activity for biohydrogenation. The extent of biohydrogenation of the UNS treatment in the current study is unknown. However, we expect that less unsaturated FA reached the duodenum than was fed, which demonstrates the powerful hypophagic effects of unsaturated FA.

There was no effect of treatment on time spent eating but UNS decreased time spent ruminating 25 min/d ($P < 0.01$) and increased time spent idle 25 min/d ($P < 0.01$). Total chewing time and time spent ruminating per kg of DMI was not different between treatments. This might be expected since cows consumed less feed for UNS treatment and would have less digesta to ruminate, which is supported by the lack of treatment effect for measures of time spent chewing per kg of DM consumed (Table 4). Although its regulation is poorly understood, time spent chewing is primarily related to dietary intake and concentration of fiber and forage fiber, and is poorly related to DMI (Allen, 1997). Total chewing time and time spent ruminating per kg of NDF intake was decreased by UNS ($P < 0.05$ and $P < 0.01$, respectively). Therefore, differences in feeding behavior observed in the current experiment cannot be attributed to differences in feed intake. Treatment diets also contained the same base ration and are not expected to differ in effectiveness of stimulating rumination, although associative effects on rumen fiber digestion and passage could have affected rumen digesta pool size, leading to changes in rumination.

Deswysen et al. (1987) reported a strong positive relationship between the number of rumen contractions and rumination time, decreased time spent ruminating may be indicative of less reticular-rumen motility. Nicholson and Omer (1983) showed that intestinal infusion of unsaturated FA decreased rumen motility of sheep. Grovum (1984)

reported almost total cessation of rumen motility after 13 h of intragastric infusion of unsaturated fat while intravenous infusion had little effect. Decreased intake and frequency of biphasic and triphasic rumen contractions were observed within 3 h of intragastric infusion of unsaturated FA (Grovum, 1984). Differences in gastric and venous infusions implicate involvement of the gut in FA depression of reticular-rumen motility. Dry matter intake was decreased and postprandial CCK was increased when diets were supplemented with calcium salts of palm oil (Choi and Palmquist, 1996), and direct intravenous infusions of CCK depressed reticular-rumen motility and intake in sheep (Grovum, 1981). These observations are consistent with CCK decreasing gut motility and centrally signaling satiety (Reidelberger, 1994). Benson and Reynolds (2001) observed increased plasma concentrations of glucagon-like-peptide-1 (**GLP-1**) with infusion of unsaturated fat (rapeseed oil). GLP-1 is a gut peptide with similar actions and secretion patterns of CCK (Hellstöm and Näslund, 2001). Benson and Reynolds did not observe a change in CCK secretion, although samples taken over time were pooled, and temporal variation could not be evaluated (Benson and Reynolds, 2001). These observations are consistent with gut peptide secretion responding to FA ingestion with subsequent effects on intake and gut motility (Reidelberger, 1994; and Hellstöm and Näslund, 2001).

Experiments testing the effect of FA on rumen motility and gut peptide secretion have used no fat controls. The effect of FA saturation on endocrine signaling and rumen motility has not been explored. Decreased rumination with UNS in the current experiment is consistent with FA saturation changing gut peptide secretion. Unsaturated FA may directly stimulate increased gut peptide secretion or change temporal release of

gut peptides relative to a meal, causing a decrease in gut motility. Absorption of FA and stimulation of gut peptide secretion likely coincide with rumination bouts between meals as the rumen creates a lag between intake of FA and their flow to the duodenum. Decreased reticulorumen motility may then decrease time spent ruminating. Decreased rumination and reticular motility may lead to increased distension from physical fill because of slower digestion and passage of digesta.

Dry matter intake response (SAT-UNS) was not related to pFCMY or pretrial fat yield. In addition, there was no relationship between pretrial parameters and chewing behavior responses (Table 6). Failure to detect a relationship of DMI with pFCMY discounts physical fill and absorbed energy as mechanisms of unsaturated FA induced hypophagia. Intake of high producing cows in a less positive energy balance is expected to be limited by physical fill, while intake of lower producing cows in a more positive energy balance is expected to be limited by absorbed fuels. Observed intake responses in the current experiment cannot be attributed entirely to either mechanism. Digestibility of saturated FA is commonly thought to be lower than unsaturated fat. However, FA digestibility cannot be determined by measuring digestibility of individual FA fed in a mixture because hindgut biohydrogenation inflates recovery of saturated FA at the expense of unsaturated fat, causing overestimation of unsaturated FA and underestimation of saturated free FA digestibility. Other experiments have concluded that the intake depression of unsaturated FA is not mediated by differences in digestibility when supplemented in FA form. The abomasal infusions previously discussed (Drackley et al. 1992; Christensen et al. 1994; and Bremmer et al. 1998) did not show differences in FA or energy digestibility when directly comparing saturated and unsaturated FA

treatments. Finally, Schauff and Clark (1989), Grummer (1988), and Palmquist (1991) directly compared calcium salts of palm FA and saturated free FA (same treatments as this experiment) and found no difference in apparent total tract digestibility of energy, lipid and FA, respectively.

Production

There were no treatment effects of FA type on yield of milk or milk components. Production response to supplemental fat is inconsistent across experiments. Chilliard (1993) reviewed the effect of fat supplementation and noted little difference in FCM in short-term experiments, presumably because of a 2-3 wk production lag observed in long-term fat studies. Experimental periods of 14 d, as used in the current experiment, may be too short to establish effects on milk yield.

Unsaturated FA treatment decreased milk protein and lactose concentration relative to SAT (protein 3.07% and 3.02%, and lactose 4.80% and 4.75% for SAT and UNS respectively). Response (SAT – UNS) of milk protein yield and concentration were positively related to milk yield (Figure 1, $P = 0.02$, $R = 0.18$; and Figure 2, $P < 0.05$, $R = 0.46$, respectively). High producing cows had larger milk protein yield responses when fed SAT compared to UNS than cows with lower milk yield. Response in individual cow FCM, milk fat yield, and fat percent were not related to pretrial production. The production level or metabolic state by response interaction that we observed for milk protein may explain the inconsistent reports of FA effects on milk protein synthesis. Non-responding cows in some experiments, but not in others, may simply dilute treatment effects or add unexplainable variation.

Decreased milk protein concentration is commonly attributed to the diluting effect of increased milk yield in some experiments. In the current experiment, there was no relationship between the response for milk protein percent (SAT – UNS) and the response for milk yield (SAT – UNS, $R^2 = 0.41$, $P = 0.23$). There was no dilution effect of milk protein observed because milk protein yield response linearly increased with increased milk yield response ($R^2 = 0.55$, $P < 0.001$). Although not observed in the current experiment, dilution of milk protein by increased milk production merits further investigation. Protein and lactose concentration of milk are both very stable and expected to be highly correlated; Wu and Huber (1994) reported a linear relationship between milk and milk protein yield with an R^2 of 0.90. Dilution of milk protein by increased milk yield would represent a deviation from normal and should not be ignored.

Emery (1972) reported that milk protein concentration decreased 0.1 to 0.3 percentage points with added fat, and DePeters and Cant (1992) reviewed the effect of fat on milk protein showing variation in published responses. Chilliard (1993) reviewed fat effect on production and noted that milk protein concentration decreased in response to fat supplementation to a greater extent in early lactation compared to peak lactation (0.8 vs. 0.5 g/kg) and in short-term experiments compared to long-term experiments (1.0 vs. 0.5 g/kg). In direct comparisons of dietary protected saturated and unsaturated FA, Grummer (1988) showed unsaturated FA decreased milk protein 0.13% compared to no fat control, and saturated fat treatment maintained milk protein. Schauff and Clark (1989) observed no effect of fat type on milk protein. Dietary FA saturation appears to be an important factor affecting milk protein response to FA treatment. Possible mechanisms

include inhibition of microbial protein production, modification of insulin signaling, and changes in the somatotrophic axis.

Unsaturated FA fed as calcium salts of palm oil are partially available for biohydrogenation, and may interfere with microbial growth rate or efficiency. Fatty acids may decrease milk protein because of decreased microbial protein yields and less protein absorbed and available for milk protein synthesis. However, feeding protected oleic acid in the form of oleamide decreased milk protein concentration and yield (Jenkins, 2000) compared to raw canola oil (high oleic acid). Free oil interferes with ruminal fermentation more than oleamide but had less of an effect on milk protein synthesis than the physiological effects of absorbed unsaturated FA.

Hyperinsulinemic-euglycemic clamp studies have identified insulin, or its stimulation of IGF, as a regulator of milk protein synthesis (McGuire et al., 1995; and Griinari et al., 1997). Increased milk protein synthesis in the clamp procedure is not solely the effect of infused glucose sparing amino acids because cows supported increased protein synthesis by increasing the extraction efficiency of essential amino acids, mammary blood flow, and glucose uptake (Mackle et al., 2000). In the present study, saturated fat increased insulin 25% as well as milk protein concentration and yield consistent with the insulin clamp model. Surprisingly, although milk protein response was related to pFCMY, insulin response (SAT – UNS) was not. More puzzling is that there was a significant negative linear relationship of milk protein response and insulin response (Figure 3). Cows with the greatest milk protein yield response (SAT – UNS) had the lowest insulin response between treatments. If insulin regulates milk protein synthesis, it is reasonable to expect that not just the plasma insulin concentration, but also

tissue sensitivity to insulin stimulation is important. Palmquist and Moser (1981) studied the relationship of dietary unsaturated fat, plasma glucose and insulin, and milk protein production. Glucose tolerance tests were used to measure insulin responsiveness and sensitivity. Cows fed calcium salts of palm oil responded to glucose infusion with more insulin secretion and had slower clearance of glucose, suggesting increased insulin resistance. The authors proposed that fat-stimulated insulin resistance may reduce amino acid transport into the mammary gland. The negative relationship between insulin and milk protein response observed in the current experiment supports the hypothesis that tissue insulin sensitivity is important to milk protein production.

Insulin stimulation of the somatotrophic axis cannot be ruled out as a possible mechanism for increasing milk protein synthesis. Molento et al. (2002) showed insulin stimulated IGF-1 production in early to mid lactation cows. They proposed that the bST to insulin ratio was an important predictor of IGF-1 production, with higher ratios correlating to higher IGF-1 concentrations. In the present study, there was a significant positive relationship between the bST/insulin response and milk protein response (Table 10), with increasing bST/insulin ratios (higher expected IGF-1 concentrations) linearly related to higher milk protein responses.

Energy Balance and Efficiency

Treatments did not affect body weight gain or body condition score changes ($P = 0.37$, $P = 0.74$, respectively). There was also no relationship between measures of energy balance and pretrial milk yield. Experimental periods were only 14 d in length, making measurement of body tissue changes difficult. The lack of effect on body weight, body

condition score, leptin, or bST indicates that cows did not change energy balance or that experimental periods were too short to observe differences.

Treatment diets contained nearly equal nutrient compositions and were considered to contain the same gross energy density. Efficiency calculated as FCM yield per kg DMI was greater for UNS than SAT cows ($P < 0.001$). This calculation does not account for changes in body energy, and treatment effects were not significant for either milk yield or body weight change. Efficiency calculated as net energy (NE_L) of body weight change plus NE_L milk production over DMI was not different between treatments. Body weight energy gain, and milk energy yield were not different between treatments, attributing any efficiency difference to changes in dry matter intake.

Calculation of marginal return or efficiency involves analysis of the response stimulated by marginal inputs. The return provided by increased dry matter intake is a more responsive and informative variable than absolute efficiency. Milk yield and milk protein yield responses (SAT – UNS) were linearly increased with increased DMI response ($P < 0.01$ and $P < 0.001$, respectively). Fat corrected milk, milk fat percent, and milk fat yield responses (SAT - UNS) were affected quadratically by increasing DMI response ($P < 0.01$). Marginal milk and milk protein yield were linearly increased and marginal milk fat yield was affected quadratically with increasing DMI response (Table 7). Increasing DMI increased production of milk and milk components. The cost of the additional production is merely the increased DMI when using marginal return. Marginal return and efficiency is gaining prominence over the absolute efficiency calculation presented and provides a more responsive decision making tool.

Plasma Metabolites and Hormones

Metabolic hormones analyzed included insulin, growth hormone, leptin, and CCK, and metabolites assayed were glucose, NEFA, and β HBA. Saturated FA increased insulin over 25% compared to UNS (12.8 vs. 10.1 μ IU/mL, $P < 0.001$). Type of FA determines insulin secretion *in vitro* with saturated and longer chain FA being more insulinotropic (Stein et al., 1997). Plasma leptin and growth hormone concentrations were not affected by treatment. Saturated FA treatment decreased NEFA over 20% compared to UNS (89.3 vs. 115.5 μ eq/l respectively, $P < 0.001$), but plasma glucose and β HBA were not affected by treatment. Decreased NEFA with no change in glucose may show different responsiveness or regulation in fat and glucose metabolism to insulin signaling. A quadratic effect of pFCM was observed on plasma NEFA. Interestingly, all cows decreased plasma NEFA when fed SAT compared to UNS. No other plasma hormone or metabolite was related to pFCMY.

A possible change in insulin sensitivity is noted using glucose to insulin ratio as a proxy for insulin stimulation of glucose uptake. Unsaturated FA had a higher glucose to insulin ratio than SAT ($P < 0.02$), but had no effect on the ratio of bST to insulin (data not shown). Plasma β HBA did not change, but it is not possible to conclude difference in FA oxidation between treatments because β HBA clearance is not known.

A quadratic effect of pFCM was observed on plasma NEFA. Interestingly, all cows decreased plasma NEFA when fed SAT compared to UNS. No other plasma hormone or metabolite was related to pFCMY.

Leptin is commonly correlated to fat cell size and body fatness. There is interest in the interaction of insulin and leptin and the ability of insulin to directly stimulate leptin

secretion. The large increase in insulin with SAT treatment provides an interesting opportunity to observe the relationship of insulin and leptin response in high producing cows. Saturated fat treatment produced a large increase in insulin but no difference in plasma leptin, and leptin response (SAT –UNS) was not related to insulin response. Plasma concentrations of insulin and leptin were not related. Plasma bST was negatively related to leptin as expected; higher bST is associated with a lower or negative energy balance and higher leptin is associated with positive energy balance (Figure 4).

CONCLUSIONS AND IMPLICATIONS

Fatty acid profile reaching the duodenum is important for predicting intake response to fat supplementation. Increasing unsaturated FA concentration of the diet decreased intake with no relationship to milk yield across cows. Unsaturated FA decreased time spent ruminating, which may be the result of reduced gut motility as previously observed in abomasal infusions. Dietary FA saturation affects insulin secretion and plasma NEFA concentration. Saturated FA increased milk protein and the magnitude of the response appears to be related to production level, insulin signaling, or IGF-1 stimulation.

Table 1. Status of 31 cows at the beginning of experiment. ¹

	Mean	SD
BW, kg	655	45
BCS	2.35	0.38
DIM	130	70
Milk yield, kg	43.7	6.3

¹ Data were collected from 32 cows for this experiment, but data from one cow was excluded due to illness.

Table 2. Ingredients and nutrient composition of treatments¹.

Nutrient	SAT	UNS
DM, % as fed		
	-----% of DM-----	
Ingredients		
Ca Soaps FA	-	57.7
Prilled FA	48.9	-
Rice Hulls	-	10.9
Limestone	19.2	-
Ground Corn	31.9	31.4
Composition		
Total FA	49.7	46.9
Calcium	6.8	7.1
FA Profile	-----% of FA -----	
C16	34.0	46.9
C18:0	46.2	4.3
C18:1	9.9	36.3
C18:2	2.54	9.31
C18:3	0.04	0.30
Unsaturated FA	13.2	46.1
C16:18 Ratio	0.58	0.94

¹ SAT- saturated fatty acids treatment of prilled fatty acids, UNS- unsaturated fatty acids treatment of calcium salts of palm oil

Table 3. Ingredient and nutrient composition of experimental diets¹.

	Pretrial	SAT	UNS
Ingredients	-----% of DM-----		
Corn silage ²	22.5	22.4	22.5
Alfalfa silage ³	18.9	20.0	20.1
Ground Corn	23.8	23.1	23.1
Whole Cottonseed	10.4	10.3	10.3
Protein mix ⁴	14.1	13.8	13.9
SAT mix ⁵	2.5	5.0	-
UNS mix ⁵	2.4	-	4.6
Mineral vitamin mix ⁶	4.4	4.5	4.5
Molasses mix ⁷	1.0	0.9	0.9
Nutrient			
DM	52.6	52.2	52.1
Total FA	7.1	7.0	6.8
% Unsaturated FA	3.8	3.3	4.0
Starch	26.6	25.5	25.7
NDF	26.0	26.4	26.9
Forage NDF	16.1	17.0	17.1
CP	18.6	17.3	17.4
Ash	5.3	5.4	5.1
Rumen-undegraded CP ⁸ (% CP)	35.7	35.6	35.6

¹ Pretrial- covariant period; SAT- saturated fatty acids treatment of prilled fatty acids, UNS- unsaturated fatty acids treatment of calcium salts of palm oil

² Corn silage contained 37.2% DM (as fed) and 39.6% NDF, 7.4% CP, 27.9% starch, and 3.0% ash on a DM basis

³ Alfalfa silage contained 30.9% DM (as fed) and 40.5% NDF, 17.0% CP, 4.2% starch, and 7.2% ash on a DM basis.

⁴ Protein mix contained 74.1% soybean meal (44% CP), 20.1% corn gluten meal, and 5.8% blood meal.

⁵ Mix composition listed in Table 2.

⁶ Mineral vitamin mix contained 16.5% vitamin E, 41.0% vitamin D, 44.4% vitamin A, 1.9% trace mineral premix, 4.1% urea, 4.6% salt, 8.4% limestone, 10.5% dicalcium phosphate, 11.7% sodium bicarbonate, and 57.9% dry ground corn as a carrier

⁷ Molasses mix was 66% DM and contained 30.3% CP.

⁸ Rumen-degraded protein estimated using values from NRC (2001).

Table 4. Effects of fatty acid saturation¹ on intake and feeding behavior.

	SAT	UNS	SE	<i>P</i>
Intake	----- (kg/d) -----			
DM	27.4	26.7	0.4	<0.01
NDF	6.93	6.88	0.09	0.39
Starch	6.79	6.62	0.10	<0.01
CP	4.57	4.46	0.06	<0.01
Total FA	4.08	3.86	0.05	<0.001
Eating time	----- min -----			
/d	210	205	6	0.33
/kg DMI	7.8	7.8	0.3	0.91
/kg NDF intake	31	30	1.0	0.40
Ruminating time				
/d	535	510	10	<0.01
/kg DMI	20	19	0.5	0.26
/kg NDF intake	77	74	1.8	0.03
Total chewing time				
/d	745	715	13	<0.01
/kg DMI	27	27	0.6	0.26
/kg NDF intake	108	104	2.3	0.02
Time spent idle				
/d	670	695	10	<0.01

¹ SAT- saturated fatty acids treatment of prilled fatty acids, UNS- unsaturated fatty acids treatment of calcium salts of palm oil

Table 5. Effects of fatty acid saturation on production¹.

	SAT	UNS	SE	<i>P</i>
Yield	----- (kg/d) -----			
Milk	41.8	42.3	1.1	0.12
3.5% FCM ³	41.8	42.4	1.2	0.11
SCM ³	39.0	39.3	1.1	0.33
Fat	1.47	1.49	0.05	0.25
Protein	1.27	1.27	0.03	0.79
Lactose	2.00	2.01	0.06	0.23
Milk Composition	----- % -----			
Fat	3.54	3.54	0.09	0.92
Protein	3.06	3.02	0.05	0.04
Lactose	4.80	4.74	0.03	<0.001
Tissue Gain				
BW change, kg/d	0.50	0.29	0.16	0.37
BCS change ⁴	0.11	0.10	0.04	0.74

¹ SAT- saturated fatty acids treatment of prilled fatty acids, UNS- unsaturated fatty acids treatment of calcium salts of palm oil

² 3.5% fat-corrected milk yield (kg/d)

³ Solids-corrected milk yield (kg/d)

⁴ Change in body condition score (BCS; five-point scale where 1 = thin to 5 = fat) over a 14-d period.

Table 6. Responses (saturated – unsaturated) of intake and production by pretrial 3.5% fat-corrected milk yield.

Response	R ²	Predictor ¹	P ²	Coefficient ³	Intercept ⁴
DMI	0.19	pFCMY ⁵	0.14	--	--
MY (kg/d)	0.39	pFCMY	0.24	--	--
3.5% FCM (kg/d)	0.19	pFCMY	0.48	--	--
Fat, %	0.06	pFCMY	0.84	--	--
Fat yield, kg	0.02	pFCMY	0.75	--	--
Protein, %	0.46	Sequence ⁶ pFCMY	<0.001 <0.05	0.174 0.049	-0.204
Protein yield, kg	0.18	Sequence pFCMY	0.48 0.02	-0.008 0.004	-0.173
Lactose, %	0.34	Sequence pFCMY (pFCMY) ²	<0.01 0.01 <0.01	0.006 0.011 0.009	1.05
Lactose yield, kg	0.66	Sequence pFCMY (pFCMY) ²	<0.001 <0.01 <0.01	-0.095 -0.049 0.0006	0.965

¹ Regression term

² P- significance of regression term

³ Coefficient of the regression term

⁴ Intercept of the regression equation

⁵ Pretrial 3.5% fat-corrected milk yield (kg/d).

⁶ Sequence parameter estimate

Table 7. Milk yield and component responses (saturated – unsaturated) by dry matter intake response (saturated - unsaturated)

Response	R ²	Predictor ¹	P ²	Coefficient ³	Intercept ⁴
MY (kg/d)	0.53	Sequence DMI Response ⁵	<0.01 <0.01	-0.98 0.82	-1.10
3.5% FCM (kg/d)	0.43	Sequence DMI Response (DMI Response) ²	0.09 0.67 0.01	-0.69 0.17 0.36	-1.26
Milk Fat, %	0.32	Sequence DMI Response (DMI Response) ²	0.17 0.03 <0.01	0.06 -0.05 0.01	0.015
Milk Fat Yield, Kg	0.35	Sequence DMI Response (DMI Response) ²	0.63 0.67 <0.01	-0.01 -0.01 0.02	-0.040
Milk Protein, %	0.41	Sequence DMI Response	<0.001 0.24	0.09 0.01	0.034
Protein yield, kg	0.39	Sequence DMI Response	0.23 <0.001	0.01 0.04	-0.023

¹ Predictor- regression term

² P – significance of regression term

³ Coefficient of the regression term

⁴ Intercept of the regression equation

⁵ DMI Response = (saturated DMI – unsaturated DMI)

Table 8. Effects of fatty acid saturation¹ on plasma hormones and metabolites.

	SAT	UNS	SE	<i>P</i>
CCK, pmol/L	12.5	14.1	0.91	0.08
Insulin, μ IU/mL	12.8	10.1	0.6	<0.001
BST, ng/mL	1.94	1.98	0.14	0.76
Leptin, ng/mL	2.17	2.56	0.09	0.18
Glucose, mg/dL	62.0	61.4	0.5	0.19
NEFA, μ eq/L	89.3	115.5	3.3	<0.001
β HBA, mg/dL	5.25	5.28	0.16	0.86
Glucose/insulin ratio, mg/ μ IU	5.50	6.50	0.28	0.15
Insulin Std. Dev.	6.53	4.76	0.48	0.01
NEFA Std. Dev.	16.9	23.8	2.0	<0.01

¹ SAT- saturated fatty acids treatment of prilled fatty acids, UNS- unsaturated fatty acids treatment of calcium salts of palm oil

Table 9. Effects of fatty acid saturation¹ on energy intake and partitioning.

	SAT	UNS	SE	<i>P</i>
Milk Energy, Mcal NE _L ²	28.7	29.9	0.77	0.33
Tissue energy gain ³ , Mcal NE _L	2.90	2.36	1.11	0.73
Efficiency, FCM/DMI	1.53	1.59	0.04	<0.001
Energy efficiency ⁴	0.54	0.55	0.02	0.76

¹ SAT- saturated fatty acids treatment of prilled fatty acids, UNS- unsaturated fatty acids treatment of calcium salts of palm oil

² NE_{L(milk)} (Mcal/d) = MY (kg) x (0.0929 x fat% + 0.0563 x true protein% + 0.0395 x lactose%) (NRC 2001).

³ NE_L gain calculated from body weight gain (NRC 2001)

⁴ (Milk energy, Mcal NE_L + Tissue energy gain, Mcal NE_L) / kg DM

Table 10. Responses (saturated – unsaturated) of energy balance and plasma metabolites and hormones

Response	R ²	Predictor ¹	P ²	Coefficient ³	Intercept ⁴
Body wt gain	0.01	pFCMY ⁵	0.64	--	--
Time spent					
Eating	0.49	pFCMY	0.29	--	--
Ruminating	0	pFCMY	0.95	--	--
Idle	0.38	pFCMY	0.72	--	--
Plasma					
Insulin, µIU/mL	0.65	pFCMY	0.84	--	--
Leptin,	0.11	pFCMY	0.54	--	--
Glucose, mg/dL	0.04	pFCMY	0.75	--	--
βHBA, mg/dL	0.05	pFCMY	0.26	--	--
Glucose/Insulin					
bST, ng/mL	0.20	Sequence pFCMY (pFCMY) ²	0.25 0.07 0.06	-0.117 0.251 -0.003	-5.054
NEFA	0.28	Sequence pFCMY (pFCMY) ²	0.46 0.02 0.01	-1.90 8.27 -0.10	-190.5
bST / Insulin	0.36	Sequence pFCMY (pFCMY) ²	<0.01 0.03 0.02	0.073 0.063 -0.00078	-1.287

¹ Predictor- regression term

² P – significance of regression term

³ Coefficient of the regression term

⁴ Intercept of the regression equation

⁵ Pretrial 3.5% fat-corrected milk yield (kg/d).

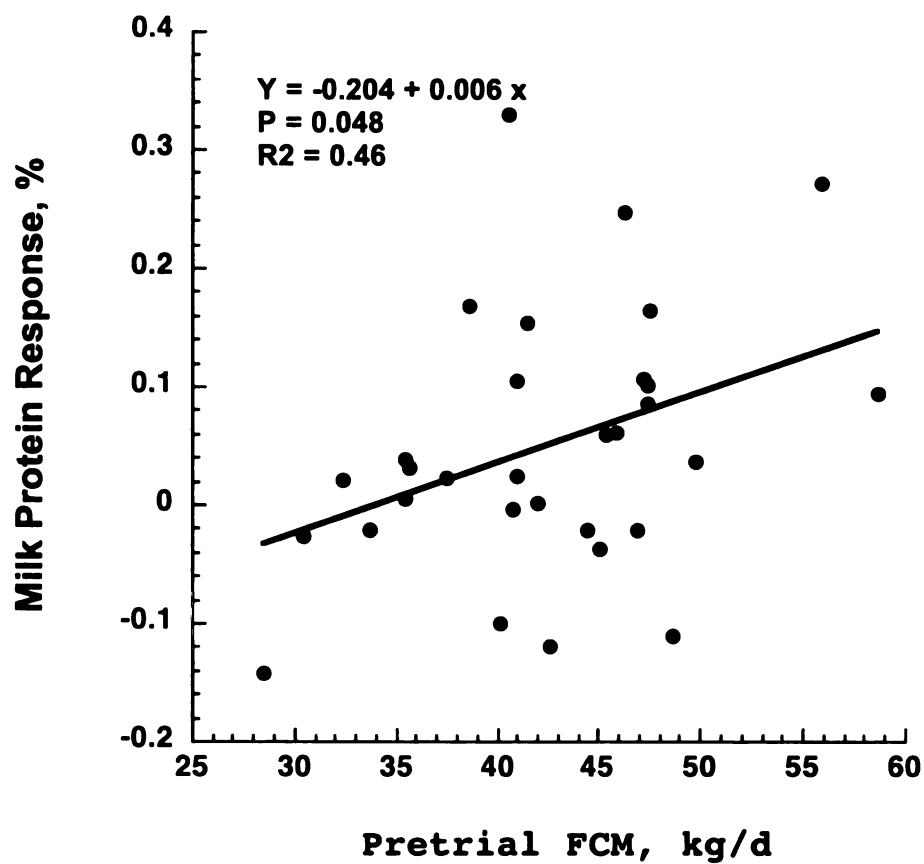


Figure 1: Milk protein percent response by pretrial fat corrected milk (FCM) yield. Relationship between milk yield over the 14 d prior to the beginning of the experiment and the response (saturated – unsaturated) in milk protein concentration to the unsaturated FA treatment.

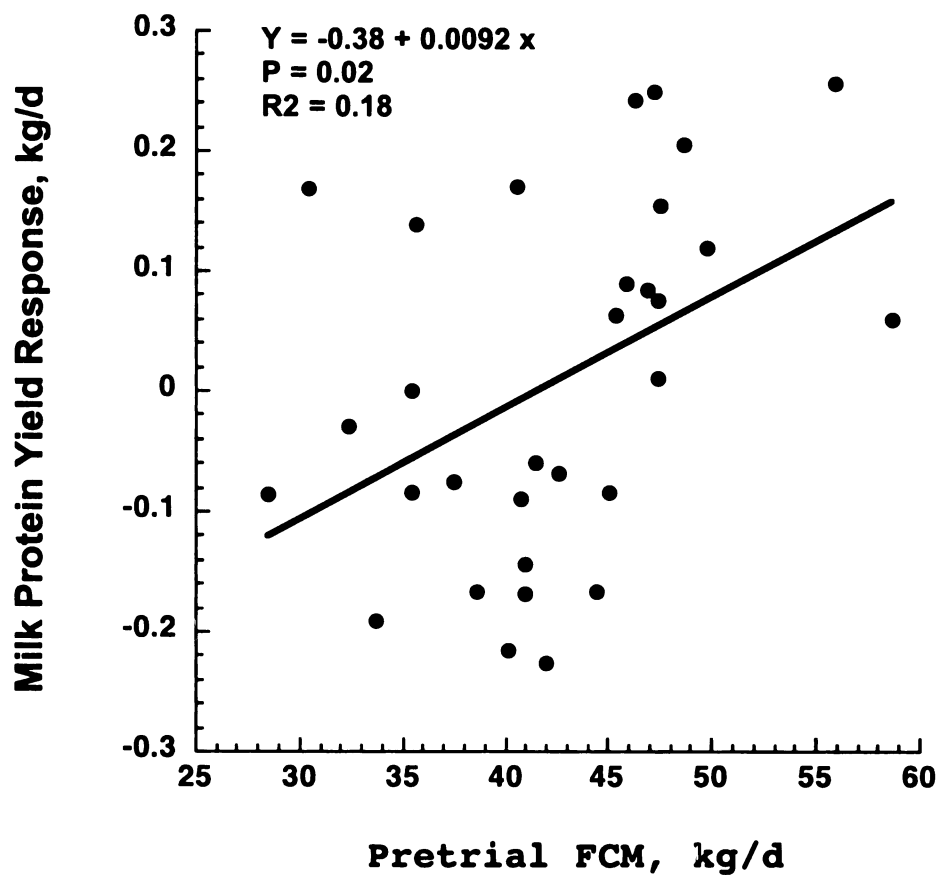


Figure 2: Milk protein yield response by pretrial fat corrected milk (FCM) yield. Relationship between milk yield over the 14 d prior to the beginning of the experiment and the response (saturated – unsaturated) in milk protein yield to the unsaturated FA treatment.

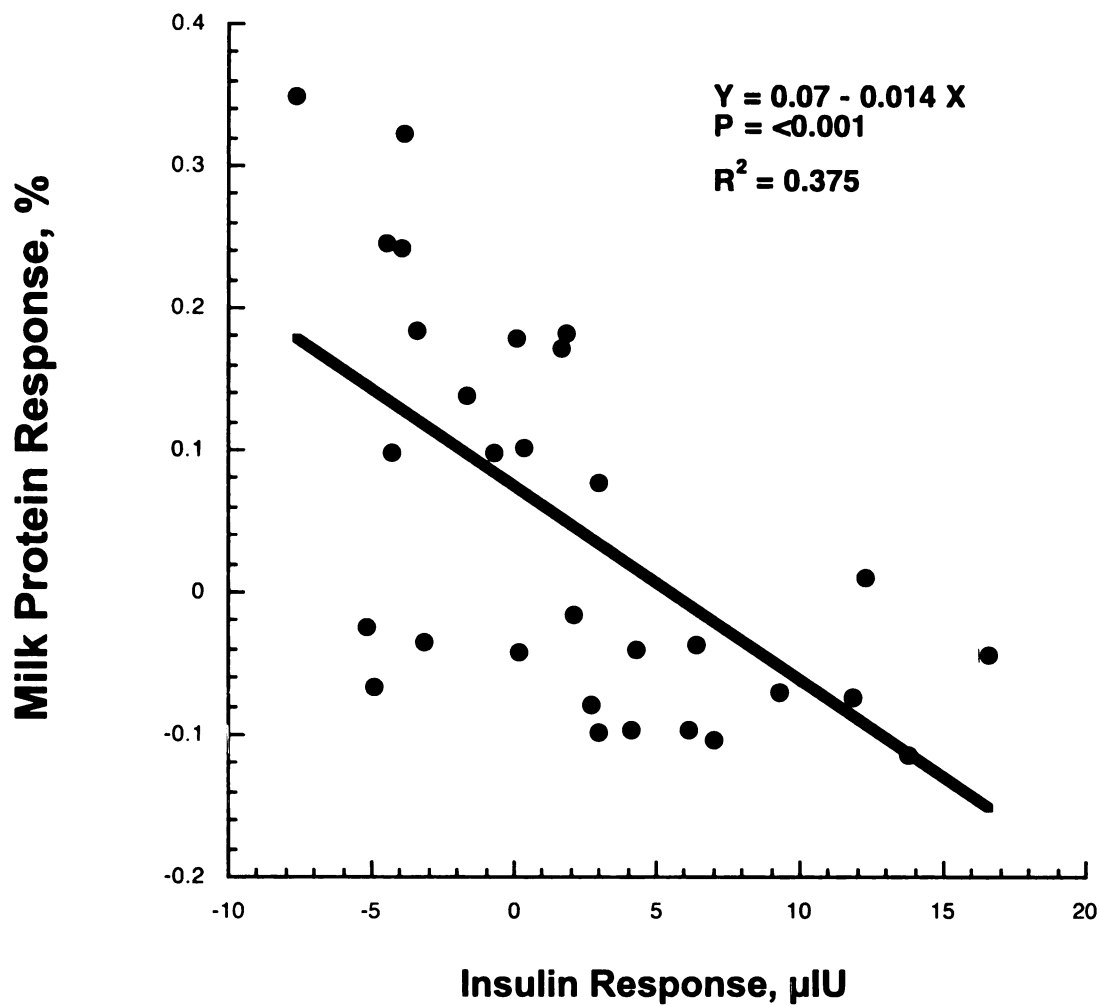


Figure 3: Milk protein percent response by insulin response. The relationship between milk protein concentration response (saturated – unsaturated) and insulin response to fatty acid saturation.

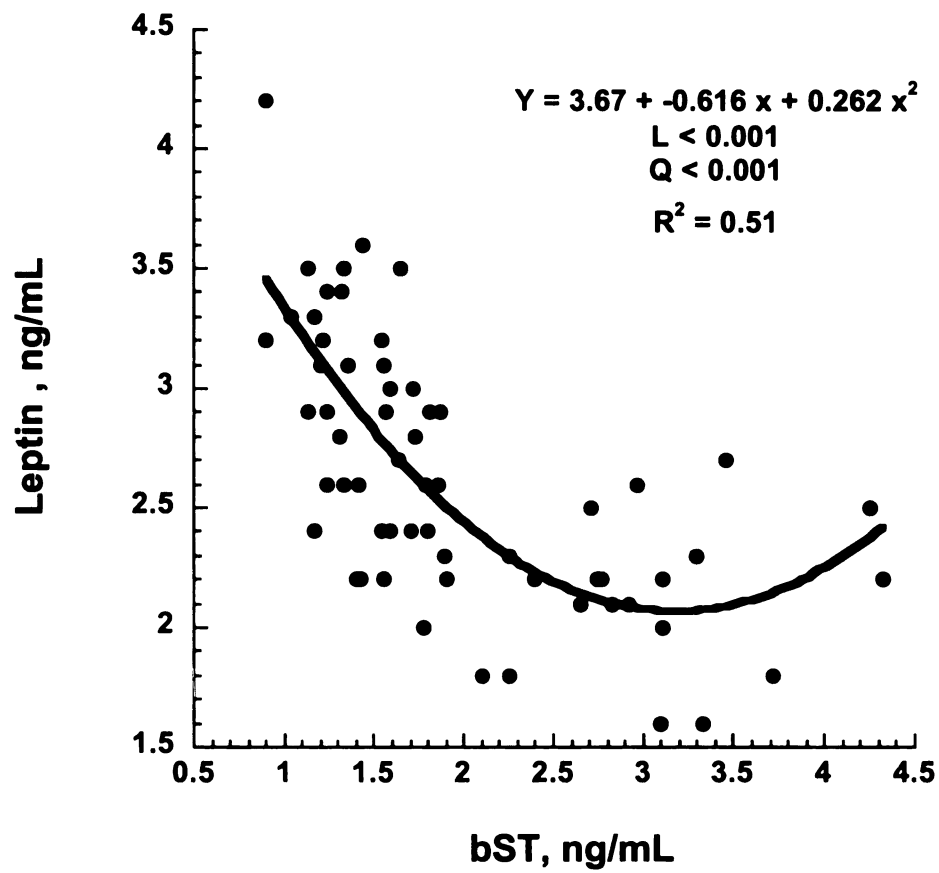


Figure 4: Relationship between mean leptin and bST concentration across animal periods.

CHAPTER 3

Effect of rumen-protected fatty acid saturation on milk yield, milk fatty acid profile, energy balance and plasma metabolites and hormones of lactating dairy cows.

ABSTRACT

Saturated and unsaturated rumen protected fat sources were evaluated for effects on yield of milk and milk components, concentration of milk components including milk fatty acid (FA) profile, and energy balance. Eight ruminally and duodenally cannulated cows and eight non-cannulated cows were used in a replicated 4x4 Latin square design with 21 d periods. Treatments were control (CON) and a linear titration of 2.5% added rumen protected FA varying in unsaturation including saturated (SAT; prilled hydrogenated free FA), 50:50 ratio of SAT and unsaturated (UNS; calcium soaps of long-chain FA), and UNS. SAT did not change milk fat concentration, but UNS linearly decreased milk fat in cannulated cows and tended to decrease milk fat in non-cannulated cows. Milk fat depression with UNS corresponded to increased milk trans-10, cis-12 conjugated linoleic acid. Milk fat profile did not change with SAT compared to CON, but UNS decreased concentration of short and medium-chain FA and increased concentration of long-chain FA. Digestible energy intake tended to decrease linearly with increasing UNS in cannulated and non-cannulated cows. Increasing UNS linearly increased empty body weight and net energy gain in cannulated cows, while plasma insulin and beta-hydroxybutyrate concentrations were decreased linearly. Efficiency of conversion of

digestible energy to milk tended to decrease linearly with UNS for cannulated cows only. Addition of rumen-protected fat did not change energy balance of cannulated cows, but decreased energy balance of non-cannulated cows. Addition of SAT provided little benefit to production and energy balance, while UNS decreased milk energy yield.

INTRODUCTION

High producing dairy cows have large energy requirements that may exceed their ability to consume dietary energy, resulting in less than maximum milk yield. Addition of fat to the diet increases energy density without increasing rumen acid production, or maintains energy density while increasing fiber for stabilization of rumen fermentation (Allen, 1997). Prilled saturated free fatty acids (FA) and calcium salts of FA are two manufactured products marketed to minimize effects of fat on ruminal fermentation. However, calcium salts of FA are not entirely protected in the rumen and dissociation of the calcium ion allows rumen biohydrogenation of unsaturated FA (Wu et al. 1991).

Traditionally, FA are considered a source of energy, but are now appreciated as biological modifiers of physiology and metabolism. Incomplete biohydrogenation of polyunsaturated FA increases duodenal flow of trans-C18:1 and conjugated linoleic acids (CLA), which have been implicated in milk fat depression through decreased lipogenic gene expression (Bauman and Griinari, 2003; Peterson et al., 2003). Milk fat depression normally occurs with little effect on dry matter intake leading to increased body weight gain in proportion to milk fat depression (Baumgard et al., 2002b). Increased body

weight gain in response to trans-10, cis-12 CLA in lactating cows is contrary to decreased body fat gain observed in growing animals (Mersmann, 2001).

The profile of FA absorbed in the duodenum can alter the FA profile of animal products, especially modifying FA saturation and CLA concentration (Grummer, 1991; and Mansbridge and Blake, 1997). Consumers are increasingly concerned about FA intake. Decreasing saturated FA intake may decrease heart disease and diabetes (Mansbridge and Blake 1997), and increasing CLA intake may decrease the incidence of cancer and obesity (Kelly, 2001). Strategies for dietary FA supplementation can be developed to alter the FA profile of meat and milk products to meet consumer demands.

The objective of this experiment was to determine effects of rumen-protected FA differing in FA saturation on milk and milk component yield, milk fatty acid profile and energy partitioning.

MATERIALS AND METHODS

This paper is the first of four papers in a series from one experiment that evaluated effects of rumen-protected FA (RPF) differing in FA saturation. This paper discusses treatment effects on milk yield, milk FA profile and energy balance, and the companion papers discuss ruminal kinetics and extent of biohydrogenation (Chapter 4), ruminal digestion kinetics and site of digestion (Chapter 5), and DMI and feeding and chewing behavior (Chapter 6). Experimental procedures were approved by the All University Committee on Animal Use and Care at Michigan State University.

Cows and Treatments

Eight ruminally and duodenally cannulated (77 ± 8.7 DIM; mean $\pm$ SD) and eight non-cannulated (106 ± 15 DIM; mean $\pm$ SD) multiparous Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center were blocked by cannulation and assigned randomly to replicated 4 x 4 Latin squares in a dose-response arrangement of treatments plus a control. Non-cannulated cows were included in the experiment to increase the number of observations for intake and milk yield. Cows were blocked by cannulation because they were selected at different times and differed in DIM, body condition score and surgical preparation. Treatments were a control diet (CON) containing no added RPF or 2.5% added RPF from saturated (SAT - prilled hydrogenated FA, Energy Booster 100[®], Milk Specialties Company Inc., Dundee, IL), intermediate mixture of saturated and unsaturated (INT), or unsaturated (UNS) FA (Ca Soaps of LCFA, Megalac-R[®], Church and Dwight Company, Inc., Princeton, NJ). Treatment periods were 21 d with the final 11 d used for sample and data collection. Surgery was performed at the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University. Immediately prior to initiation of the experiment, mean empty BW (ruminal digesta removed) of cannulated cows was 516 ± 33 kg (mean $\pm$ SD) and mean BW of non-cannulated cows was 638 ± 51 kg (mean $\pm$ SD).

Treatment mix composition is shown in Table 2. Treatment mixes included limestone and rice hulls to balance for calcium and FA concentration and 50% ground corn as a carrier. The base ration was formulated to provide 2.5% rumen available FA from cottonseed, as would be expected in commercial diets, and treatments were formulated to provide 2.5% rumen-protected FA as commonly recommended.

Experimental diets contained 40% forage (66:33 corn silage: alfalfa silage), 13.5% whole cottonseed, dry ground corn, premixed protein supplement (soybean meal, corn gluten meal, and blood meal), a mineral and vitamin mix, and 2.5% added rice hulls (CON), saturated FA (SAT), 50:50 mix of saturated and unsaturated fat (INT) or unsaturated FA (UNS) treatment (Table 3). All diets were fed as a total mixed rations. Final diet FA concentration and composition is shown in Table 3.

Data and Sample Collection

Throughout the experiment, cows were housed in tie-stalls and fed once daily (0900 h) at 115% of expected intake. Amounts of feed offered and orts were weighed for each cow daily. Samples of all diet ingredients (0.5 kg) and orts from each cow (12.5%) were collected daily on d 11 to 14 and combined into one sample to represent four days for digestibility determination (d 11-14). Cows were milked twice daily in their stalls during the feeding behavior monitoring phase (d 16 to 19) and in a milking parlor during the rest of each period. Milk yield was measured at each milking on d 11-19, and milk was sampled at each milking on d 16 to 19.

Methods for determining fecal output and digestibility for cannulated cows are described in Chapter 5. Indigestible NDF (iNDF) was used as a marker to calculate total tract fecal flow for non-cannulated cows. Fecal samples (1,000 g) were collected every 9 h from d 12 to d 14 yielding eight samples representing every 3 h of a 24-hour period to account for diurnal variation.

Sample and Statistical Analysis

Feed and fecal samples were processed and analyzed as described in Chapter 5. Milk samples were composited based on milk fat yield and centrifuged at 17,800 x g for 30 min at 8°C. Fat cake (300-400 mg) was extracted according to Hara and Radin (1978) and methyl esters were formed according to Christie (1982) as modified by Chouinard et al. (1999). FA were quantified by GC (Model 8500, Perkins-Elmer Corp, Norwalk, CT), using a SP-2560 capillary column (100m X 0.20 mm id with 0.02- μ m film thickness; Supelco, Bellefonte, PA). Oven temperature was 140°C for 5 min, then ramped 4°C/min to 240°C and held for 15 min. Helium flow was 20 cm/sec. Milk samples were analyzed for fat, true protein, and lactose with infrared spectroscopy by Michigan DHIA (East Lansing).

Commercial radioimmunoassay kits were used to determine plasma concentration of insulin (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA), and glucagon (Glucagon kit GL-32K, St. Charles, MO). Commercial enzyme kits were used for analysis of glucose (Glucose kit #510; Sigma Chemical Co., St. Louis, MO), NEFA (NEFA C-kit; Wako Chemicals USA, Richmond, VA), and β -hydroxybutyrate (β HBA; kit #310-A; Sigma Chemical Co., St. Louis, MO).

Energy values were calculated as follows:

DE intake = GEI x GE digestibility [GE digestibility as reported in Chapter 4]

NE_L intake- calculated from DE through ME according to NRC 2001.

Milk NE_L (Mcal/d) = MY (kg) x [0.0929 x (Fat %) + 0.0563 x (True Protein%) + 0.0395 x (Lactose%)] (NRC, 2001) ;

NE_M = 0.080 x BW^{0.75} (NRC, 2001); and

$$NE_L \text{ balance} = NE_L \text{ intake} - NE_M - \text{Milk } NE_L$$

All data were analyzed using the fit model procedure of JMP® (Version 5, SAS Institute, Cary, NC) according to the following model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk}$$

where

μ = overall mean,

C_i = random effect of cow ($i = 1$ to 8),

P_j = fixed effect of period ($j = 1$ to 4),

T_k = fixed effect of treatment ($k = 1$ to 4),

e_{ijk} = residual error.

There was a significant block by treatment effect for milk production and other variables of primary interest ($P < 0.10$) so cannulated and non-cannulated cow data was separated for presentation. Period by treatment interaction was evaluated, but was removed from the statistical model when not significant ($P > 0.10$). Period by treatment interaction was not significant for any variable of primary interest; variables with significant interactions are noted in the tables. Data points with Studentized Residuals greater than three were considered outliers and excluded from analysis. Few points were excluded in analysis and rarely more than one per response variable. Preplanned contrasts included the effect of addition of RPF (CON vs. SAT, INT and UNS), linear effect of increasing concentration of unsaturated fat [L (SAT vs. UNS)], and quadratic effect of increasing concentration of unsaturated fat [Q (INT vs. SAT and UNS)]. The preplanned contrasts do not allow individual comparison of each fat treatment to the control. Protected LSD was used for mean separation in the discussion when the model

treatment effect was significant. Pearson correlation coefficients were determined between cow-period observations for some parameters. Average parameters for each block presented in Table 1 were determined by including block in the above model. Treatment effects, linear and quadratic responses, and correlations were declared significant at $P < 0.05$, and tendencies were declared at $P < 0.10$.

Data from two cow-periods were excluded from statistical analysis. One cannulated cow developed clinical mastitis on d 19 of period 3, rumen samples, body weight and body condition score was not collected for this period. Data previously collected in this period was included in our analysis. The cow did not fully recover and data from period 4 was not used.

RESULTS AND DISCUSSION

Characteristics of cows within each block are presented in Table 1. Cannulated cows were 30d earlier in lactation at the start of the experiment, with lower BW and greater milk yield than non-cannulated cows. Although blocks only slightly differed in DIM, there was a large difference in BCS indicating differences in metabolic state.

Diets were based on the same forage and concentrates and differed only in addition of FA treatment mix (Table 2). The CP concentration of the diets averaged 16.1%, which was lower than the target CP formulated for 17.8% because a number of dietary ingredients contained lower concentrations of CP during the experiment than measured before the experiment began. Treatments were formulated to contain the same calcium concentrations using limestone, and rice hulls were used to take the place of FA in the CON to maintain approximately the same fermentable and digestible carbohydrate

concentration (Table 2). Treatments differed in FA concentration and profile. Control diet contained 5.5 % FA and RPF diets contained 8.3, 8.1 and 7.8 % FA for SAT, INT and UNS. Treatment mixes (Table 2) were formulated based on manufacturer's product specifications. Calcium salts of long-chain FA contained much lower FA than expected. To compensate for the lower FA concentration of UNS, a greater concentration of UNS mix was included in the UNS and INT treatments and CON mix (rice hulls only) was used to compensate for the increased inclusion rate in CON, SAT and INT. The small FA concentration difference among the final RPF treatments is attributed to variation during the experiment. Dietary unsaturated FA density increased from SAT to UNS treatment (3.9, 4.4 and 4.9 % for SAT, INT, and UNS). Increased unsaturated FA from SAT to UNS included increased C18:1, C18:2 and C18:3 and decreased C18:0 (Chapter 4). Addition of RPF increased the C16:C18 ratio, but the ratio was not changed within RPF.

Milk and Milk Component Yield

Rumen-protected FA did not affect milk yield of cannulated cows (Table 4) or non-cannulated cows (Table 5). However, within RPF treatments, milk yield was reduced linearly with increasing UNS for cannulated cows only. Increasing UNS linearly decreased milk fat concentration of cannulated cows and tended to decrease milk fat concentration of non-cannulated cows. The linear reduction in milk yield and milk fat concentration with increasing UNS linearly decreased fat-corrected milk and milk fat yield for cannulated cows. Yields of milk and fat-corrected milk as well as concentrations of milk fat and lactose were not different for SAT compared to CON for

either block of cows. Yield and concentration of milk protein was not affected by treatment for cannulated or non-cannulated cows and milk urea nitrogen was linearly decreased with UNS for cannulated cows only.

Milk Fatty Acid Profile

Milk FA profile of cannulated and non-cannulated cows was consistent with previous reports during milk fat depression (Baumgard, 2002a; and Peterson, 2003). Addition of SAT did not increase cis-9, trans-11 CLA or trans-C18:1 FA concentration, but UNS increased their concentration in cannulated and non-cannulated cows. In the cannulated cows, trans-10, cis-12 CLA was increased by SAT and increasing UNS linearly increased trans-10, cis-12 CLA. Non-cannulated cows did not increase trans-10, cis-12 CLA with SAT, but UNS increased the CLA compared to control. Milk fat concentration had a strong quadratic relationship with trans-10, cis-12 CLA [$Y = 3.50 - 17.41 + 131.6(X - 0.03)^2$; $R^2 = 0.60$], cis-9, trans-11 CLA [$Y = 3.58 - 27.99 + 214.65(X - 0.0204)^2$; $R^2 = 0.66$], and trans-C18:1 [$Y = 4.03 - 0.27 + 0.02(X - 3.74)^2$; $R^2 = 0.56$] in agreement with others (Peterson et al. 2002; Bauman and Griinari, 2003), although only trans-10, cis-12 CLA has been demonstrated to induce milk fat depression (Baumgard et al. 2000). Addition of SAT had very little effect on milk FA profile, but increasing UNS decreased concentration of short and medium chain FA and increased concentration of long-chain FA. Milk fat depression, induced by increased duodenal flow of trans-10, cis-12 CLA, is mediated through decreased gene expression of lipogenic enzymes, leading to decreased mammary *de novo* FA synthesis (Baumgard et al. 2002a; and Peterson et al. 2003). The observed FA profile changes are consistent with decreased FA synthesis

causing a decrease in concentration of short and medium chain FA. Addition of UNS FA increased the unsaturated proportion of C18 FA, but also increased trans-C18:1 concentration. Increased unsaturated FA and CLA with UNS should increase consumer appeal, but increased total trans-C18:1 is expected to negatively affect consumer appeal because of the association of trans-C18:1 and heart disease. Although we recognize the importance of separation of trans-C18:1 isomers they could not be separated with the FA analysis procedure used.

Energy Intake and Balance

Cannulated cows

Rumen-protected FA decreased DE intake and increasing UNS FA tended ($P = 0.06$) to linearly decrease DE intake, but NE_L intake was not affected by treatment (Table 8). Calculation of NE_L intake accounts for increased efficiency of converting DE from FA to NE. Saturated FA treatment did not affect milk yield, but INT and UNS decreased NE_L milk because of lower milk yield and milk fat concentration with increasing UNS. Empty BW gain and calculated net energy of tissue gain were increased with increasing UNS but BCS was not affected by treatment. Energy balance, NE_L milk, and NE maintenance as a fraction of energy intake were not affected by treatment. Simple efficiency calculated as NE_L milk as a fraction of DE intake tended to decrease with increasing UNS FA ($P = 0.10$). But NE_L for production (tissue gain plus milk) as a fraction of DE intake was not changed by treatment.

Fatty acids may change energy balance through modification of multiple physiological processes that affect intake, digestibility, metabolic efficiency, or

production, complicating prediction of energy balance. Treatment changed nutrient partitioning; UNS decreased milk and milk fat yield compared to CON and SAT, and increasing UNS linearly increased BW gain resulting in no difference in energy output. Tyrrell and Moe (1972) observed decreased efficiency of ME utilization for milk synthesis during milk fat depression, consistent with the tendency for decreased efficiency of converting DE to NE_L milk, because of increased energy use for tissue gain. Milk production and BW gain are both homeorhetically controlled. Feed intake is normally expected to decrease with increasing BW gain through chemostatic regulation. Increased BW gain may be physiologically directed to regain body condition lost in early lactation, and increased BW gain may not feedback on energy intake depending on metabolic state. Milk fat depression induced by biohydrogenation intermediates normally does not result in decreased intake (Baumgard et al., 2002a). Increased tissue energy gain as a result of decreased milk energy output, instead of decreased energy intake, is unexpected because it demonstrates a disconnect of production and intake leading to an imbalance in energy homeostasis (Chapter 6). Although it is possible that biohydrogenation intermediates that induce milk fat depression may also increase BW gain in dairy cows, this is opposite of the effect of CLA in monogastrics (Mersmann, 2001). Milk fat depression increases availability of acetate that is either directed or demanded by adipose tissue to increase tissue gain. Increased BW gain may represent directed growth or it may represent disposal of a metabolite to correct an imbalance in metabolites.

Metabolic control of the increased body weight gain is not well understood. Milk fat depression of 25 to 50% resulted in no change in plasma glucose, insulin and leptin

concentration or insulin stimulated glucose clearance, but did result in 24 to 33% reduced lipolytic response to an epinephrine challenge (Baumgard et al., 2002a). Gaynor et al. (1996) observed no effect of abomasal infusion of cis or trans-C18:1 on disappearance rates of glucose, insulin secretion following a glucose challenge, and appearance rates of NEFA and triglycerides after a norepinephrine challenge. In the current study, SAT increased plasma insulin concentration compared to CON, but UNS had no effect. Furthermore, increasing SAT linearly increased insulin concentration. We have previously reported increased plasma insulin with saturated FA compared to calcium salts of palm oil (Chapter 2), consistent with *in vitro* insulintropic effects of saturated FA (Stein et al., 1997). There was no difference among treatments for plasma glucagon, and β -HBA was linearly decreased with increasing UNS concentration presumably because of lower FA intake. A period by treatment interaction was observed for plasma glucose and NEFA concentration so treatment effects cannot be determined. Romo et al. (1996) measured energy metabolism during cis and trans-C18:1 FA infusion, observing increased production of milk energy with cis-C18:1 FA, but failed to detect differences in energy expenditure or tissue retention. Increased body weight gain and little change in intake during milk fat depression represents a failure in energy balance regulation that cannot be attributed to homeostatic signaling or regulation of lipid and glucose metabolism.

Non-cannulated cows

Observation of nutrient intake, ruminal digestion kinetics and site of digestion of cannulated cows is presented in companion papers (Chapter 4-6). Non-cannulated cow DM and OM intakes were not affected by treatment. Intakes of NDF and starch were

decreased and intake of total FA was increased by addition of RPF. Greater NDF and lower FA intake for CON compared to RPF was because rice hulls were included in place of FA for the CON diet. Addition of RPF increased total tract digestibility of NDF and pdNDF, and tended to increase OM digestibility (Table 9). Increasing UNS tended to quadratically increase total tract NDF digestibility and tended to quadratically decrease total tract starch digestibility. Changes in nutrient digestibility with UNS tended to increase the amount of NDF and pdNDF digested in the total tract with a quadratic response across RPF treatments.

Rumen-protected FA did not increase DE or NE intake in non-cannulated cows, and increasing UNS tended to linearly decrease DE and NE_L intake (Table 10). There were no effects of treatment on NE_L milk production, BW gain, or NE_L tissue gain, although RPF tended to increase BCS. Observing BW as opposed to rumen empty BW increases error and bias due to variation and treatment effect on rumen digesta weight. Although BCS tended to increase, calculated energy balance for cows decreased with RPF. Rumen-protected FA also increased milk energy and maintenance energy as a percent of energy intake. Treatment did not change efficiency of milk production or energy utilization. Large differences in energy balance are not expected because plasma insulin, glucagon, glucose, and β -HBA were not affected by treatment.

Cannulated and non-cannulated cows responded differently to treatment. Milk fat concentration was significantly depressed by UNS for cannulated cows but only tended to be decreased by UNS for non-cannulated cows. Less significant milk fat depression and milk CLA concentration indicates less duodenal trans-FA flow or decreased sensitivity to trans-FA isomers in the mammary gland. Duodenal trans-FA flow may be decreased by

more effective protection of PUFA or more complete biohydrogenation of trans-FA. Fatty acid protection and biohydrogenation may differ between the blocks because differences in intake or passage rate. Non-cannulated cows were had much more adipose tissue (1.2 BCS greater) and had lower milk yield (5.7 kg) and were expected to be in a different metabolic state. Metabolic state may interact with metabolic and physiologic response to FA biohydrogenation intermediates.

CONCLUSION

Increasing unsaturated FA treatment decreased milk fat yield and intake of digestible energy. Milk fat depression was consistent with the biohydrogenation theory of milk fat depression with decreased milk fat concentration associated with increased trans-10, cis-12 conjugated linoleic acid and lower concentrations of short and medium chain fatty acids. Cows experiencing milk fat depression increased body weight gain. Increased body weight gain may be because of the type of fuels available and incomplete intake compensation to maintain energy homeostasis.

Table 1. Parameter means¹ for cannulated and non-cannulated cows used in the experiment.

Parameter	Cannulated	Non-cannulated	P ²
Pretrial DIM	77	106	<0.001 ²
BW	614	677	<0.01 ³
BCS	1.9	3.1	<0.001 ³
Milk	45.6	39.9	0.08 ³
FCM	40.7	38.7	0.54 ³

¹ All means except pretrial DIM are the mean over the entire experiment.

² Linear contrast of cannulated vs. non-cannulated

Table 2. Composition of treatment mixes¹.

Nutrient	CON	SAT	UNS
	-----% of DM-----		
Ingredients			
Ca Soaps FA	-	-	57.5
Prilled FA	-	50.5	-
Rice Hulls	50.5	-	10.9
Limestone	16.7	16.7	-
Ground Corn	32.7	32.7	31.6
Nutrient			
Total FA	1.6	58.5	43.4
Unsaturated FA	1.0	6.1	23.6

¹ Treatments were CON- control with supplemental rumen-protected fatty acids (FA), SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

Table 3. Ingredient and nutrient composition of experimental diets¹.

	CON	SAT	INT	UNS
Ingredients	-----% of DM ² -----			
Corn silage ³	24.6	24.7	24.7	24.6
Alfalfa silage ⁴	12.6	12.6	12.6	12.6
Ground Corn	28.7	28.8	28.8	28.7
Whole Cottonseed	13.5	13.5	13.5	13.5
Protein mix ⁵	10.5	10.6	10.5	10.5
Mineral vitamin mix ⁶	4.3	4.3	4.3	4.3
CON Mix ⁷	5.7	0.5	0.2	-
SAT Mix ⁷	-	5.0	2.5	-
UNS Mix ⁷	-	-	2.9	5.7
Nutrient				
DM	55.6	55.7	55.7	55.7
OM	92.6 ^c	92.9 ^b	93.1 ^a	93.1 ^a
Total FA	5.5 ^d	8.3 ^a	8.1 ^b	7.8 ^c
Unsaturated FA	3.6 ^d	3.9 ^a	4.4 ^b	4.9 ^c
Starch	30.8 ^a	30.3 ^{ab}	30.5 ^{bc}	30.7 ^c
NDF	29.1 ^a	27.3 ^d	27.5 ^c	27.7 ^b
Indigestible NDF	11.2 ^a	9.7 ^b	10.0 ^b	9.9 ^c
Forage NDF	16.9 ^c	17.0 ^a	16.9 ^{bc}	17.0 ^b
CP	16.2 ^a	16.1 ^b	16.1 ^b	16.1 ^b
Rumen-undegraded CP ⁸	5.1 ^a	4.8 ^d	5.1 ^b	4.9 ^c
% NDF from forage	57.4 ^d	61.4 ^a	60.9 ^b	60.5 ^c
GE MCal/Kg	4.55 ^b	4.72 ^a	4.72 ^a	4.71 ^a

¹ Treatments were CON- control with supplemental rumen-protected fatty acids (FA), SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² Means with different superscripts differ by P<0.05

³ Corn silage contained 34.7% DM (as fed), and 43.4% NDF, 8.4% CP, 10.7% indigestible NDF, 24.1% starch, and 4.8% ash on a DM basis.

⁴ Alfalfa silage contained 36.3% DM (as fed) and 48.1% NDF, 16.2% CP, 25.7% indigestible NDF, 2.6% starch, and 9.7% ash on a DM basis.

⁵ Protein mix contained 74.1% soybean meal, 20.1% corn gluten meal, and 5.8% blood meal.

⁶ Mineral vitamin mix contained 12.7% sodium bicarbonate, 11.5% limestone, 5.5% salt, 2.2% trace mineral premix, 2.0% urea, 2.0% dicalcium phosphate, 0.6% vitamin D, 0.48% vitamin A, 0.12% vitamin E, and 62.9% dry ground corn as a carrier

⁷ Mix composition listed in Table 2

⁸Rumen-degraded protein estimated using values from NRC (2001).

Table 4. Effects of dietary rumen-protected fatty acids on milk production of cannulated cows.

	Treatment LS Means ¹					<i>P</i> ²			
	CON	SAT	INT	UNS	SE	Trt	RPF	L	Q
Yield, kg/d									
Milk	47.0	46.6	45.2	43.7	2.7	0.06	0.10	0.02	0.95
3.5% FCM ²	43.8	42.3	40.2	37.0	2.7	0.01	0.01	0.01	0.71
Milk fat	1.45	1.37	1.26	1.10	0.10	0.00	0.01	<0.001	0.68
Milk protein	1.33	1.34	1.30	1.30	0.06	0.62	0.61	0.26	0.66
Milk lactose	2.33	2.27	2.20	2.14	0.14	0.10	0.07	0.08	0.95
Milk solids	4.11	4.06	3.94	3.86	0.22	0.19	0.14	0.11	0.85
Milk composition, %									
Fat	3.06	2.93	2.78	2.43	0.19	<0.001	0.01	<0.001	0.40
Protein	2.84	2.88	2.89	2.96	0.08	0.20	0.14	0.15	0.47
Lactose	4.95	4.9	4.83	4.83	0.05	0.00	<0.001	0.01	0.16
Solids	8.77	8.75	8.67	8.74	0.09	0.42	0.36	0.88	0.17
Milk Urea N, mg/dL									
	16.1	16.4	15.6	14.2	0.81	0.04	0.29	0.01	0.63

¹ Treatments were CON- control with supplemental rumen-protected fatty acids (FA), SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² Trt: treatment effect, RPF: effect of rumen-protected FA, L: linear effect of saturation, and Q: quadratic effect of saturation.

³ 3.5% fat-corrected milk.

Table 5. Effects of dietary rumen-protected fatty acids on milk production of non-cannulated cows.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	Trt	RPF	L	Q
Yield, kg/d									
Milk	39.4	39.8	39.5	40.8	2.1	0.54	0.46	0.38	0.37
3.5% FCM ²	38.9	39.1	38.0	39.1	2.2	0.81	0.86	0.99	0.35
Milk fat	1.34	1.35	1.30	1.32	0.10	0.81	0.67	0.69	0.44
Milk protein	1.25	1.25	1.21	1.26	0.05	0.58	0.88	0.83	0.18
Milk lactose	1.91	1.91	1.85	1.95	0.10	0.61	0.93	0.56	0.23
Milk Solids	3.53	3.54	3.43	3.59	0.18	0.59	0.91	0.64	0.21
Milk composition, %									
Fat	3.45	3.43	3.31	3.25	0.23	0.17	0.15	0.08	0.84
Protein	3.18	3.16	3.11	3.11	0.07	0.21	0.12	0.19	0.49
Lactose	4.82	4.79	4.75	4.79	0.06	0.11	0.07	0.93	0.08
Solids	8.95	8.90	8.80	8.85	0.11	0.01	0.01	0.25	0.05
Milk Urea N, mg/dL	16.7	16.8	16.8	16.5	0.61	0.92	0.95	0.56	0.72

¹ Treatments were CON- control with supplemental rumen-protected fatty acids (FA), SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² Trt: treatment effect, RPF: effect of rumen-protected FA, L: linear effect of saturation, and Q: quadratic effect of saturation.

³ 3.5% fat-corrected milk.

Table 6. Effects of dietary rumen-protected fat on milk fatty acid profile for cannulated cows.

Fatty Acid	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	Trt	RPF	L	Q
	g/100 g fatty acids								
6:0	2.21	2.29	1.59	1.61	0.16	0.001	0.02	<0.001	0.05
8:0	1.33	1.28	0.89	0.83	0.09	<0.001	<0.001	<0.001	0.03
10:0	3.09	2.85	1.99	1.84	0.19	<0.001	<0.001	<0.001	0.01
12:0	3.43	3.15	2.35	2.26	0.18	<0.001	<0.001	<0.001	<0.01
14:0	11.0	10.7	8.9	8.6	0.36	<0.001	<0.001	<0.001	0.02
15:0	1.12	1.15	0.85	0.86	0.08	0.003	0.03	0.002	0.09
16:0	28.3	30.1	29.2	28.4	0.82	0.18	0.26	0.06	0.94
16:1, cis	1.53	1.62	1.62	1.72	0.18	0.63	0.33	0.47	0.76
17:0	0.67	0.74	0.59	0.51	0.02	<0.001	0.003	<0.001	0.06
18:0	10.8	10.9	11.0	9.15	0.65	0.02	0.41	0.006	0.13
18:1, trans	3.16	2.96	4.14	6.27	0.39	<0.001	0.004	<0.001	0.29
18:1, cis-9	18.5	18.9	21.0	19.6	0.72	0.007	0.01	0.23	0.006
18:2, cis-9,cis-12	2.71	2.66	3.10	3.65	0.11	<0.001	0.002	<0.001	0.67
18:3	0.22	0.19	0.21	0.23	0.009	<0.001	0.30	<0.001	0.70
20:0	0.12	0.13	0.13	0.11	0.006	0.03	0.56	0.008	0.19
cis-9,trans-11 CLA	0.01	0.02	0.03	0.05	0.005	<0.001	<0.001	<0.001	0.13
trans-10, cis-12 CLA	0.02	0.03	0.04	0.08	0.006	<0.001	<0.001	<0.001	0.006
Total									
<C16	23.7	22.8	17.7	17.2	0.91	<0.001	<0.001	<0.001	0.003
C18	36.3	36.2	40.3	40.1	1.16	0.004	0.02	0.003	0.07
Uns/Sat C18	0.70	0.70	0.73	0.77	0.01	<0.001	0.004	<0.001	0.52
Yield	-----g/d-----								
<C16	34.1	31.7	24.1	19.4	3.2	<0.001	<0.001	<0.001	0.48
C16	43.8	44.0	41.1	33.5	3.6	0.003	0.08	<0.001	0.38
>C16	54.8	51.7	56.2	46.3	4.3	0.05	0.27	0.11	<0.05

¹ Treatments were CON- control with supplemental rumen-protected fatty acids (FA), SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² Trt: treatment effect, RPF: effect of rumen-protected FA, L: linear effect of saturation, and Q: quadratic effect of saturation.

Table 7. Effects of dietary rumen-protected fatty acids on milk fatty acid profile for non-cannulated cows.

Fatty Acid	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	Trt	RPF	L	Q
g/100 g fatty acids									
6:0	2.35	2.05	1.69	1.89	0.22	0.08	0.03	0.52	0.19
8:0	1.46	1.25	1.03	1.10	0.13	0.01	0.004	0.23	0.18
10:0	3.49	3.04	2.47	2.51	0.30	<0.001	<0.001	0.03	0.15
12:0	3.89	3.49	2.89	2.83	0.29	<0.001	<0.001	0.007	0.17
14:0	11.5	11.0	10.0	9.3	0.34	<0.001	<0.001	<0.001	0.49
15:0	1.21	1.27	1.05	0.88	0.10	<0.001	0.009	<0.001	0.62
16:0	29.2	30.4	29.9	28.8	0.69	0.02	0.28	0.004	0.53
16:1, cis	1.49	1.45	1.41	1.38	0.14	0.59	0.27	0.43	0.97
17:0	0.70	0.80	0.65	0.46	0.03	<0.001	0.03	<0.001	0.51
18:0	10.5	10.9	10.9	10.0	0.47	0.06	0.78	0.02	0.14
18:1, trans	2.61	2.88	3.43	4.52	0.60	<0.001	0.005	<0.001	0.44
18:1, cis-9	17.4	18.0	19.4	19.2	0.82	0.02	0.01	0.07	0.18
18:2, cis-9,cis-12	2.72	2.58	2.85	3.69	0.12	<0.001	0.005	<0.001	0.02
18:3	0.21	0.17	0.21	0.24	0.009	<0.001	0.34	<0.001	0.76
20:0	0.11	0.14	0.13	0.12	0.006	0.001	0.001	0.005	0.37
cis-9,trans-11 CLA	<0.01	0.01	0.02	0.02	0.008	0.10	0.06	<0.10	0.70
trans-10, cis-12 CLA	0.01	0.02	0.02	0.03	0.01	<0.10	0.03	0.28	0.61
Total									
<C16	25.5	23.8	20.4	19.7	1.3	<0.001	<0.001	0.001	0.15
C18	34.2	35.1	37.6	38.9	1.2	<0.001	<0.001	<0.001	0.41
Uns/Sat C18	0.69	0.69	0.71	0.74	0.01	<0.001	0.005	<0.001	0.51
Yield									
<C16	34.3	32.4	27.0	27.0	3.2	0.002	0.003	0.01	0.13
C16	41.6	43.6	41.0	40.3	3.3	0.36	0.99	<0.10	0.57
>C16	48.8	49.7	51.2	53.6	3.5	0.12	0.11	0.07	0.81

¹ Treatments were CON- control with supplemental rumen-protected fatty acids (FA), SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² Trt: treatment effect, RPF: effect of rumen-protected FA, L: linear effect of saturation, and Q: quadratic effect of saturation.

Table 8. Effects of dietary rumen-protected fatty acids on energy balance and efficiency for cannulated cows.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	Trt	RPF	L	Q
Intake									
DE ³ , Mcal/d	78.0	75.3	74.0	70.1	3.1	0.07	0.04	0.06	0.59
NE _L ⁴ , Mcal/d	42.3	41.7	41.3	39.2	1.8	0.38	0.32	0.20	0.61
Production									
Milk NE _L ⁵ , Mcal/d	29.8	28.6	27.5	25.8	1.67	<0.01	<0.01	<0.01	0.68
Empty BW change, kg / d	0.21	0.11	0.49	0.94	0.24	0.08	0.28	0.02	0.89
NE _L ⁶ Empty BW gain, / d	1.06	0.43	2.14	4.16	1.03	0.07	0.32	0.02	0.90
BCS change, /21d	0.05	0.04	0.13	0.08	0.04	0.42	0.51	0.43	0.17
Balance									
NE _L balance ⁷ , Mcal/d	-5.5	-5.6	-4.2	-4.7	1.4	0.85	0.68	0.63	0.52
Milk NE _L , %NE _L intake	43.0	45.3	43.8	46.8	2.0	0.29	0.20	0.48	0.21
NE _{Maint} , %NE _L intake	69.8	68.9	66.8	65.4	2.9	0.61	0.37	0.34	0.91
Efficiency									
NE _L Milk / DE Intake	0.40	0.41	0.38	0.38	0.02	0.31	0.54	<0.10	0.53
NE _L Prod / DE Intake	0.42	0.42	0.41	0.44	0.02	0.86	0.70	0.69	0.53

¹ Treatments were CON- control with supplemental rumen-protected fatty acids (FA), SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² Trt: treatment effect, RPF: effect of rumen-protected FA, L: linear effect of saturation, and Q: quadratic effect of saturation.

³ Digestible energy intake as reported (Chapter 5)

⁴ NE_{L(intake)} = DMI (kg) x (0.0245 x TDN(%)) (NRC 1989).

⁵ NE_{L(milk)} (Mcal/d) = MY (kg) x (0.0929 x fat% + 0.0563 x true protein% + 0.0395 x lactose%) (NRC, 2001).

⁶ NE_{L(empty body weight change)} calculated according to NRC 2001

⁷ NE_{L(intake)} – NE_{L(maintenance)} – NE_{L(milk)}, where NE_{L(maintenance)} = 0.080 x BW^{0.75} (NRC 2001)

⁸ (NE_L Milk Yield + NE_L Body Weight Gain) / DE Intake

Table 9. Effects of dietary rumen-protected fatty acids on intake and total tract digestion for non-cannulated cows.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	Trt	RPF	L	Q
Intake, kg/d									
DM	26.7	26.3	25.4	25.4	0.97	0.26	0.12	0.27	0.54
OM	24.7	24.4	23.6	23.6	0.91	0.35	0.18	0.30	0.54
NDF	7.7	7.2	7.1	7.1	0.24	0.02	0.003	0.52	0.68
pdNDF	4.7	4.7	4.5	4.6	0.16	0.62	0.46	0.50	0.39
Starch	8.4	7.8	7.7	7.8	0.33	0.08	0.01	0.88	0.53
Total FA	1.4	2.2	2.0	2.0	0.07	<0.001	<0.001	0.004	0.54
C16 FA	0.28	0.51	0.48	0.46	0.02	<0.001	<0.001	0.01	0.71
C18 FA	0.99	1.40	1.31	1.28	0.05	<0.001	<0.001	0.06	0.004
TT Digested, kg/d									
OM	16.5	17.1	16.3	16.0	0.68	0.31	0.97	0.08	0.61
NDF	3.2	3.5	3.3	3.5	0.15	0.02	0.01	0.078	0.05
pdNDF	3.2	3.5	3.3	3.5	0.15	0.02	0.01	0.78	0.05
Starch	7.8	7.4	7.3	7.2	0.30	0.09	0.02	0.43	0.76
Total FA	1.0	1.6	1.4	1.4	0.06	<0.001	<0.001	0.09	0.35
C16 FA	0.21	0.39	0.36	0.35	0.01	<0.001	<0.001	0.03	0.37
C18 FA	0.71	0.98	0.92	0.94	0.04	<0.001	<0.001	0.38	0.34
TT Digested, % Intake									
OM	66.6	70.1	69.0	68.1	1.1	0.21	0.08	0.23	0.95
NDF	42.0	48.7	46.9	50.3	1.2	<0.001	<0.001	0.31	0.07
pdNDF	68.8	75.0	73.3	77.4	1.8	0.01	0.003	0.32	0.16
Starch	93.6	94.6	94.7	92.9	0.39	0.007	0.27	0.003	0.06
Total FA	71.3	70.8	70.6	73.7	1.3	0.29	0.80	0.11	0.29
C16 FA	75.7	76.0	74.5	76.4	1.1	0.54	0.99	0.77	0.16
C18 FA	71.6	69.7	70.3	73.9	1.4	0.15	0.86	0.04	0.35

¹ Treatments were CON- control with supplemental rumen-protected fatty acids (FA), SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² Trt: treatment effect, RPF: effect of rumen-protected FA, L: linear effect of saturation, and Q: quadratic effect of saturation.

Table 10. Effects of dietary rumen-protected fatty acids on energy balance and efficiency for non-cannulated cows.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	Trt	RPF	L	Q
Intake									
DE ³ , Mcal/d	77.4	83.8	78.9	78.2	3.4	0.17	0.25	0.07	0.44
NE _L ⁴ , Mcal/d	42.2	47.5	44.4	43.8	2.0	0.07	0.06	0.06	0.45
Production									
Milk NE _L ⁵ , Mcal/d	26.8	26.9	26.0	26.9	1.4	0.70	0.76	0.99	0.26
Empty BW change, kg / d	1.6	0.6	2.0	1.6	0.7	0.46	0.58	0.83	0.14
NE _L ⁶ BW change, / d	11.8	4.5	15.3	6.3	4.9	0.41	0.59	0.80	0.12
BCS change, /21d	0.05	0.14	0.17	0.17	0.05	0.27	0.06	0.63	0.78
Balance									
NE _L balance ⁷ , Mcal/d	3.52	1.76	-0.50	-1.53	1.6	0.07	0.04	0.09	0.72
Milk NE _L , %NE _L intake	64.2	57.3	58.8	61.4	2.7	0.10	0.04	0.15	0.82
NE _{Maint} , %NE _L intake	45.2	40.4	42.0	42.4	2.0	0.12	0.03	0.30	0.73
Efficiency									
NE _L Milk / DE Intake	0.35	0.32	0.33	0.34	0.01	0.27	0.17	0.16	0.73
NE _L Prod / DE Intake	0.50	0.38	0.52	0.43	0.06	0.39	0.43	0.59	0.15

¹ Treatments were CON- control with supplemental rumen-protected fatty acids (FA), SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² Trt: treatment effect, RPF: effect of rumen-protected FA, L: linear effect of saturation, and Q: quadratic effect of saturation.

³ Digestible energy intake as reported (Chapter 5)

⁴ NE_{L(intake)} = calculated from DE through ME according to NRC (2001)

⁵ NE_{L(milk)} (Mcal/d) = MY (kg) x (0.0929 x fat% + 0.0563 x true protein% + 0.0395 x lactose%) (NRC, 2001).

⁶ NE_{L(empty body weight change)} calculated according to NRC 2001

⁷ NE_{L(balance)} – NE_{L(maintenance)} – NE_{L(milk)}, where NE_{L(maintenance)} = 0.080 x BW^{0.75} (NRC 2001).

⁸ (NE_L Milk Yield + NE_L Body Weight Gain) / DE Intake

Table 11. Effects of dietary rumen protected fatty acids on plasma metabolites and hormones.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	Trt	RPF	L	Q
Cannulated Cows									
Insulin ³ , μ IU/ml	6.9	8.3	6.8	5.8	0.86	0.04	0.92	0.006	0.78
Glucagon, pg/ml	114.3	100.8	109.5	107.8	7.1	0.30	0.17	0.31	0.38
Glucose, ^{4,5} mg/dl	58.2	57.6	58.3	58.3	0.95	- ⁵	-	-	-
NEFA, ^{3,5} mM	74.7	64.1	76.0	68.0	3.0	-	-	-	-
β -HBA, ³ (mg/dl)	4.9	5.1	4.7	4.5	0.22	0.19	0.58	0.04	0.79
Non-cannulated cows									
Insulin ³ , μ IU/ml	9.2	8.6	9.0	9.2	1.2	0.95	0.78	0.61	0.93
Glucagon, pg/ml	124.4	125.6	130.6	128.7	9.9	0.85	0.54	0.69	0.62
Glucose, ⁴ mg/dl	56.0	57.7	57.0	57.7	1.2	0.49	0.16	0.98	0.53
NEFA, ^{3,5} mM	62.6	60.0	62.9	59.7	4.6	-	-	-	-
β -HBA, ³ (mg/dl)	5.4	4.8	5.0	5.1	0.25	0.34	0.12	0.39	0.85

¹ Treatments were CON- control with supplemental rumen-protected fatty acids (FA), SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² Trt: treatment effect, RPF: effect of rumen-protected FA, L: linear effect of saturation, and Q: quadratic effect of saturation.

³ Measured for a composite of 8 samples collected over 3 d, representing 3-h intervals of a 24-h day.

⁴ Calculated using 8 samples collected over 3 d, representing 3-h intervals of a 24-h day.

⁵ Significant Period by Treatment interaction.

CHAPTER 4

Kinetic model of rumen biohydrogenation: effects of rumen-protected fatty acid saturation on fractional rate of biohydrogenation and duodenal fatty acid flow in lactating dairy cows.

ABSTRACT

Saturated and unsaturated rumen protected fatty acid sources were evaluated for effects on fractional rate and extent of rumen biohydrogenation and duodenal fatty acid (FA) flow. Eight ruminally and duodenally cannulated multiparous Holstein cows (77 ± 12 DIM, mean $\pm$ SD) were used in a replicated 4x4 Latin square design with 21 d periods. Treatments were control and a linear titration of 2.5% added rumen-protected FA (RPF) varying in saturation: saturated (SAT; prilled hydrogenated free FA), intermediate mix of SAT and unsaturated (UNS; calcium soaps of long-chain FA), and UNS FA. A simple model of rumen FA metabolism is proposed that allows calculation of first order fractional rate of FA biohydrogenation and FA passage after determination of ruminal FA pool size and duodenal flux. Rumen protected FA increased rumen FA turnover rate. Passage rates of C16:0, C18:0 and total C18 were linearly decreased and trans-C18:1 fractional passage rate was quadratically affected with increasing UNS. Increasing UNS increased extent of C18:2 and C18:3 biohydrogenation, and decreased extent of 18:1 and trans-18:1 biohydrogenation. Calcium salts failed to protect polyunsaturated FA from rumen biohydrogenation despite a mean ruminal pH of 6.0, and UNS decreased rumen

biohydrogenation of trans-C18:1 leading to increased duodenal flow. This model allows a mechanistic description of rumen biohydrogenation and determination of extent of C18:1 biohydrogenation.

INTRODUCTION

Dietary fat serves a number of functions in lactating dairy cows including substrate for energy, components for cellular structure, and second messengers. Some fatty acids (FA) are biologically active with the ability to modify reproductive efficiency (Staples et al., 1998), milk fat synthesis (Bauman and Griinari, 2003), and metabolism (Drackley, 2000). Prilled, hydrogenated free fatty acids (FA) and calcium salts of FA are two commercially developed products marketed to minimize effects of fat on ruminal fermentation. Fatty acids bound to calcium ions are unavailable for bacterial uptake, but FA become available by dissociation of the calcium ion. Dissociation of the calcium ion is affected by pH and the binding affinity of the FA, with greater dissociation at lower pH, especially with more highly unsaturated FA (Sukhija and Palmquist, 1990).

Unsaturated FA available for uptake are partially biohydrogenated in the rumen leading to the production of trans-FA isomers and saturated FA (Harfoot and Hazlewood, 1988). Increasing FA saturation decreases the toxic effects of unsaturated FA on bacteria. Harfoot (1982) proposed that the goal of biohydrogenation by ruminal microbes is the production of saturated FA for incorporation into their plasma membranes. The high lipolytic capacity of ruminal microbes increases the pool of unsaturated free FA; if bacteria did not hydrolyze esterified FA there would be very low levels of available FA

in the rumen. Recognizing this goal of biohydrogenation may provide insight into biohydrogenation capacity and control.

Allen (2000) proposed that the extent of biohydrogenation is determined by the characteristics of the fat source, retention time in the rumen, and characteristics of the microbial population. Using simple enzyme kinetic theory, total biohydrogenation is determined by pool size of available FA, rumen retention time, and bacterial hydrogenation capacity that is a function of bacteria concentration, microbial population, and rumen environment. Microbial biohydrogenation is a multistep process for which the kinetics are not well documented. Beam et al. (2000) presented a schematic of lipid metabolism in the rumen that included lipolysis, isomerization and hydrogenation, resulting in formation of saturated FA. Hydrogenation of linolenic and linoleic acid results in the formation of trans monounsaturated FA after the formation of trans-diene intermediates that are rapidly metabolized (Harfoot and Hazelwood, 1988).

Biohydrogenation of oleic acid also includes formation a number of trans-C18:1 intermediates (Mosley et al. 2002). Trans-C18:1 can be hydrogenated to stearic acid or passed to the duodenum. Numerous *in vitro* studies have demonstrated efficient biohydrogenation of C18:2 and C18:3, but have noted decreased capacity for trans-C18:1 biohydrogenation, especially with increased polyunsaturated FA (PUFA; Beam et al. 2000), decreased pH (Martin and Jenkins, 2002) and decreased dietary fiber (Harfoot and Hazlewood, 1988).

Fatty acid profile reaching the duodenum is determined by FA metabolism in the rumen. The profile of absorbed FA can be altered to maximize animal efficiency and increase value of animal products by manipulating FA metabolism in the rumen if the

kinetics of rumen FA metabolism are understood. The objective of this experiment was to determine effects of protected FA differing in FA saturation on ruminal biohydrogenation and duodenal FA flow and more mechanistically describe the process of biohydrogenation.

MATERIALS AND METHODS

This is the second of four papers in a series from one experiment that evaluated effects of rumen-protected fat sources differing in fat saturation. This paper discusses treatment effects on rumen FA biohydrogenation and duodenal flow, and the companion papers focus on milk yield, milk FA profile and energy balance (Chapter 3), ruminal and post-ruminal nutrient digestion (Chapter 5), and intake, and feeding and chewing behavior (Chapter 6). Experimental procedures were approved by the All University Committee on Animal Use and Care at Michigan State University.

Cows and Treatments

Eight early lactation (77 ± 8.7 DIM; mean $\pm$ SD) ruminally and duodenally cannulated multiparous Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center were assigned randomly to replicated 4 x 4 Latin squares in a dose-response arrangement of treatments plus a control. Treatments were diets containing no added protected fat or 2.5% rumen protected FA (**RPF**) from saturated (**SAT**- prilled hydrogenated free FA, Energy Booster 100[®], Milk Specialties Company Inc.), an intermediate mixture of saturated and unsaturated (**INT**), or unsaturated FA (**UNS**- Ca Soaps of LCFA, Megalac-R[®], Church and Dwight Company, Inc., Princeton, NJ). Treatment periods were 21 d with the final 11 d used for sample and data collection.

Cows were ruminally and duodenally cannulated prior to calving and assigned randomly to treatment sequence. Surgery was performed at the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University. Immediately prior to initiation of the experiment, empty BW (ruminal digesta removed) was 516 ± 33 kg (mean $\pm$ SD).

Treatment mix and diet composition are reported in Chapter 3. Experimental diets contained 40% forage (66:33 corn silage: alfalfa silage), 13.5% whole cottonseed, dry ground corn, premixed protein supplement (soybean meal, corn gluten meal, and blood meal), a mineral and vitamin mix, and 2.5% added rice hulls (CON), saturated FA (SAT), 50:50 mix of saturated and unsaturated fat (INT) or unsaturated FA (UNS). All diets were fed as a total mixed ration.

Data and Sample Collection

Throughout the experiment, cows were housed in tie-stalls and fed once daily (0900 h) at 115% of expected intake. Amounts of feed offered and orts were weighed for each cow daily. Samples of all diet ingredients (0.5 kg) and orts from each cow (12.5%) were collected daily on d 11 to 14 and combined into one sample to represent four days for digestibility determination (d 11-14). Indigestible NDF was used as a marker to calculate duodenal flow for the cannulated cows. Duodenal samples (1,000 g) were collected every 9 h from d 12 to d 14 yielding eight samples representing every 3 h of a 24-hour period to account for diurnal variation.

Ruminal contents were evacuated manually through the ruminal cannula at 1350 h (4.5 h after feeding) on d 20, and at 0700 h (2 h before feeding) on d 21 of each period.

Total ruminal content mass and volume were determined. During evacuation, 10% aliquots of digesta were separated to allow accurate sampling. Aliquots were squeezed through a nylon screen (1 mm pore size) to separate into primarily solid and liquid phases. All samples were frozen immediately at -20°C .

Sample and Statistical Analysis

Forages and orts were ground with dry ice in a Wiley[®] mill (6mm screen; Authur H. Thomas, Philadelphia, PA), sub-sampled and lyophilized (Tri-Philizer™ MP, FTS Systems, Stone Ridge, NY) for analysis of DM concentration. Dried forage, orts, and RPF were reground in a Wiley[®] mill with a 1mm screen, dry ice was ground with the RPF to prevent fat from melting in the grinder. Concentrates were ground in a cyclone mill with a 2 mm screen (Udy Mill, Seedburo Equipment Co, Chicago, IL). Rumen liquid and solid subsamples were lyophilized, ground in a Wiley[®] mill with a 1mm screen, and recombined according to the original ratio of solid and liquid DM. Duodenal samples were thawed, combined, and filtered into primarily solid and liquid phases using nylon mesh (1 mm pore size) to minimize sampling errors due to segregation of samples into solid and liquid phases. Both phases were weighed, and sub-samples were taken from each phase. Liquid and solid sub-samples were lyophilized, ground in a Wiley[®] mill with a 1mm screen, and recombined by weight according to the original ratio of solid and liquid DM. A portion of all samples was placed in a Whirl Pac bag (NASCO, Fort Atkinson, WI) flushed with nitrogen gas and frozen for FA analysis.

Concentrations of all nutrients except DM were expressed as percentages of DM determined by drying at 105°C in a forced-air oven for more than 8 h. Indigestible NDF

was estimated as NDF residue after 240-h *in vitro* fermentation (Goering and Van Soest, 1970). Rumen fluid for the *in vitro* incubations was collected from a non-pregnant dry cow fed only alfalfa hay. Feeds and rumen and duodenal digesta FA were extracted according to Sukhija and Palmquist (1988). Ruminal pool sizes (kg) of nutrients were determined by multiplying the concentration of each component by the ruminal digesta DM mass (kg).

A simple model was developed to calculate biohydrogenation rate of unsaturated FA in the rumen (Figure 1). The model assumes that unsaturated FA are not oxidized, but are hydrogenated and appear in a less saturated pool, pool sizes are representative of steady state conditions and biohydrogenation follows first-order kinetics. Ruminal turnover, fractional passage rate (k_p) and fractional biohydrogenation rate (k_b) for each FA (FA_i) pool were calculated using the following equations:

Turnover rate in the rumen (% h⁻¹) =

$$\frac{(\text{FA}_i \text{ flow into pool from intake and biohydrogenation, g/h} / \text{Ruminal FA}_i \text{ pool, g})}{\times 100}$$

Fractional passage rate from the rumen (% h⁻¹) =

$$(\text{Duodenal FA}_i \text{ flow, g/h} / \text{Ruminal FA}_i \text{ pool, g}) \times 100$$

Fractional disappearance rate from the rumen (% h⁻¹) =

$$(\text{Rumen FA}_i \text{ turnover rate, g/h} / \text{Rumen FA}_i \text{ Pool, g}) \times 100$$

Fractional biohydrogenation rate from the rumen ((% h⁻¹)=

Fractional FA_i disappearance rate (% h⁻¹) - Fractional FA_i passage rate (% h⁻¹)

Extent of biohydrogenation (% biohydrogenated) =

= Fractional FA_i biohydrogenation rate (% h⁻¹) / [Fractional FA_i biohydrogenation rate (% h⁻¹) + Fractional FA_i passage rate (% h⁻¹)]

Total biohydrogenation index (Proportion of double bonds, %) was calculated according to Tice et al. (1994) =

$$100 - [100 * (\text{Duodenal } ((\text{C18:1} + (\text{C18:2} * 2) + (\text{C18:3} * 3)) / \text{Total C18}) / \text{Intake } (\text{C18:1} + (\text{C18:2} * 2) + (\text{C18:3} * 3)) / \text{Total C18}))]$$

C18:3 biohydrogenation index (Proportion of C18:3, %) =

$$100 - [100 * (\text{Duodenal } (\text{C18:3} / \text{Total C18}) / \text{Intake } (\text{C18:3} / \text{Total C18}))]$$

C18:2 biohydrogenation index (Proportion of C18:2, %) =

$$100 - [100 * (\text{Duodenal } (\text{C18:2} / \text{Total C18}) / \text{Intake } (\text{C18:2} / \text{Total C18}))]$$

All data were analyzed using the fit model procedure of JMP® (Version 5, SAS Institute, Cary, NC) according to the following model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk}$$

where

μ = overall mean,

C_i = random effect of cow (i = 1 to 8),

P_j = fixed effect of period ($j = 1$ to 4),

T_k = fixed effect of treatment ($k = 1$ to 4),

e_{ijk} = residual error.

Period by treatment interaction was evaluated, but was removed from the statistical model when not declared significant ($P > 0.10$). Period by treatment was not significant for any variable of primary interest; variables with significant interactions are noted in the tables. Data points with Studentized Residuals greater than 3.0 were considered outliers and excluded from analysis. Very few observations were excluded and rarely more than one per response variable. Preplanned contrasts include the effect of addition of RPF (CON vs. SAT, INT and UNS), linear effect of increasing concentration of unsaturated fat [L (SAT vs. UNS)], and quadratic effect of increasing concentration of unsaturated fat [Q (INT vs. SAT and UNS)]. The preplanned contrasts do not allow individual comparison of each fat treatment to the control. Protected LSD was used for mean separation when treatment was significant. Pearson correlation coefficients were determined between cow-period observations for some parameters. Treatment effects, linear and quadratic responses, and correlations were declared significant at $P < 0.05$, and tendencies were declared at $P < 0.10$.

One cow developed clinical mastitis on d 19 of period 3, and her rumen was not evacuated. Data collected in this period before d19 for this cows was included in statistical analysis. The cow did not fully recover and data from period 4 was not included in the dataset.

RESULTS AND DISCUSSION

Fatty Acid Intake and Duodenal Flow

Fatty acid intake and duodenal flow are reported in Table 1. Fatty acid intake is a function of dietary FA concentration and daily DMI. Rumen protected FA treatment increased total FA intake. Increasing UNS linearly decreased total FA intake primarily because of a large decrease in DMI (Chapter 5) but also from slightly lower FA concentration. Saturated FA increased intake of C16:0 and C18:0, while UNS increased intake of cis-18:1, 18:2 and 18:3.

Dietary FA are biohydrogenated by bacteria in the rumen, and animal response is highly dependent on the resulting FA profile reaching the duodenum. Dietary treatments were selected to maximize the difference in duodenal unsaturated FA flow, especially polyunsaturated FA (PUFA), using commercially available products. Saturated FA treatment increased FA flow by increasing dietary FA density with little effect on DMI, while UNS failed to increase duodenal FA flow compared to control because DM intake was depressed. Within RPF, increasing UNS linearly decreased duodenal FA flow. Duodenal flow of C16:0 and C18:0 was increased by SAT compared to CON, but was not affected by UNS. Increasing SAT linearly increased C18:0 flow, with SAT delivering nearly twice the flux of C18:0 to the duodenum as UNS. In contrast, duodenal flow of cis-C18:1 was not different between SAT and CON, but flows were increased by INT and UNS. Increasing UNS quadratically affected duodenal flow of trans-18:1 FA and linearly increased cis-18:1 FA. Although treatments were expected to drastically change duodenal PUFA flow, treatment did not affect flow of C18:2 FA reaching the

duodenum, and C18:3 FA was linearly decreased by UNS. UNS treatment failed to increase duodenal flow of C18:2 and C18:3 FA because of intake depression and incomplete protection of unsaturated FA. Addition of RPF increased duodenal flow of saturated FA for SAT treatment and monounsaturated FA for UNS treatment in this experiment.

Rumen protected FA increased duodenal C16:0 concentration, and UNS linearly increased C16:0. Increasing UNS linearly decreased duodenal C18:0 concentration and linearly increased cis- and trans-C18:1, C18:2 and C18:3 concentration. Using mean separation, SAT did not change, but UNS increased trans-C18:1 and C18:2 concentration relative to CON. Saturated FA decreased concentration of C18:3, but UNS did not differ from control.

Duodenal flow of CLA isomers could not be detected with the analytical procedures used. The FA extraction and methylation procedure of Sukhija and Palmquist (1988) causes partial transformation of CLA with only 57.4 and 54.9% methylated recovery of cis-9, trans-11 and trans-10, cis-12 CLA respectively (Duckett et al. 2002). However, duodenal flow of CLA is extremely low relative to total VFA; Piperova et al. (2002) reported 1.0 to 1.8 g/d flow of total CLA with 0.24 to 0.53 g/d flow of trans-9, cis-11 and 0.05 to 0.26 g/d flow of trans-10, cis-12 CLA. Similar CLA flow results were reported in steers (0.63 to 1.2 g/d CLA; Duckett et al., 2002), and in sheep (0.12 to 0.20 g/d cis-9, trans-11 CLA; Kucuk et al, 2001).

The large synthesis of cis-9, trans-11 in the mammary gland is a major contributor to total milk CLA secretion (7.2-9.1 g/d CLA), making rumen contribution of total CLA small compared to *de novo* synthesis (Piperova et al., 2002). However, some CLA

isomers are secreted in smaller amounts than duodenal flow suggesting the importance of rumen synthesis for some isomers.

Indigestible NDF was used as the flow marker for calculation of duodenal flow. Chromic oxide was originally intended for use as an external marker but resulted in unrealistically high duodenal flow possibly because of inadequate subsampling procedures for duodenal liquid. Indigestible NDF is prone to treatment bias as duodenal FA might affect *in vitro* fermentation for determination of indigestible residue. To insure that this was not the case, we also calculated duodenal and fecal flow using acid detergent-sulfuric acid lignin and 120 hr indigestible ADF after ether extraction. Results were similar for all markers and iNDF was chosen for calculation of duodenal flows.

Rumen Pool and Turnover

Rumen FA pool size is the mean of the pool size before and after feeding, and is assumed to represent steady state pool size (Table 3). Rumen protected FA increased rumen pool size of total and C16:0 FA. Saturated FA linearly increased C18:0 pool relative to UNS, while UNS was not different from CON. The rumen trans-C18:1 pool was linearly increased by UNS, but treatment did not affect cis-18:1, 18:2 or 18:3 pool sizes. We were unable to find previous research reporting FA pool sizes in the rumen. However, Abughazaleh et al. (2002, 2003) reported 2.6-8.7% trans-11 C18:1, 0.09-0.26% cis-9,trans-11 CLA, and up to 0.13 % trans-10,cis-11 CLA as a percentage of total FA in rumen grab samples. Fatty acid profile of grab samples from the rumen may not represent true FA profile of rumen contents because FA may associate differently with liquid and solid fractions of the rumen, biasing the sample. In the current study, trans-

C18:1 FA ranged from 5.4 to 8.4% of total FA, and CLA was not detected, possibly because of limitations of our FA analysis method. Conjugated linoleic acid rumen pools may be of minimal importance in discussion of rumen FA biohydrogenation, but trans-C18:1 is an important pool for modeling ruminal biohydrogenation because of its much greater size.

Kinetics of Biohydrogenation

Biohydrogenation is traditionally reported as the proportion of unsaturated FA or double bonds removed in the rumen (Wu et al., 1991, and Tice et al. 1994). Although this is an index of rumen metabolism it fails to mechanistically describe FA biohydrogenation. Also, this method is inadequate for calculation of biohydrogenation of monounsaturated FA because appearance from biohydrogenation of more PUFA is not determined. Rate of biohydrogenation may be determined by *in vitro* batch and continuous culture systems (Wu and Palmquist, 1991, and Beam et al. 2000), although both may have limited application to normal rumen fermentation. *In situ* methods have also been employed (Enjalbert et al. 2003), but have limited application because of FA entrance and exit from the *in situ* bags.

Methods for modeling rumen carbohydrate digestion (Allen and Mertens, 1998) can be applied to rumen FA metabolism. The pool and flux method determines first order rumen passage and digestion rates using duodenal flow and rumen pool size (Firkins et al., 1998). Measuring FA pool size and duodenal passage allows calculation of rumen retention time and determination of the fractional rate of FA biohydrogenation, assuming all disappearance of FA is because of biohydrogenation and not from oxidation

or absorption. The model also allows calculation of the extent of biohydrogenation of individual FA calculated from passage and biohydrogenation rates.

Determination of fractional passage and biohydrogenation rates are complicated by heterogeneous pools (ex. free in rumen, adsorbed to feed, associated with metal ion, etc), and determination of first order kinetics rely on the assumption that enzyme concentration is not limiting. In addition, FA entry rate for some pools is from biohydrogenation in addition to intake. Two versions of a simplified model were developed to calculate rumen FA biohydrogenation rates while accounting for appearance of FA from biohydrogenation (Figure 1). Model A (Figure 1a) assumes biohydrogenation of C18:3 and C18:2 directly to C18:1, and biohydrogenation of C18:1 to C18:0. Model B (Figure 1b) partitions C18:1 to cis- and trans-C18:1 FA pools and assumes biohydrogenation of C18:3, C18:2 and cis-C18:1 to trans-18:1, and biohydrogenation of trans-C18:1 to C18:0. The C18:3 and C18:2 pools are cis isomer pools only, representing cis-9,cis-12 C18:2 and cis-9,cis-12,cis-15 C18:3, reference to C18:3 and C18:2 simply refers to these FA in the remainder of the paper.

Harfoot and Hazlewood (1988) provide detailed description of FA biohydrogenation, including the production of trans-diene intermediates during biohydrogenation of C18:3 and C18:2. Ruminal pools of these intermediates were not detectable in the current experiment, and others have reported low rumen concentration of trans-diene FA (AbuGhazaleh et al. 2002 and 2003). Biohydrogenation intermediates have very high rates of biohydrogenation as indicated by high rates of C18:2 and C18:3 biohydrogenation compared to very small pool sizes of biohydrogenation intermediates.

Production of trans-diene intermediates and more complex pathways are recognized, but were not expected to improve model utility.

Biohydrogenation of cis-C18:1 to C18:0 was traditionally believed to occur by direct biohydrogenation without formation of intermediates (Harfoot and Hazlewood, 1988). However, increasing rumen available oleic acid has been shown to increase trans-C18:1 FA concentration in rumen digesta (AbuGhazaleh et al., 2003), concentration in rumen digesta and duodenal flow (Kalscheur et al. 1997) and concentration in milk (DeLuca and Jenkins, 2000; Jenkins, 2000; Kalscheur et al. 1997, 2003; and AbuGhazaleh et al. 2003). Mosely et al. (2002) observed production of a number of trans-C18:1 intermediates during *in vitro* biohydrogenation of cis-9 C18:1. *In vivo* observation of increased trans-C18:1 with cis-18:1 treatments may be from cis-C18:1 inhibition of biohydrogenation. Direct inhibition by cis-18:1 has not been explored, but increasing C18:2 concentration decreases biohydrogenation *in vitro* (Beam et al. 2000). *In vitro* production of trans-C18:1 from cis-C18:1 provides strong evidence for formation of the trans-C18:1 intermediate. Direct formation of C18:0 from cis-C18:1 requires a kinetic approach to verify as both paths result in the formation of C18:0. The presence of the direct path and partitioning of biohydrogenation between the direct and indirect route is not known and may vary with bacterial population and rumen environment. In addition, Proell et al. (2002) observed 15% formation of cis-18:1 from labeled trans-9 18:1 in *in vitro* batch culture, although it appears to be a slow reaction resulting in only a slight enrichment of the cis-18:1 pool after 48 h of incubation. In addition, trans-9 C18:1 is a very small pool in the rumen, which combined with the slow rate, limits appreciable formation of cis-C18:1 in the rumen. Data concerning formation of cis-C18:1 from trans-

C18:1 FA present in the rumen in higher concentrations is not available. Reverse reactions expected to result in very small fluxes are expected to be of little consequence because of dilution by the large forward flux and are ignored in these simple models. Therefore, model B assumes all biohydrogenated cis-18:1 enters the trans-18:1 pool.

The models assume that disappearance of FA is because of biohydrogenation and not absorption or oxidation. Loss of FA from the rumen through absorption and oxidation are often overlooked or considered minimal. Ruminant infusion of radioactive labeled linoleic acid and diversion of duodenal flow showed minimal plasma recovery of label (Jenkins, 1993). However, ruminal loss of FA is commonly observed in digestion studies; Jenkins (1993) reported FA loss in 15 of 47 published studies. Although this could be attributed to flow marker bias, Doreau and Chillard (1997) discussed evidence supporting FA oxidation and absorption in the rumen, citing *in vitro* oxidative capacity of rumen epithelium and possible oxygen transfer across the epithelium to bacteria capable of oxidative metabolism. Rumen digestion or absorption of C18:3, C18:2 and cis-C18:1 will inflate the biohydrogenation rate of trans-C18:1 because of an overestimation of influx to the trans-C18:1 pool. Wu et al. (1991) proposed calculation of biohydrogenation as a proportion of total C18 to correct for rumen loss of FA. The same could be done for the kinetic models by setting C18 intake equal to duodenal C18 flow by calculating individual C18 FA intake as duodenal total C18 flow multiplied by the C18 proportion of the FA fed. However, actual observed intake and duodenal flow values were used in model parameterization in the present study. Fractional rumen disappearance of total and C16 FA was not changed by treatment, but disappearance rate of C18 tended to increase with increasing UNS. Apparent rumen digestion of C16 FA

was increased with RPF, and increasing UNS RPF tended to increase total and C18 FA (Chapter 5). Rumen turnover of total, C18, and C16 FA was increased by RPF (Table 3). Dietary FA in the control treatment were fed as whole cottonseed that would be expected to be retained in the rumen fiber mat increasing ruminal retention time. Rumen protected FA fed in granular form with smaller particle size are expected to flow from the rumen faster than FA associated with cottonseed because they are less likely to be retained in the fibrous mat. Increasing UNS had no effect on rumen FA turnover.

Rumen protected FA did not affect fractional passage rate of total FA, C18:2 or C18:3 from the rumen (Table 4). Fractional passage rate of total C18 FA was linearly increased by SAT, but was not different for UNS compared to CON. Increasing UNS linearly decreased C16:0, C18:0 and total C18 fractional passage rate, did not affect fractional passage rate of cis-C18:1, and affected fractional passage of trans-C18:1 quadratically with the highest value for INT. Differences in FA passage rates within RPF may represent different FA pool types, association with different fractions, or associative effects on rumen passage of other nutrient pools; fractional passage rate of indigestible NDF from the rumen increased linearly with increasing SAT (Chapter 5).

Fractional rate of biohydrogenation of individual FA in the rumen is an important mechanistic measure as it reports FA biohydrogenation as a proportion of the available FA. Increasing UNS had no effect on C18:2 biohydrogenation but linearly increased fractional biohydrogenation rate of C18:3 (Table 4). Increased fractional rate of C18:3 biohydrogenation with UNS identified poor rumen protection by calcium salts. More highly unsaturated FA are expected to have a higher pKa (Sukhija and Palmquist, 1990) increasing dissociation of the calcium FA complex as pH decreases. However, ruminal

pH in this experiment was moderate and not affected by treatment with a mean of 6.0 (Chapter 6).

Addition of SAT did not affect rate of biohydrogenation of total C18:1, trans-C18:1 and cis-C18:1 compared to CON. However, UNS linearly decreased rate of biohydrogenation of C18:1 and trans-C18:1, and tended ($P = 0.08$) to increase rate of cis-C18:1 biohydrogenation. A potential bias of increased rumen FA loss with UNS may have increased the observed fractional rate of C18:1 biohydrogenation.

Biohydrogenation of trans-18:1 is considered the rate limiting step of rumen biohydrogenation (Harfoot and Hazelwood 1988), allowing trans-C18:1 to accumulate in the rumen. Biohydrogenation of trans-C18:1 cannot be determined by previous measurements of rumen FA metabolism, but the proposed models allow determination of C18:1 biohydrogenation. Increased duodenal flow of trans-C18:1 for UNS is from greater intake of unsaturated FA (particularly cis-C18:1), increasing inflow to the trans-C18:1 pool by biohydrogenation, and a decreased rate of biohydrogenation and increased passage rate of trans-C18:1 from the rumen..

Extent of Biohydrogenation

Extent of biohydrogenation of C18:1 and more specifically trans-C18:1 was linearly decreased by increasing concentration of unsaturated FA (Table 5). There was no difference in extent of biohydrogenation of cis-C18:1, but biohydrogenation of C18:2 and C18:3 were increased by increasing unsaturated FA. In agreement, biohydrogenation index calculated according to Tice et al. (1994) shows a linear increase in the number of double bonds destroyed with increasing UNS. Modification of the index to calculate

biohydrogenation of individual PUFA showed that biohydrogenation of C18:3 tended to increase linearly and C18:2 tended to be affected quadratically with increasing UNS.

Model Simplifications and Assumptions

Determination of first order kinetics by the pool and flux method relies on the assumption of homogenous pools and that the entire pool is available for biohydrogenation. This could cause errors in the parameters because not all FA are in the same form, and FA form affects availability for bacterial uptake. Fatty acids that are esterified, associated with a metal ion, or simply adsorbed to feed particle are not available for bacterial uptake and subsequent biohydrogenation. In the current study, RPF were non-esterified but differed in association with metal cations. A more realistic representation of available FA pool is presented in Figure 2, and represents a submodel of biohydrogenation for each individual FA. Each FA pool can be subdivided first into esterified and free FA, and secondly into available and unavailable pools within esterified and unesterified pools. Esterified FA undergo lipolysis by bacteria to yield free FA, however not all esterified FA are physically available to bacteria. The availability of esterified FA depends on their location within plant cellular structure, seed coats, within pellets, or adsorbed to particles. The lag for availability represents hydration and breakdown of feed particle making esterified FA available for bacterial hydrolysis. Fatty acids produced from hydrolysis are expected to enter the available free FA pool. Free or unesterified FA also enter the rumen directly by feed intake. Unavailable free FA represents FA adsorbed to or protected by feed particles and FA associated with metal cations. Nutritionists have recognized that the addition of metal cations such as calcium

can partially alleviate FA inhibition of fiber digestion through formation of an insoluble salt that blocks FA uptake by microbes (Palmquist and Jenkins, 1980). Increasing saturation and chain length of the FA increases the amount and strength of salt formed (Jenkins and Palmquist, 1982). The formation of the metal salts is determined by the binding affinity of the cation and the dissociation constant of the fatty acid. Fatty acid binding to metal cations is partially dependent on pH of the rumen and the pK_a of the FA. Sukhija and Palmquist (1990) determined the pK_a for calcium salts of stearate, tallow, palm FA and soy oil to be 4.5, 4.5, 4.6 and 5.6 respectively. These pK_a values are somewhat misleading since they are determined for a mixture of FA. Soy oil contains a much higher concentration of unsaturated FA than the other treatments, and demonstrates the high pK_a of unsaturated FA. Variation in rumen pH is expected to change the amount of FA available for bacterial uptake and biohydrogenation as FA salts can be formed and destroyed in the rumen. The pool size of FA available for biohydrogenation by the model represented in Figure 2 is a function of the rate of esterified FA availability (k_{lag}), the rate of lipolysis (k_{lip}), rate of free FA availability (k_{avl}), rate of complexing as a salt (k_c), rate of passage (K_p) and rate of biohydrogenation (k_b). Although total esterified and free FA pools can be determined and may represent a significant improvement in representing biohydrogenation, the available and unavailable pools of each fraction cannot be realistically evaluated.

Differences in the fractional biohydrogenation rate calculated using the simplified models (Figure 1) indicates that the pools are not homogeneous and differ in availability, or that rates are affected by the microbial population and are not first-order. Future

research should resolve the relative influence of each on rate of FA biohydrogenation in the rumen.

CONCLUSION

Calculation of individual FA biohydrogenation with a simplified model allows a more mechanistic description of rumen FA biohydrogenation. The model described increased duodenal trans-C18:1 FA flow as the result of increased ruminal production of trans-C18:1 and decreased trans-C18:1 biohydrogenation. Future ability to predict duodenal FA profile depends on observation and analysis of individual steps of FA metabolism. Modeling biological systems requires assumptions and simplification of unknown or undeterminable events. Assumptions for this model must be tested in the future. It is our hope that this model will be challenged and improved with new experimental data. More complex models may be developed in the future to model production of individual trans isomers, although flux through such pathways may require isotope labeling.

Figure 1

Panel A

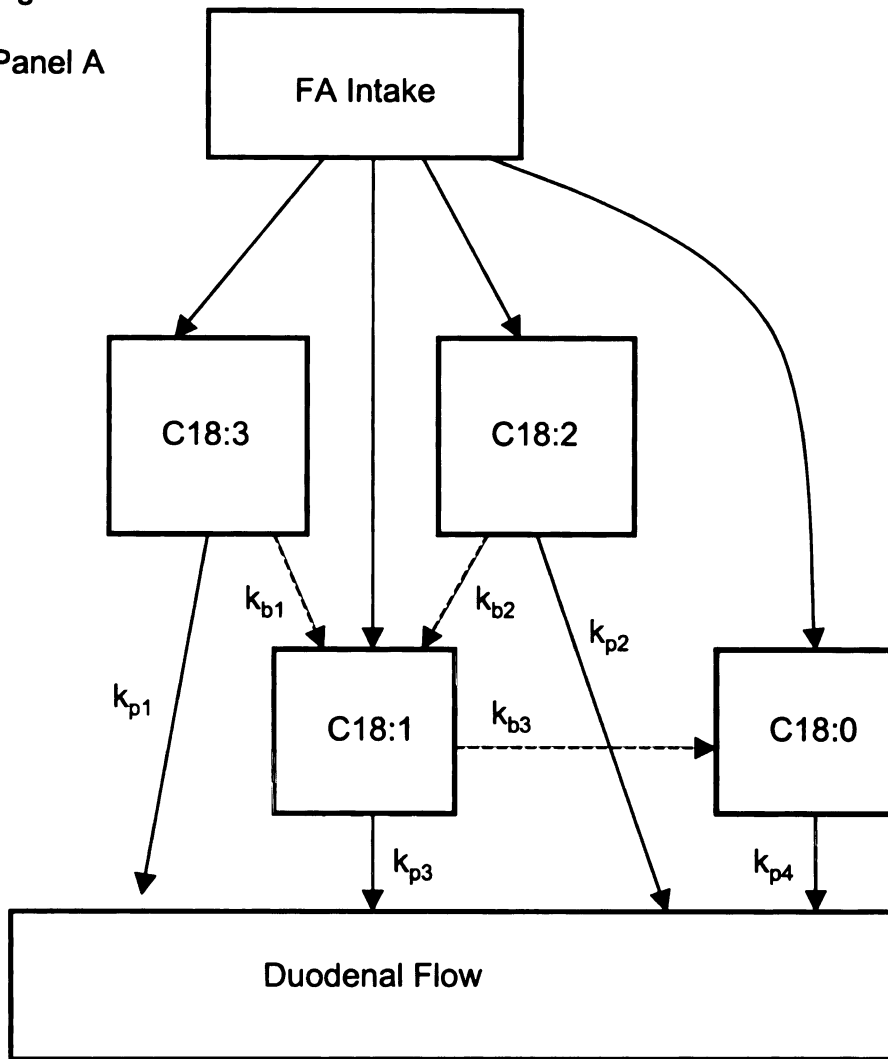


Figure 1: Panel A. Simple model of rumen biohydrogenation for calculation of fractional passage and biohydrogenation rates

Figure 1 (Cont.)

Panel B

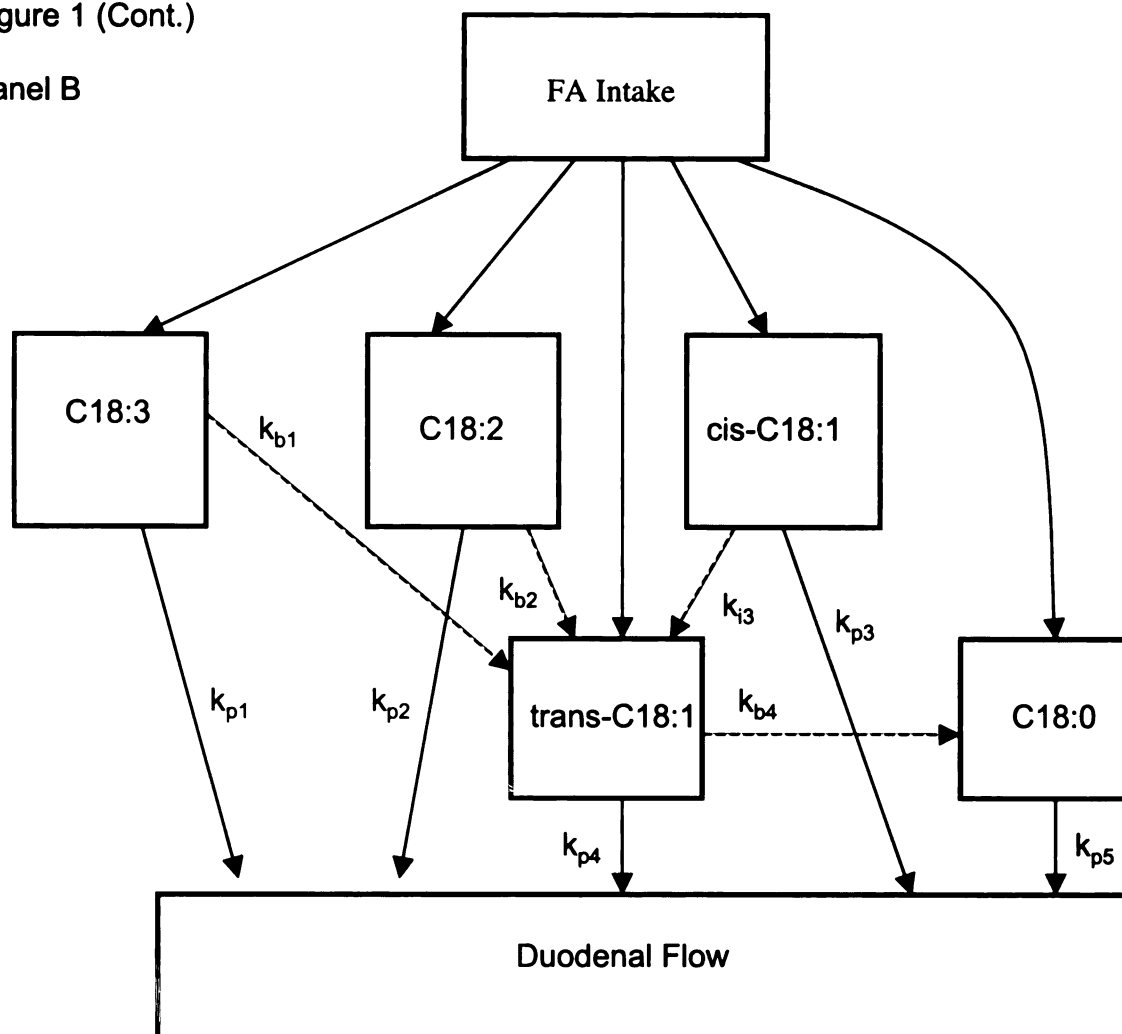


Figure 1. Simplified model of rumen biohydrogenation for calculation of fractional passage and biohydrogenation rates. The model allows calculation of rumen FA fractional biohydrogenation and passage rates while accounting for appearance of FA from biohydrogenation. The simple model (Panel A) assumes biohydrogenation of C18:3 (k_{b1}) and C18:2 (k_{b2}) directly to C18:1, and biohydrogenation of C18:1 (k_{b3}) to C18:0. The second model partitions C18:1 to cis- and trans-C18:1 FA pools. The more complex model in Panel B assumes biohydrogenation of C18:3 (k_{b1}), and C18:2 (k_{b2}) to trans-C18:1, and isomerization of cis-C18:1 (k_{i3}) to trans-C18:1. Finally, trans-C18:1 (k_{b3}) is biohydrogenated to C18:0. The C18:3 and C18:2 pools represent cis-9,cis-12 C18:2 and

cis-9,cis-12,cis-15 C18:3 and do not contain trans isomers. Production of trans-diene intermediates is ignored due to their small pools relative to their large flux. Each FA pool is available for passage and each passes at its own rate signified by different subscript (k_{p1-5}).

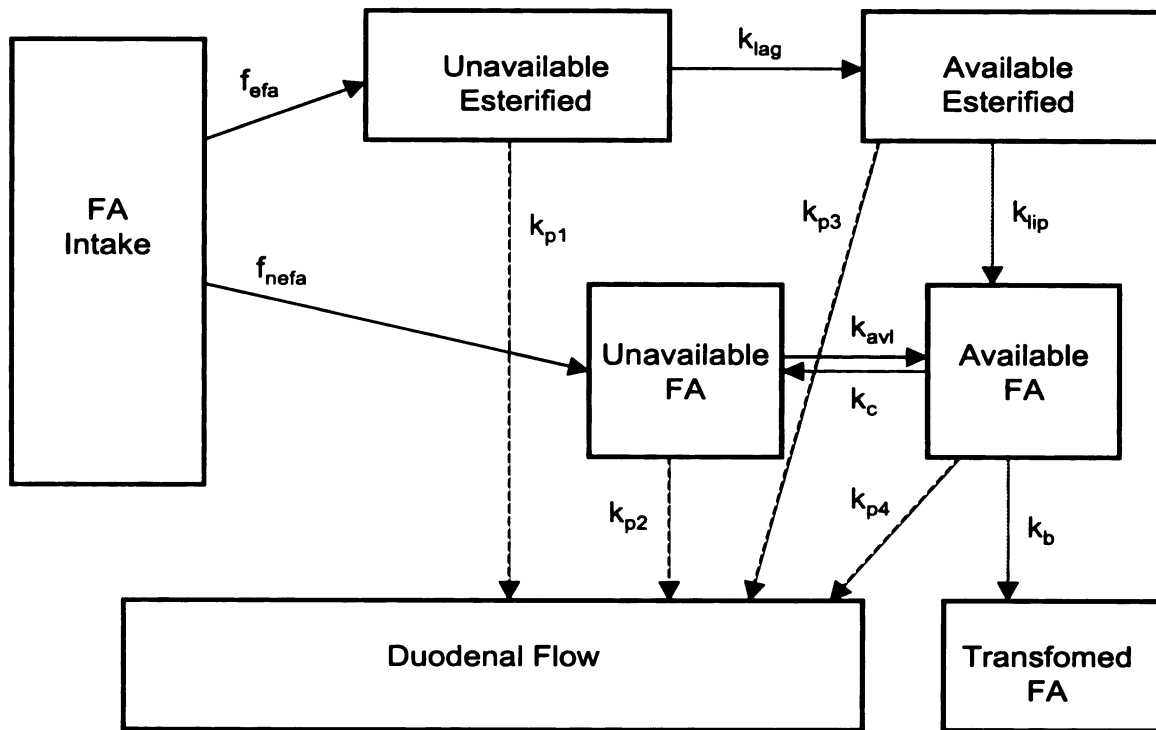


Figure 2: Model describing fatty acids (FA) available for bacterial uptake in the rumen recognizing heterozygous FA pools. Fatty acid intake is partitioned into an esterified FA fraction (f_{efa}) and a non-esterified FA fraction (f_{nefa}). Unavailable FA include FA in cellular structures, adsorbed to feed particles, protected in seedcoats, and bound to metal cations. A lag function (k_{lag}) represents the rate that esterified FA become available for bacterial lipolysis, and k_{lip} is the rate that available esterified FA are hydrolyzed to free FA. Hydrolyzed FA enter the available FA pool. Unavailable FA become available by breakdown of feed particle in digestion and dissociation of metal cations due to pH, and available FA can become unavailable by complexing with a metal cation (k_c). Available FA are taken up by microbes and biohydrogenated, transforming them to different FA or FA isomer. The rate of biohydrogenation is represented as k_b . All FA pools are available for passage to the duodenum and pass at different rates signified by different subscripts (k_{p1-4}).

Table 1. Fatty acid composition of diets

	CON	SAT	INT	UNS
Fatty Acid	-----% of Total FA ² -----			
C14:0	0.5 ^d	1.4 ^a	1.0 ^b	0.6 ^c
C16:0	18.8 ^c	22.6 ^b	22.9 ^{ab}	23.1 ^b
C18:0	2.6 ^c	17.6 ^a	10.5 ^b	3.0 ^c
C18:1 trans-11	0.05 ^c	0.40 ^a	0.27 ^b	0.12 ^c
C18:1 cis-9	15.6 ^b	12.0 ^c	15.6 ^b	19.4 ^a
C18:1 cis-11	0.69 ^b	0.60 ^c	0.69 ^b	0.78 ^a
C18:2	46.6 ^a	31.3 ^d	35.1 ^c	39.3 ^b
C18:3	2.8 ^a	1.8 ^d	2.1 ^c	2.3 ^b
> C20	1.4 ^a	1.3 ^b	1.3 ^c	1.2 ^d
Total FA, % DM	5.5 ^d	8.3 ^a	8.1 ^b	7.8 ^c
Unsaturated FA, % DM	3.6 ^d	3.9 ^a	4.4 ^b	4.9 ^c
C16:C18 ratio	0.28 ^b	0.36 ^a	0.36 ^a	0.36 ^a

¹ Treatments were CON- control with no supplemental rumen-protected fatty acids (FA), SAT- saturated FA from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated FA fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

³ Means with different superscripts differ by P<0.05

Table 2. Effects of rumen-protected fatty acids varying in saturation on FA intake, duodenal flow, and biohydrogenation.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Intake, g/d									
Total FA	1500	2100	2000	1800	70	<0.001	<0.001	<0.001	0.68
C16:0	280	490	460	430	20	<0.001	<0.001	0.003	0.97
C18:0	40	380	210	50	10	<0.001	<0.001	<0.001	0.19
C18:1 trans	1.2	12	6.9	2.7	0.4	<0.001	<0.001	<0.001	0.39
C18:1 cis	240	270	320	360	12	<0.001	<0.001	<0.001	0.21
C18:2	690	670	700	710	30	0.09	0.77	0.02	0.28
C18:3	41	40	44	47	1	<0.001	0.04	<0.001	0.50
Duodenal Flow, g/d									
Total FA	1400	2000	1900	1500	120	<0.001	0.003	<0.001	0.25
C16:0	290	440	430	350	30	<0.001	<0.001	0.007	0.29
C18:0	580	880	680	470	53	<0.001	0.07	<0.001	0.89
C18:1 trans	160	170	280	260	24	<0.001	0.003	0.001	0.02
C18:1 cis	75	84	100	100	6.3	<0.001	<0.001	0.003	0.15
C18:2	94	100	99	97	6.6	0.57	0.28	0.37	0.99
C18:3	7.4	7.8	7.6	6.6	0.4	0.16	0.87	0.04	0.41
Duodenal Composition, % Total FA									
C16:0	20.4	22.3	22.8	23.4	0.33	<0.001	<0.001	0.03	0.88
C18:0	41.1	44.6	36.7	30.9	1.4	<0.001	0.03	<0.001	0.53
C18:1 trans	11.1	8.5	15.0	18.2	1.4	<0.001	0.07	<0.001	0.31
C18:1 cis	5.2	4.2	5.4	6.9	0.16	<0.001	0.11	<0.001	0.47
C18:2	6.7	5.1	5.3	6.4	0.28	<0.001	0.003	0.002	0.15
C18:3	0.53	0.39	0.41	0.47	0.02	0.001	<0.001	0.02	0.39

¹ Treatments were CON- control with no supplemental rumen-protected fatty acids (FA), SAT- saturated FA from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated FA fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

Table 3. Effects of rumen-protected fatty acids varying in saturation on ruminal pools and turnover rates

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Ruminal Pool, g									
Total FA	920	1040	1040	960	40	0.04	0.02	0.10	0.29
C16:0	200	230	240	230	10	0.001	<0.001	0.82	0.24
C18:0	280	390	330	260	20	<0.001	<0.05	<0.001	0.99
C18:1 trans	56	56	75	80	4.7	<0.001	0.001	<0.001	0.06
C18:1 cis	75	79	85	90	6.6	0.24	0.15	0.15	0.99
C18:2	160	170	170	150	15	0.82	0.86	0.52	0.61
C18:3	4.5	4.8	4.8	4.3	0.4	0.64	0.70	0.29	0.57
Rumen Turnover, % h⁻¹									
Total FA	6.8	8.1	7.9	7.8	0.32	0.03	0.005	0.60	0.93
C16	6.0	8.1	7.9	7.7	0.32	0.001	<0.001	0.44	0.94
C18	7.2	8.1	8.1	8.1	0.34	0.13	0.02	0.90	0.98

¹ Treatments were CON- control with no supplemental rumen-protected fatty acids (FA), SAT- saturated FA from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated FA fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

Table 4. Effects of rumen-protected fatty acids varying in saturation on rates of passage from the rumen and rates of biohydrogenation.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Passage Rate (K_p), % h⁻¹									
Total FA	6.4	7.3	7.4	6.5	0.36	0.14	0.16	0.11	0.26
C16:0	6.3	7.5	7.2	6.2	0.40	0.06	0.12	0.03	0.47
C18:0	8.4	9.6	8.7	7.6	0.62	0.11	0.78	0.02	0.86
C18:1-trans	5.0	5.2	7.1	6.3	0.56	0.04	0.07	0.16	0.04
C18:1-cis	4.2	4.7	4.9	4.8	0.31	0.37	0.11	0.85	0.57
C18:2	2.4	2.6	2.5	2.6	0.19	0.67	0.28	0.89	0.62
C18:3	7.1	6.9	6.9	7.1	0.65	0.99	0.90	0.88	0.91
Total C18	6.5	7.5	7.4	6.6	0.33	<0.05	0.07	0.04	0.36
Digestion Rate, % h⁻¹									
TFA	0.31	0.60	0.74	1.43	0.37	0.17	0.15	0.11	0.54
C16	-0.20	0.81	0.77	1.6	0.46	0.05	0.02	0.19	0.41
C18	0.72	0.69	0.97	1.64	0.35	0.20	0.35	0.07	0.66
Biohydrogenation Rate (k_b), % h⁻¹									
Model A									
C18:3	31.1	28.2	34.2	38.9	2.3	0.02	0.31	0.003	0.81
C18:2	15.0	14.6	16.1	16.7	1.08	0.44	0.50	0.15	0.76
C18:1	20.6	20.0	15.8	15.7	1.44	0.01	0.02	0.02	0.18
Model B									
C18:1 trans	48.4	47.5	34.1	33.4	3.4	<0.001	0.004	0.001	0.07
C18:1 cis	9.4	10.1	11.3	12.0	0.79	0.09	0.06	0.08	0.78

¹ Treatments were CON- control with no supplemental rumen-protected fatty acids (FA), SAT- saturated FA from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated FA fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

Table 5. Effects of rumen-protected fatty acids varying in saturation on extent of biohydrogenation and biohydrogenation index.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Biohydrogenation Extent (% Intake)									
C18:1	73.4	71.5	61.9	63.6	2.8	0.01	0.02	0.04	0.09
C18:1 trans	90.5	90.1	82.0	83.0	2.1	0.006	0.02	0.01	0.06
C18:1 cis	69.0	68.0	69.1	71.6	1.8	0.41	0.74	0.12	0.74
C18:2	86.4	84.5	86.1	86.6	0.77	0.07	0.33	0.02	0.42
C18:3	81.8	80.2	82.9	84.9	1.1	0.03	0.47	0.005	0.78
Biohydrogenation Index, (% intake) ³									
Total C18	72.0	69.6	64.7	61.8	1.5	0.001	0.001	<0.001	0.55
C18:3	79.6	78.4	80.8	81.1	1.1	0.24	0.68	0.07	0.42
C18:2	84.9	83.1	84.6	83.1	0.70	0.16	0.12	0.85	<0.10

¹ Treatments were CON- control with no supplemental rumen-protected fatty acids (FA), SAT- saturated FA from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated FA fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

³ Calculated according to Tice et al. 1994.

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CHAPTER 5

Effects of rumen-protected fatty acid saturation on ruminal and total tract nutrient digestion in lactating dairy cows

ABSTRACT

Saturated and unsaturated rumen-protected fat sources were evaluated for effects on ruminal digestion kinetics, and ruminal and post-ruminal nutrient digestion. Eight early lactation ruminally and duodenally cannulated cows (77 ± 12 DIM, mean $\pm$ SD) were used in a replicated 4x4 Latin square design with 21 d periods. Treatments were control (CON) and a linear titration of 2.5% added rumen-protected fatty acids varying in unsaturation; saturated (SAT; prilled hydrogenated free FA), 50:50 mix of SAT and unsaturated (UNS; calcium soaps of long-chain FA), and UNS. The base ration included 37.2% forage and 13.5% cottonseed. SAT linearly decreased ruminal digestibility of DM and OM because of a linear reduction in ruminal neutral detergent fiber (NDF) digestibility. The reduction in ruminal NDF digestibility was because of a linear decrease in digestion rate and a linear increase in passage rate of potentially digestible NDF for SAT treatment. Digestibility was not different between treatments because of compensatory post-ruminal digestion. Ruminal FA and C18 FA digestibility tended to increase linearly with UNS, and post-ruminal C18 FA digestibility increased with UNS. SAT linearly decreased ruminal OM digestibility and decreased intestinal long-chain FA

digestibility, although differences in FA digestibility may be partially explained by FA intake.

INTRODUCTION

Fat is commonly included in diets of dairy cows to increase energy density. Rumen available fatty acids (FA), from oilseeds, byproducts and tallow, can be used to increase dietary FA concentration up to 3 percentage units with minimal negative effects on microbial growth and rumen function (NRC, 2001). However, unprotected unsaturated FA are absorbed by rumen bacteria and can be toxic to the microbes unless saturated by biohydrogenation (Harfoot, 1981).

Prilled FA and calcium salts of FA are two commercially available rumen-protected fat (RPF) sources commonly used to increase dietary FA concentration. Prilled FA are highly saturated FA developed to decrease interference with microbial fermentation. Calcium salts of long-chain FA are FA complexed with a calcium ion making them insoluble. Microbes cannot absorb FA as calcium salts and FA salts have little effect on microbial fermentation. However, the complex dissociates as ruminal pH decreases allowing microbial uptake and biohydrogenation (Wu et al., 1991).

Rumen-protected FA are often prescribed to increase daily energy intake, although the ability of the cow to increase daily energy intake depends on the energy density of the diet, diet digestibility and site of nutrient digestion. Associative effects of FA may shift site of nutrient digestion from the rumen to the intestine possibly reducing diet digestibility. We previously reported that UNS increased trans-FA duodenal flow

through increased biohydrogenation of polyunsaturated FA and decreased rates of biohydrogenation and passage of trans-18:1 (Chapter 4). Saturated FA treatment increased C16:0 and C18:0 flow to the duodenum and had little effect on ruminal metabolism of FA compared to control.

The objective of this experiment was to determine the effects of RPF saturation on ruminal digestion kinetics and site and extent of nutrient digestion. We hypothesized that the more unsaturated RPF source would decrease feed intake, increasing ruminal retention time and ruminal OM digestibility compared to the saturated RPF treatment.

MATERIALS AND METHODS

This paper is the third of a series of four papers from one experiment that evaluated effects of RPF differing in FA saturation. This paper discusses treatment effects on ruminal digestion kinetics and site of digestion, and the companion papers focus on milk production, milk FA profile and energy balance (Chapter 3), ruminal kinetics and extent of biohydrogenation (Chapter 4), and feed intake and feeding and chewing behavior (Chapter 6). Experimental procedures were approved by the All University Committee on Animal Use and Care at Michigan State University.

Treatments and Cows

Eight ruminally and duodenally cannulated multiparous Holstein cows (77 ± 8.7 DIM; mean $\pm$ SD) from the Michigan State University Dairy Cattle Teaching and Research Center were used in a replicated 4 x 4 Latin square design experiment. Cows were randomly assigned to treatment sequence. Treatments were a control diet (CON)

containing no added RPF or 2.5% RPF from saturated (SAT, prilled hydrogenated free FA, Energy Booster 100[®], Milk Specialties Company Inc., Dundee, IL), intermediate mixture (50:50) of saturated and unsaturated (INT), or unsaturated (UNS, Ca Soaps of LCFA, Megalac-R[®], Church and Dwight Company, Inc., Princeton, NJ) FA. Treatment periods were 21 d with the final 11 d used for sample and data collection. Cows were surgically prepared prior to calving and duodenal cannulas were soft gutter type made of tygon and vinyl tubing (Crocker et al., 1998). The duodenum was fistulated proximal to the pylorus region and prior to the pancreatic duct and cannulas were placed between the 10th and 11th ribs as described by Robinson et al. (1985). Both ruminal and duodenal surgeries were performed at the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University. Immediately prior to initiation of the experiment, empty BW (ruminal digesta removed) of cows was 516 ± 33 kg (mean $\pm$ SD).

Diet components and composition are presented in a companion paper (Chapter 3). Briefly, experimental diets contained 40% forage (66:33, corn silage: alfalfa silage), 13.5% whole cottonseed, dry ground corn, premixed protein supplement (soybean meal, corn gluten meal, and blood meal), a mineral and vitamin mix, and 2.5% added rice hulls (CON), saturated FA (SAT), 50:50 mix of saturated and unsaturated FA (INT) or unsaturated FA (UNS). The base diet contained 5.5% FA with 2.5% FA from whole cottonseed. All diets were fed as a total mixed rations.

Data and Sample Collection

Cows were housed and fed as previously described (Chapter 2). Indigestible NDF was used as a marker to calculate duodenal flow and ruminal digestibility and chromic oxide was used as a marker to calculate digestibility in the total tract. Gelatin capsules (1.5 oz., Tropac Inc., Airfield, NJ) containing 5 g of chromic oxide and ground spelt hulls (Wiley® mill, 2 mm screen; Authur H. Thomas, Philadelphia, PA) were dosed through the ruminal cannula at 0700, 1500, and 2300 h (total of 15 g Cr₂O₃/d) from 7 to 14 d with a priming dose of 3X on d 7. Duodenal samples (1,000 g), fecal samples (500 g), and rumen fluid samples (100 ml) were collected every 9 h from d 12 to d 14 so that 8 samples were taken for each cow each period, representing every 3 h of a 24-hour period to account for diurnal variation. Rumen fluid samples were obtained by combining and straining digesta from 5 different sites in the rumen. All samples were immediately frozen at -20° C.

Ruminal contents were evacuated manually through the ruminal cannula at 1350 h (4.5 h after feeding) on d 20 and at 0700 h (2 h before feeding) on d 21 of each period. Total ruminal content mass and volume were determined. During evacuation, 10% aliquots of digesta were separated to allow accurate sampling. Aliquots were squeezed through a nylon screen (1 mm pore size) to separate into primarily solid and liquid phases. Samples were taken from both phases for determination of nutrient pool size.

Sample and Statistical Analysis

Diet ingredients, orts, rumen contents and duodenal digesta were processed as previously described (Chapter 3). Briefly, forages and orts were coarse ground with dry

ice, lyophilized (Tri-Philizer™ MP, FTS Systems, Stone Ridge, NY) and then finely ground. Rumen solid and liquid fractions were lyophilized and recombined based on original DM ratio of solid and liquid fractions. Duodenal digesta was similarly split into solid and liquid fractions, subsampled, lyophilized and recombined based on the DM ratio of the fractions. Fecal samples were lyophilized, ground using a Willey mill® (1mm screen; Authur H. Thomas, Philadelphia, PA) and combined on an equal DM basis into one sample per cow per period. A portion of all samples were placed in a Whirl Pac™ bag (Nasco, Fort Atkinson, WI) flushed with nitrogen gas and frozen for FA analysis to minimize FA oxidation.

All dried samples were analyzed for DM, ash, NDF, 240-hour *in vitro* indigestible NDF (iNDF), potentially digestible NDF (pdNDF), CP, starch, gross energy (GE), and FA concentration and profiles. Ash concentration was determined after 5 h oxidation at 500° C in a muffle furnace. Concentration of NDF was according to Van Soest et al. (1991, method A). Indigestible NDF was estimated as NDF residue after 240-h *in vitro* fermentation (Goering and Van Soest, 1970). Rumen fluid for *in vitro* incubations was collected from a non-pregnant dry cow fed only alfalfa hay. Fraction of pdNDF was calculated by difference (1.00 – iNDF). Crude protein was analyzed according to Hach et al. (1987). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide. Glucose concentration was measured using a glucose oxidase method (Glucose kit #510; Sigma Chemical Co., St. Louis, MO), and absorbance was determined with a micro-plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Gross energy was assayed by bomb calorimeter (Parr Instrument Inc., Moline, IL). Rumen fluid was analyzed for concentration of major VFA

and lactate by HPLC (Waters Corp., Milford, MA). Fatty acids were extracted according to Sukhija and Palmquist (1988), and quantified by GC (Model 8500, Perkins-Elmer Corp, Norwalk, CT), using a SP-2560 capillary column (100m X 0.20 mm id with 0.02- μ m film thickness; Supelco, Bellefonte, PA). Oven temperature was 140°C for 5 min, then ramped 4°C/min to 240°C and held for 15 min. Helium flow was 20 cm/sec. Concentrations of all nutrients except DM were expressed as percentages of DM determined by drying at 105° C in a forced-air oven for more than 8 h.

Fecal samples were analyzed for concentration of chromium. Samples were digested with phosphoric acid (Williams et al., 1962), and chromium was quantified by flame atomic absorption spectrometry (SpectraAA 220, Varian, Victoria, Australia) according to manufacturer's recommendation. Nutrient intake was calculated using the composition of feed offered and refused on d 11-14. Ruminal pool sizes of nutrients were determined by multiplying the concentration of each component by the ruminal digesta DM mass. Turnover rate in the rumen, passage rate from the rumen, and ruminal digestion rate of each component were calculated according to Oba and Allen (2003).

To determine differences between treatments, all data were analyzed using the fit model procedure of JMP[®] (Version 5, SAS Institute, Cary, NC) according to the following model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk}$$

where

μ = overall mean,

C_i = random effect of cow (i = 1 to 8),

P_j = fixed effect of period (j = 1 to 4),

T_k = fixed effect of treatment ($k = 1$ to 4), and

e_{ijk} = residual error.

Period by treatment interaction was evaluated, but was removed from the statistical model when not significant ($P > 0.10$). Period by treatment interaction was not significant for any variable of primary interest; variables with significant interactions are noted in the tables. Data points with Studentized Residuals greater than three were considered outliers and excluded from analysis. Few points were excluded in analysis and rarely more than one per response variable. Preplanned contrasts included the effect of addition of RPF (CON vs. SAT, INT and UNS), linear effect of increasing concentration of unsaturated fat [L (SAT vs. UNS)], and quadratic effect of increasing concentration of unsaturated fat [Q (INT vs. SAT and UNS)]. The preplanned contrasts do not allow individual comparison of each fat treatment to the control. Protected LSD was used for mean separation in the discussion when the model treatment effect was significant. Pearson correlation coefficients were determined between cow-period observations for some parameters. Treatment effects, linear and quadratic responses, and correlations were declared significant at $P < 0.05$, and tendencies were declared at $P < 0.10$. For reasons previously described (Chapter 4), rumen pool data from one period and all data from one cow-period was excluded from statistical analysis.

RESULTS AND DISCUSSION

Treatment diet content and composition were previously presented (Chapter 3). Diets contained the same base ration and differed in 2.5% RPF or rice hulls. Control diet contained 5.5% FA and RPF diets contained 8.3, 8.1 and 7.8 % FA for SAT, INT and

UNS. Dietary unsaturated FA density increased from SAT to UNS treatment (3.9, 4.4 and 4.9 % for SAT, INT, and UNS).

Intake

Addition of RPF decreased DMI, and increasing UNS linearly decreased intake within RPF. Intake of other nutrients including NDF, pdNDF, starch and CP followed the same response pattern. Intake and feeding behavior are discussed in a companion paper (Chapter 5).

Flow Marker

Indigestible NDF was used as the flow marker for calculation of duodenal flow and chromic oxide was used for determination of fecal flow. Chromic oxide was also intended for prediction of duodenal flow, but resulted in unrealistically high duodenal flow possibly because of inadequate subsampling procedures for duodenal liquid. Use of indigestible NDF has the potential to cause treatment bias because of effects of duodenal FA on *in vitro* fermentation for determination of indigestible residue. To insure that this was not the case, we also calculated duodenal and fecal flow for all cow periods using acid detergent-sulfuric acid lignin as well as 120 hr indigestible ADF after ether extraction. These markers provided results similar to flow and digestibility calculated with iNDF. Chromic oxide was used as the total tract fecal marker because it was not affected by subsampling.

Ruminal Carbohydrate Digestion

SAT linearly decreased apparent ruminal DM and OM digestibility (Table 2) because of a reduction in ruminal NDF digestibility (Table 3). Apparent ruminal digestibility of starch was not affected by FA saturation. Increasing SAT concentration reduced the amount of DM and tended to reduce the amount of OM and NDF digested in the rumen, but did not affect the amount of starch digested. Addition of RPF did not affect ($P>0.58$) apparent ruminal digestibility of DM, OM, NDF, or starch but decreased the amounts of OM and starch apparently digested in the rumen (Tables 4, 5, and 6).

Rumen-protected FA treatments decreased ruminal VFA concentration and changed VFA profile by decreasing acetate and increasing propionate concentrations (Table 5). Total ruminal VFA concentration tended to decrease linearly with increasing SAT, and the molar proportion of acetate tended to decrease and branch chain VFA proportion increased with increasing SAT. Decreased total VFA and acetate concentration is consistent with the reduction in apparent ruminal OM digestibility.

The reduction in ruminal OM digestibility with SAT was not expected, because saturated FA are not expected to interfere with microbial fermentation. *In vitro* fermentations with addition of free stearic acid at 10% of DM was not different from esterified stearic acid, although fermentation was decreased with free palmitic and oleic FA (Chalupa et al., 1984). Although higher concentrations of free stearic acid, ranging from 10-20% of DM, did result in a linear decrease of VFA production (Chalupa et al., 1984), these are much higher FA concentrations than observed in the rumen in this experiment (Chapter 3), and the authors noted low metal cation concentration in the buffer that were expected to limit formation of FA salts. Most reports of prilled,

hydrogenated free FA have shown no effect on total tract nutrient digestion in lactating dairy cows (Schauff and Clark, 1989; Palmquist, 1991; Elliott et al. 1996). However, there is some evidence of a reduction in ruminal or total tract digestibility by prilled, hydrogenated FA. Grummer (1988) observed decreased total tract NDF digestibility with a low inclusion rate (0.68 kg/d) of prilled, hydrogenated FA compared to a low fat control, although ruminal VFA concentration and *in situ* DM and NDF digestion rates were not affected, and there were no effects on digestibility with a high FA inclusion rate (0.91 kg/d). Saturated free FA compared to low fat and esterified saturated FA treatment decreased ruminal OM digestibility in steers (Elliott et al., 1997), and total tract DM digestibility of lactating cows (Eastridge and Firkins, 1991). In the current study, it appears that SAT decreased ruminal digestibility via alterations of rumen passage rates.

Ruminal Digestion Kinetics

Saturated RPF decreased the fractional digestion rate and linearly increased the fractional passage rate of pdNDF (Table 6). Increasing passage rate and decreasing digestion rate resulted in a linear decrease of up to 24% for ruminal digestibility of pdNDF by SAT (Table 3). Contrary to its effects on pdNDF, SAT linearly decreased iNDF passage rate. Opposite effects on pdNDF and iNDF passage rates might be related to SAT effects on chewing behavior. SAT linearly increased rumination time per day and rumination time per kg DMI, and SAT cows ruminated over 50 min more per day than CON and UNS (Chapter 5). Size and density of digesta particles are constraints to passage from the rumen, and are affected by chewing and fermentation (Allen, 1996). Increased rumination is expected to increase particle size reduction rate as well as

increase rumen movements and sorting of small particles entrapped in the fibrous mat increasing their rate of escape from the rumen (Allen, 1996). Increased rumination for SAT may have increased escape of particles containing more rapidly digested NDF, explaining the greater passage rate of pdNDF and a slower digestion rate for the pdNDF remaining in the rumen. The slower rate of fermentation may increase iNDF buoyancy in the rumen and decrease iNDF passage rate (Allen, 1996).

Ruminal FA turnover was increased by RPF treatment, no differences were detected for fractional rates of passage and digestion of total FA. Ruminal turnover of DM, OM, NDF, pdNDF, and starch were not affected by RPF or FA saturation. Ruminal FA pool size, and FA biohydrogenation and passage kinetics are reported in companion papers (Chapter 3).

Post-Ruminal Digestion

Treatment did not affect post-ruminal digestibility of OM, NDF, pdNDF and starch as a percent of duodenal flow (Tables 2, 3, 4). Differences in the amount of duodenal flow decreased the amount of OM and tended to decrease the amount of starch digested post-rationally with increasing UNS. SAT linearly decreased post-ruminal digestibility of DM and OM as a percent of intake, representing a shift in site of digestion away from the rumen and towards the intestine. The changes in post-ruminal digestion are consistent with compensatory nutrient digestion in the intestine and hindgut.

Total Tract Digestion

Rumen-protected FA and FA saturation had no effect on total tract DM, OM, NDF or starch digestibility because of compensatory post-ruminal digestion. Saturated FA numerically decreased DM, OM, NDF and pdNDF total tract digestibility compared to INT and UNS providing support for decreased ruminal digestibility, especially because total tract and ruminal digestibilities were determined using independent markers. Although FA digestibility linearly decreased with increasing SAT (Table 8), the effect was too small to affect the digestible energy concentration of the diets (Table 7).

Fatty Acid Digestion

Rumen-protected FA increased and UNS linearly decreased total, C16, and C18 FA intake (Tables 8 and 9). Intake and duodenal flow of individual FA were described in Chapter 4, it was reported that UNS linearly decreased duodenal C18:0 concentration and linearly increased duodenal C18:1, C18:2 and C18:3 concentration. Rumen-protected FA did not affect total or C18 ruminal FA digestibility, but increased C16 FA ruminal digestibility. Increasing UNS tended to linearly increase ruminal digestibility of total and C18 FA.

Loss of dietary FA from the rumen through absorption across the rumen wall and oxidative metabolism is often considered minimal, and bacterial synthesis of FA is commonly expected to produce a net positive flow of FA through the rumen. Low ruminal absorption and metabolism of FA was first concluded with minimal plasma recovery of labeled carbon after ruminal infusion of radioisotope labeled linoleic acid while diverting nutrients with a reentrant cannula (Jenkins, 1993). In addition, microbial

FA synthesis was reported during *in vitro* fermentation (Wu and Palmquist, 1991). In contrast to the net positive FA flow expected, Jenkins (1993) observed that ruminal FA loss was reported for 15 out of 47 published treatment means. Regression analysis predicted an 8 percent loss of FA intake, and up to a 30 percent FA loss was reported in the dataset (Jenkins, 1993). Ferlay et al. (1993) reported a 14% increase in FA flow with control diet and 36.7 and 21.3% ruminal FA loss with rapeseed FA fed as calcium salts and triglycerides respectively. Ruminal FA loss may be because of flow marker bias under-predicting duodenal flow. However, Doreau and Chilliard (1997) proposed that negative ruminal FA flux is not due to flow marker bias, but caused by absorption and oxidation of FA, especially with higher fat diets. Rumen epithelium and bacteria adhering to the rumen wall may be capable of oxidative metabolism of FA (Doreau and Chilliard, 1997). Doreau and Chilliard (1997) noted that higher FA concentration diets experience greater FA loss and hypothesize that FA are less adsorbed to feed particles leading to increased contact with the rumen wall and increased opportunity for absorption and oxidation. In addition, increased dietary FA concentration decreases microbial FA synthesis (Jenkins, 1993), which may allow detection of ruminal FA digestion. Variation in reported rumen FA loss may be because of bacterial FA synthesis, especially in low FA diets. Bacteria incorporate dietary FA into their plasma membranes and bacterial FA cannot be considered entirely from microbial production, so true FA digestibility occasionally reported in the literature has little meaning. Simple digestion studies cannot partition duodenal FA flow into dietary and microbial synthesized fractions limiting the ability to determine the extent of rumen FA synthesis and digestion.

Investigations of ruminal FA loss have not considered effects of FA saturation. Increased ruminal disappearance of unsaturated FA observed in the current study may represent increased metabolism of unsaturated FA, and many reports of ruminal FA loss were unsaturated FA treatments (Ferlay et al., 1993; and Wu et al., 1991). It is logical that unsaturated FA may be more highly oxidized in the rumen because they are less hydrophobic than saturated FA and are more dispersed in the rumen allowing increased contact with ruminal bacteria. In addition, ruminal bacteria absorb unsaturated FA during biohydrogenation. Unsaturated FA that are absorbed in excess of bacterial requirements for cellular membranes might be oxidized to eliminate their toxic effect. Fatty acid saturation and concentration in the diet might explain the inconsistent ruminal FA loss noted by Jenkins (1993). The increasing occurrence of ruminal FA loss reported in digestion studies merits investigation of ruminal FA metabolism.

Digestibility of total FA as a percent of duodenal FA flow tended to decrease and C16 FA digestibility decreased with RPF treatments. Increasing SAT tended to decrease total FA digestibility and decreased C18 FA digestibility as a proportion of FA flowing to the duodenum. Total tract digestibility of total FA was not affected by RPF treatments but increasing SAT linearly reduced total, C16 and C18 FA digestibility. SAT increased duodenal flow of FA but UNS did not change FA flow compared to CON because of decreased intake and increased ruminal FA loss. Within RPF, increasing SAT linearly increased duodenal FA flow because of increased intake, less ruminal FA disappearance and unintentionally higher dietary FA concentration (see discussion of treatments in Chapter 3). Amount of total and C16 FA digested post-ruinally tended to increase with increasing SAT. Post-ruinal digestion of total and C18 FA as a percent of intake was

not different across treatment, as a result of differences in ruminal digestion. This shows equal efficiency of capturing dietary FA energy between SAT and UNS. Treatment did not affect total tract total and C16 FA digestibility but decreased total tract digestibility of C18 FA for non-cannulated cows on the same diets (Chapter 3). Saturated FA are considered less digestible than unsaturated FA. Differences in FA digestibility reported in the literature are biased because of ruminal FA oxidation, confounding of esterification, biohydrogenation in the large intestine and oxidation of unsaturated FA prior to analysis. Total tract digestibility of esterified FA is lower than unesterified FA (Elloitt et al., 1994; Elloitt et al., 1999), and triglyceride digestibility decreases with increasing saturation (Pantoja et al., 1996; Pantoja et al., 1995). Elliot et al. (1999) observed that highly saturated triglycerides are more resistant to ruminal and intestinal lipolysis, resulting in lower digestibility. The low pH of the duodenum prohibits lipase function, and esterified FA are not hydrolyzed until the jejunum, decreasing the opportunity for FA absorption (Noble, 1981). Decreased ruminal lipolysis of saturated FA increases duodenal flow of esterified FA. The belief that saturated FA are less digestible may be an erroneous conclusion based on decreased total tract digestibility of saturated esterified FA causing decreased ruminal lipolysis and increased duodenal flow of less digestible esterified FA.

Sample handling and preparation may bias digestibility calculation because of partial oxidation of FA (Palmquist, personal communication). In the current study, samples were flushed with nitrogen gas and frozen to minimize sample oxidation. However, the methylation procedure of Sukhija and Palmquist (1988) may also cause partial loss of unsaturated FA. Oxidation of unsaturated FA from improper storage and

sample preparation decreases unsaturated FA concentration, leading to an over-prediction of unsaturated FA digestibility compared to saturated FA, which are much less prone to oxidation.

Data for total tract and intestinal digestibility of individual FA supports decreased digestibility of saturated FA compared to unsaturated FA. However, measures for individual FA are meaningless because of FA biohydrogenation and synthesis in the large intestine (Merchen et al., 1997). Digestibility of individual FA only be determined with duodenally and ileally cannulated cows, although the cost and complexity of multiple intestinal cannulation has prevented such measures. However, digestibility of unsaturated FA can be compared to saturated FA by observing total, C16 or C18 FA digestibility between treatments differing in duodenal FA profile. Christensen et al. (1994) and Bremmer et al. (1998) measured digestibility of total FA for abomasally infused free FA and observed no difference between saturated and unsaturated FA treatments. Schauff and Clark (1989), Grummer et al. (1988) and Palmquist (1991) directly compared calcium salts of palm oil and prilled, saturated free FA, finding no difference in total tract digestibility of energy, lipid and FA. Elliott et al. (1996) observed 8-percentage units lower total tract FA digestibility with prilled FA compared to calcium salts of palm oil, although treatments were not compared in the statistical contrasts. Doreau and Chilliard (1997) summarized 64 treatment groups reporting FA digestibility in the small intestine or the lower tract, finding no difference between C16 and C18 FA, and observed only slight differences between lower tract saturated and unsaturated C18 FA digestibility (77, 85, 83 and 76% for 0, 1, 2 and 3 double bonds respectively).

Lastly, true loss of ruminal FA discounts the value of measurement of total tract digestibility. Increased ruminal digestion may increase total tract FA digestibility without increasing energy available to the animal. If unsaturated FA are more highly degraded in the rumen, increased unsaturated FA digestion in the total tract might not increase intestinal FA absorption. The energy efficiency of ruminally digested FA is not known, but considerable energy loss is expected because of bacterial maintenance energy requirements and loss of energy in chemical transformation. Digestion studies have reported substantial ruminal FA digestion (Wu et al. 1991; and Ferlay et al. 1993), which may have a large effect on energy absorption, especially considering the high energy value of FA. Merchen et al. (1997) proposed that fat digestion experiments should utilize duodenally cannulated animals for observation of duodenal FA profile and calculation of ruminal and post-ruminal digestibility as common in starch and fiber digestion studies.

Unsaturated FA treatment linearly increased digestibility of C18 FA flowing to the duodenum 7.6 percentage units compared to SAT, and we have previously reported 4.2 percentage unit increase in total tract digestibility with non-cannulated cows on the same diets (Chapter 3). In the current experiment, post-ruminal C18 FA digestibility of UNS was not different from control but was decreased by SAT. Decreased FA digestibility of SAT cannot be directly attributed to lower digestibility of C18:0 as CON and SAT did not differ in duodenal C18:0 composition. Fatty acid digestion across RPF saturation may be a result of the amount of duodenal FA or DM flow. Schauff and Clark (1992) reported decreased FA digestibility as the FA content of the diet was increased with dietary calcium salts of palm oil, but Jenkins (1999) reported linearly increased total tract FA digestibility when feeding oleamide from 0 to 5% of the diet. Palmquist (1991)

discussed decreased FA digestibility with increasing duodenal flow and reported drastic decreases in true and marginal true digestibility with increasing FA intake, noting a 4.4 percentage unit decrease in marginal true FA digestibility (digestibility of each increment of fatty acid consumed) per 100 g of FA intake. Weisberg et al. (1992a and 1992b) observed decreased FA digestibility when increasing FA intake from 500 to 1000 g/d of palmitic and stearic acid and when increasing FA intake from tallow. Analysis of the relationship between FA digestibility and FA intake is confounded by FA form and saturation used to increase FA intake, making conclusions difficult.

The associative effects of amount of feed intake, digesta passage rate and diet composition on FA digestibility has received little attention. Grum et al. (1996) reported a large decrease (>23.9 percentage units) in C18 FA digestibility with increased concentrate feeding with and without tallow. Elliott et al. (1995) observed increased total tract FA digestibility in diets that replaced ground corn with soyhulls. Weisbjerg et al. (1992b) did not observe any difference in tallow FA intake at low (8.6 kg) and high (12.6 kg) DMI, although both intake levels were considerably lower than observed in the current study. In the current study, increasing DMI tended to increase total FA digestibility of duodenal flow ($R^2 = 0.10$, $P < 0.10$). Associative effects of dietary carbohydrates on FA digestibility are not known but may include level of DM intake, and duodenal digesta flow rate and composition.

CONCLUSION

Saturated FA treatment decreased ruminal NDF digestibility possibly because increased rumination resulted in a faster passage of more rapidly fermentable NDF.

Saturated FA treatment also decreased FA digestibility, although it is not possible to discern if it is because of FA composition or increased flow of FA to the duodenum. Addition of rumen-protected FA may not increase energy intake because of decreased DM intake and negative associative effects on ruminal digestion.

Table 1. Ingredient and nutrient composition of experimental diets¹.

	CON	SAT	INT	UNS
Ingredients	-----% of DM-----			
Corn silage ²	24.6	24.7	24.7	24.6
Alfalfa silage ³	12.6	12.6	12.6	12.6
Ground Corn	28.7	28.8	28.8	28.7
Whole Cottonseed	13.5	13.5	13.5	13.5
Protein mix ⁴	10.5	10.6	10.5	10.5
Mineral vitamin mix ⁵	4.3	4.3	4.3	4.3
CON Mix ⁶	5.7	0.5	0.2	-
SAT Mix ⁶	-	5.0	2.5	-
UNS Mix ⁶	-	-	2.9	5.7
Nutrient				
DM	55.6	55.7	55.7	55.7
OM	92.6 ^c	92.9 ^b	93.1 ^a	93.1 ^a
Total FA	5.5 ^d	8.3 ^a	8.1 ^b	7.8 ^c
Unsaturated FA	3.6 ^d	3.9 ^a	4.4 ^b	4.9 ^c
Starch	30.8 ^a	30.3 ^{ab}	30.5 ^{bc}	30.7 ^c
NDF	29.1 ^a	27.3 ^d	27.5 ^c	27.7 ^b
Indigestible NDF	11.2 ^a	9.7 ^b	10.0 ^b	9.9 ^c
Forage NDF	16.9 ^c	17.0 ^a	16.9 ^{bc}	17.0 ^b
CP	16.2 ^a	16.1 ^b	16.1 ^b	16.1 ^b
Rumen-undegraded CP ⁷	5.1 ^a	4.8 ^d	5.1 ^b	4.9 ^c
% NDF from forage	57.4 ^d	61.4 ^a	60.9 ^b	60.5 ^c
GE MCal/Kg	4.55 ^b	4.72 ^a	4.72 ^a	4.71 ^a

¹ Treatments were CON- control with no supplemental rumen-protected fatty acids (FA), SAT- saturated FA from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated FA fed as Ca soaps of LCFA.

² Corn silage contained 34.7% DM (as fed), and 43.4% NDF, 8.4% CP, 10.7% indigestible NDF, 24.1% starch, and 4.8% ash on a DM basis.

³ Alfalfa silage contained 36.3% DM (as fed) and 48.1% NDF, 16.2% CP, 25.7% indigestible NDF, 2.6% starch, and 9.7% ash on a DM basis.

⁴ Protein mix contained 74.1% soybean meal, 20.1% corn gluten meal, and 5.8% blood meal.

⁵ Mineral vitamin mix contained 12.7% sodium bicarbonate, 11.5% limestone, 5.5% salt, 2.2% trace mineral premix, 2.0% urea, 2.0% dicalcium phosphate, 0.6% vitamin D, 0.48% vitamin A, 0.12% vitamin E, and 62.9% dry ground corn as a carrier

⁶ Mix Composition listed in Table 1

⁷ Rumen-degraded protein estimated using values from NRC (2001).

Table 2. Effects of rumen protected fatty acids varying in saturation on digestion of DM and OM.

	Treatment LS Means ¹						P ²		
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
DM									
Intake, kg/d	27.1	25.4	24.5	23.0	0.93	<0.001	<0.001	<0.001	0.50
Apparently ruminally digested									
kg/d	8.0	6.1	7.1	7.5	0.68	0.05	0.06	0.03	0.65
%	29.7	23.7	29.0	33.2	2.5	0.01	0.64	0.002	0.82
Apparent total tract digested									
kg/d	17.7	16.5	16.2	15.3	0.7	0.008	0.003	0.04	0.61
%	65.6	65.1	66.0	66.7	1.2	0.73	0.77	0.28	0.93
OM									
Intake, kg/d	5.0	23.6	22.8	21.3	0.87	<0.001	<0.001	<0.001	0.45
Apparently ruminally digested									
kg/d	8.5	6.8	7.5	7.8	0.64	0.05	0.03	0.07	0.70
%	34.1	28.6	33.1	37.1	2.4	0.02	0.58	0.002	0.92
Passage to duodenum, kg/d	16.4	16.7	15.3	13.5	0.76	<0.001	0.06	<0.001	0.75
Apparent post-ruminal digested									
kg/d	8.0	8.6	7.6	6.5	0.65	0.07	0.52	0.01	0.97
% of intake	32.3	37.0	33.4	30.3	2.6	0.18	0.63	0.03	0.92
% of duodenal passage	48.5	51.3	49.6	47.9	2.4	0.69	0.67	0.27	0.99
Apparent total tract digested									
kg/d	16.5	15.4	15.2	14.3	0.6	0.01	0.004	0.06	0.52
%	66.3	65.6	66.8	67.4	1.2	0.68	0.85	0.24	0.82

¹ Treatments were CON- control with no rumen-protected fatty acids (FA), SAT- saturated FA from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated FA fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

Table 3. Effects of rumen protected fatty acids varying in saturation on digestion of total NDF and potentially digestible NDF (pdNDF).

	Treatment LS Means ¹						P ²		
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
NDF									
Intake, kg/d	7.5	6.8	6.7	6.1	0.6	<0.001	<0.001	0.005	0.37
Ruminally digested									
kg/d	2.2	1.7	1.9	2.0	0.17	0.13	0.08	0.10	0.87
%	28.8	24.6	27.8	32.2	2.0	0.04	0.78	0.006	0.80
Passage to duodenum, kg/d	5.3	5.1	4.9	4.2	0.18	<0.001	0.004	<0.001	0.29
Post-ruminally digested									
kg/d	0.81	0.99	1.00	0.55	0.22	0.36	0.89	0.13	0.37
% of intake	10.8	15.0	14.5	8.9	3.2	0.42	0.59	0.15	0.51
% of duodenal flow	15.0	19.5	20.1	12.8	4.1	0.50	0.61	0.23	0.43
Total tract digested									
kg/d	3.0	2.70	2.85	2.56	0.19	0.36	0.18	0.57	0.29
% of intake	39.9	39.6	42.5	41.1	2.5	0.78	0.67	0.63	0.43
pdNDF³									
Intake, kg/d	4.5	4.4	4.2	4.0	0.18	0.02	0.03	0.02	0.69
Ruminally digested									
kg/d	2.2	1.7	1.9	2.0	0.17	0.13	0.08	0.10	0.87
%	47.8	38.5	43.9	50.5	3.0	0.03	0.30	0.006	0.86
Passage to duodenum, kg/d	2.3	2.6	2.4	1.9	0.12	0.004	0.92	<0.001	0.56
Post-ruminally digested									
kg/d	0.66	0.77	0.95	0.50	0.19	0.36	0.73	0.28	0.17
% of intake	15.5	18.6	22.8	13.1	4.3	0.40	0.59	0.34	0.19
% of duodenal passage	26.4	28.8	40.5	26.7	0.72	0.47	0.50	0.82	0.16
Total tract digested									
kg/d	2.8	2.5	2.8	2.5	0.19	0.38	0.25	0.90	0.19
% of intake	62.8	57.1	65.7	63.6	3.6	0.31	0.86	0.17	0.19

¹ Treatments were CON- control with no rumen-protected fatty acids (FA), SAT- saturated FA from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated FA fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.³ Potentially digestible NDF.

Table 4. Effects of rumen protected fatty acids varying in saturation on digestion of starch.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Intake, kg/d	8.5	7.6	7.4	7.0	0.30	<0.001	<0.001	0.001	0.45
Apparently ruminally digested									
kg/d	5.0	4.3	4.6	4.2	0.36	0.02	0.007	0.67	0.11
%	58.2	55.7	61.6	59.7	3.5	0.39	0.78	0.24	0.21
Passage to duodenum, kg/d	3.5	3.4	2.8	2.8	0.26	0.04	0.04	0.06	0.23
Apparent post-ruminal digested									
kg/d	3.0	2.9	2.4	2.4	0.26	0.07	0.09	0.07	0.17
% of intake	35.8	38.8	32.5	34.9	3.6	0.37	0.89	0.26	0.18
% of duodenal flow	84.8	87.1	83.6	86.0	1.8	0.38	0.63	0.59	0.12
Apparent total tract digested									
kg/d	8.0	7.2	7.0	6.6	0.3	<0.001	<0.001	0.003	0.59
%	94.0	94.5	94.2	94.5	0.43	0.67	0.35	0.95	0.44

¹ Treatments were CON- control with no rumen-protected fatty acids (FA), SAT- saturated FA from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated FA fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

Table 5. Effects of rumen protected fatty acids varying in saturation on ruminal fermentation.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Total VFA, mM	140	133	135	138	2.0	<0.05	<0.001	<0.10	0.92
Lactate, mM	0.10	0.50	0.19	0.67	0.17	0.07	0.005	0.45	<0.05
VFA, mol/100 mol									
Acetate	52.5	49.3	49.8	50.2	0.50	<0.001	<0.001	0.09	0.87
Propionate	32.1	33.9	34.0	33.4	0.53	0.007	<0.001	0.30	0.34
Butyrate	11.1	11.5	11.4	11.8	0.25	0.17	<0.001	0.32	0.26
Branched-chain VFA	4.7	5.3	4.8	4.7	0.17	0.04	<0.001	0.01	0.42
Acetate: Propionate	1.64	1.46	1.47	1.51	0.04	<0.001	<0.001	0.19	0.61

¹ Treatments were CON- control with no rumen-protected fatty acids (FA), SAT- saturated FA from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated FA fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

Table 6. Effects of rumen protected fatty acids varying in saturation on ruminal digestion kinetics.

	Treatment LS Means ¹				P ²				
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Ruminal Turnover, rate, %/h									
DM	7.8	7.4	7.3	7.6	0.27	0.57	0.32	0.43	0.58
OM	7.8	7.4	7.4	7.7	0.27	0.53	0.27	0.46	0.56
NDF	4.0	3.6	3.8	3.9	0.16	0.30	0.19	0.17	0.92
pdNDF	5.1	4.9	4.8	4.8	0.33	0.92	0.53	0.87	0.86
INDF	3.1	2.5	2.8	3.0	0.14	0.03	0.04	0.02	0.96
Starch	34.5	32.7	31.8	29.5	2.4	0.49	0.25	0.34	0.79
Total FA	6.8	8.1	7.9	7.8	0.32	0.03	0.005	0.60	0.93
Ruminal Passage, rate, %/h									
Starch	14.8	14.5	12.5	11.7	1.6	0.29	0.22	0.14	0.71
pdNDF	2.6	3.1	2.7	2.4	0.22	0.16	0.66	0.03	0.81
INDF	3.1	2.5	2.8	3.0	0.15	0.04	0.06	0.02	0.74
Total FA	6.4	7.3	7.4	6.5	0.36	0.14	0.16	0.11	0.26
Ruminal digestion, rate %/h									
Starch	19.8	18.0	20.4	17.8	1.8	0.59	0.58	0.91	0.22
pdNDF	2.4	1.8	2.2	2.4	0.2	0.12	0.21	0.04	0.81
Total FA	0.31	0.60	0.74	1.43	0.37	0.17	0.15	0.11	0.54

¹ Treatments were CON- control with no supplemental rumen-protected fatty acids (FA), SAT- saturated FA from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated FA fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

³ Potentially digestible NDF.

Table 7. Effects of rumen protected fatty acids varying in saturation on digestion of energy.

	Treatment LS Means ¹						P ²		
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Energy									
Intake, Mcal GE/d	122.8	119.8	115.4	107.8	4.4	0.001	0.001	0.001	0.47
Apparent total tract digested									
Mcal/d	78.0	75.3	74.0	70.1	3.1	0.07	0.04	0.06	0.59
%	63.7	62.9	64.1	65.1	1.2	0.52	0.80	0.15	0.94

¹ Treatments were CON- control with no rumen-protected fatty acids (FA), SAT- saturated FA from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated FA fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

Table 8. Effects of rumen protected fatty acids varying in saturation on digestion of FA.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Total FA									
Intake, kg/d	1.5	2.1	2.0	1.8	0.07	<0.001	<0.001	<0.001	0.68
Apparently ruminally digested									
kg/d	0.06	0.17	0.16	0.32	0.09	0.23	0.15	0.20	0.43
%	4.0	7.3	7.9	18.4	4.9	0.15	0.19	0.09	0.38
Passage to duodenum, kg/d	1.4	2.0	1.9	1.5	0.12	<0.001	0.003	<0.001	0.25
Apparent post-ruminal digested									
kg/d	1.0	1.3	1.3	1.0	0.11	0.11	0.12	0.08	0.31
% of intake	69.1	59.6	63.0	56.7	5.2	0.33	0.12	0.66	0.42
% of duodenal passage	71.5	63.8	67.9	69.3	2.5	0.11	0.09	0.08	0.62
Apparent total tract digested									
kg/d	1.1	1.4	1.4	1.4	0.06	<0.001	<0.001	0.16	0.79
%	73.1	66.9	70.6	75.1	1.6	<0.001	0.15	0.001	0.80

¹ Treatments were CON- control with no rumen-protected fatty acids (FA), SAT- saturated FA from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated FA fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

Table 9. Effects of rumen protected fatty acids varying in saturation on digestion of C16 and C18 fatty acids.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Total C16 Fatty Acid									
Intake, kg/d	0.28	0.50	0.47	0.44	0.02	<0.001	<0.001	0.001	0.96
Apparently ruminally digested									
kg/d	-0.01	0.05	0.04	0.09	0.03	0.06	0.02	0.23	0.32
%	-3.8	9.1	8.0	20.0	6.2	0.05	0.02	0.16	0.34
Passage to Duodenum, kg/d	0.30	0.45	0.43	0.35	0.03	<0.001	<0.001	0.006	0.28
Apparent post-ruminal digested									
kg/d	0.23	0.31	0.30	0.24	0.03	0.08	0.07	0.08	0.38
% of intake	81.6	62.8	64.8	56.0	6.5	<0.05	<0.01	0.41	0.47
% of duodenal passage	77.9	68.4	70.1	69.5	2.6	0.06	0.008	0.74	0.71
Apparent total tract digested									
kg/d	0.22	0.36	0.34	0.33	0.02	<0.001	<0.001	0.12	0.85
%	77.9	71.9	73.2	76.0	1.6	0.03	0.02	0.04	0.66
Total C18 Fatty Acid									
Intake, kg/d	1.0	1.4	1.3	1.2	0.05	<0.001	<0.001	<0.001	0.55
Apparently ruminally digested									
kg/d	0.09	0.13	0.14	0.13	0.05	0.30	0.27	0.16	0.53
%	9.6	8.8	10.7	20.3	4.5	0.19	0.46	0.06	0.46
Passage to Duodenum, kg/d	0.9	1.2	1.2	0.9	0.07	<0.001	0.005	<0.001	0.25
Apparent post-ruminal digested									
kg/d	0.64	0.78	0.79	0.66	0.07	0.16	0.15	0.14	0.30
% of intake	64.0	57.3	60.7	55.9	4.7	0.57	0.26	0.82	0.46
% of duodenal passage	70.4	62.4	67.5	70.0	2.5	0.05	0.15	0.02	0.64
Apparent total tract digested									
kg/d	0.75	0.91	0.91	0.89	0.04	<0.001	<0.001	0.73	0.60
%	73.5	66.1	70.9	76.2	1.6	<0.001	0.11	<0.001	0.89

¹ Treatments were CON- control with no rumen-protected fatty acids (FA), SAT- saturated FA from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated FA fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

CHAPTER 6

Effect of rumen-protected fatty acid saturation on feed intake, and feeding and chewing behavior of lactating dairy cows.

ABSTRACT

Saturated and unsaturated rumen-protected fat sources were evaluated for effects on feed intake, meal patterns and chewing behavior. Eight ruminally and duodenally cannulated cows were used in a replicated 4x4 Latin square design with 21 d periods. Treatments were control and a linear titration of 2.5% added rumen-protected fatty acids (**RPF**) varying in unsaturation; saturated (**SAT**; prilled hydrogenated free FA), 50:50 ratio of SAT and unsaturated (**UNS**; calcium soaps of long-chain FA), and **UNS**. Dry matter intake for SAT was not different from control while **UNS** linearly decreased DMI. Wet weight of ruminal digesta decreased with **RPF**, and decreased linearly with increasing **UNS**. Treatment did not change meal number, meal length or time between meals, but increasing **UNS** decreased meal size within **RPF**. Time spent ruminating was greater for SAT compared to **CON** and was linearly increased by SAT. Increasing unsaturated FA flow to the duodenum decreases feed intake by decreasing meal size, and increasing saturated FA flow to the duodenum increases rumination time per day by increasing rumination bout length.

INTRODUCTION

High producing dairy cows have high energy requirements that may exceed their ability to consume dietary energy on some diets, resulting in less than maximum milk yield. Addition of fat to the diet increases energy density without increasing rumen acid production, or maintains energy density while increasing fiber for stabilization of rumen fermentation (Allen, 1997). Prilled saturated free fatty acids (FA) and calcium salts of FA are two manufactured products marketed to minimize effects of fat on ruminal fermentation. However, calcium salts of FA are not entirely protected in the rumen and dissociation of the calcium ion allows rumen biohydrogenation of unsaturated FA (Wu et al. 1991).

Intake is highly regulated by animal nutrient requirement and metabolic state, and by the type and temporal pattern of fuels absorbed. A meta-analysis of treatment means from the literature indicated different hypophagic effects of fat supplements differing in FA source, form, and type (Allen, 2000). Within commonly fed rumen-protected fat (RPF) sources, calcium salts of palm oil linearly decreased DMI with increasing dietary concentration, while hydrogenated FA did not affect DMI (Allen, 2000). Fatty acids reaching the duodenum have been proposed to have different hypophagic effects (Drackley, 1992), and abomasal FA infusion has consistently demonstrated unsaturated FA hypophagia (Benson and Reynolds, 2001). Although many experiments observe daily DMI, few have observed feeding and chewing behavior. Daily intake is a function of meal size and intermeal interval. Prilled and hydrogenated free FA and calcium soaps of long-chain FA were selected to provide the largest difference in unsaturated FA,

especially poly-unsaturated FA (PUFA) flow to the duodenum possible with commonly available feed ingredients. Calcium salts of long-chain FA increased duodenal flow of monounsaturated FA, but failed to increase PUFA flow in this study (Chapter 3). The objective of this experiment was to determine effects of rumen-protected FA saturation on feed intake and feeding and chewing behavior of lactating dairy cows. We hypothesized that increasing UNS would decrease intake.

MATERIALS AND METHODS

This paper is the fourth of four papers in a series from one experiment that evaluated effects of RPF differing in FA saturation. This paper discusses treatment effects on DMI and feeding and chewing behavior, and the companion papers focus on milk yield, milk FA profile and energy balance (Chapter 3), rumen kinetics of FA biohydrogenation (Chapter 4), and ruminal and post-ruminal nutrient digestion (Chapter 5). Experimental procedures were approved by the All University Committee on Animal Use and Care at Michigan State University.

Cows and Treatments

Eight ruminally and duodenally cannulated multiparous Holstein cows (77 ± 8.7 DIM; mean $\pm$ SD) from the Michigan State University Dairy Cattle Teaching and Research Center were used in a replicated 4 x 4 Latin square design experiment. Cows were randomly assigned to treatment sequence. Treatments were a control diet (CON) containing no added RP- FA or 2.5% added RPF from saturated (SAT - prilled hydrogenated FA, Energy Booster 100[®], Milk Specialties Company Inc., Dundee, IL),

intermediate mixture of saturated and unsaturated (INT), or unsaturated (UNS) FA (Ca Soaps of LCFA, Megalac-R[®], Church and Dwight Company, Inc., Princeton, NJ).

Treatment periods were 21 d with the final 11 d used for sample and data collection.

Surgery was performed at the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University. Immediately prior to initiation of the experiment, empty body weight (ruminal digesta removed) of cows was 516 ± 33 kg (mean $\pm$ SD).

Treatment mix composition and diet composition are reported in Chapter 3.

Experimental diets contained 40% forage (66:33, corn silage: alfalfa silage), 13.5% whole cottonseed, dry ground corn, premixed protein supplement (soybean meal, corn gluten meal, and blood meal), a mineral and vitamin mix, and 2.5% added rice hulls (CON), saturated FA (SAT), 50:50 mix of saturated and unsaturated fat (INT) or unsaturated FA (UNS). All diets were fed as a total mixed ration.

Data and Sample Collection

Throughout the experiment, cows were housed in tie-stalls and fed once daily (1100 h) at 115% of expected intake. Amounts of feed offered and orts were weighed for each cow daily. Samples of all diet ingredients (0.5 kg) and orts from each cow (12.5%) were collected daily on d 11 to 14 and d 16-19 and combined into one sample to represent four days for digestibility analysis (d 11-14) and four days for feeding behavior observation (d 16-19).

Feeding behavior was monitored from d 16 to d 19 (96 h) of each period using a computerized data acquisition system (Dado and Allen, 1993). Data of chewing

activities, feed disappearance, water consumption and rumen pH were recorded for each cow every 5 sec. When chewing equipment malfunctioned for an individual cow during a 24-h period (1100h to 1100h), chewing behavior was deleted for that day. The system successfully collected 77% of the total chewing behavior data (average 3.1d per cow per period). The feeding behavior analysis procedure allowed determination of meal size and length for days missing short periods of chewing data. This intervention allowed analysis of meal parameters for 88% of the observation days. Feeding behavior was determined according to Dado and Allen (1993) with the following modifications. Potential meals were identified at the 75th percentile of the running standard deviation of the manger weight to account for differences in baseline variation between data files. Minimum meal size was 1 kg (as fed) and minimum continuous meal length was 30 s. Meals parameters determined to be outliers (outside the 10th and 90th percentile) were manually verified and corrected if determined to be in error. Daily means were calculated for number of meal bouts per day, interval between meals, meal size, eating time, ruminating time, and total chewing time. These response variables were calculated as daily means and averaged for each period. Indwelling rumen pH probes were calibrated daily. Data was discarded for the probe if calibration drifted ± 0.10 pH units at either pH 4.0 or pH 7.0. Seventy-eight percent of the cow days met the criteria. Ruminal contents were evacuated manually through the ruminal cannula at 1350 h (4.5 h after feeding) on d 20 and at 0700 h (2 h before feeding) on d 21 of each period as described in Chapter 3.

Sample and Statistical Analysis

Diet ingredients, orts, rumen contents and duodenal digesta were processed as previously described (Chapter 3). Overall DM and nutrient intake was calculated as the means of digestibility (d 11-14) and feeding behavior (d16-19) observations. The ratio of chewing activity to DM and nutrient intake utilized intake from feeding behavior observation only. Ruminal pool sizes (kg) of OM, NDF, iNDF, and starch were determined by multiplying the concentration of each component in DM by the ruminal digesta DM weight (kg).

Hunger and satiety ratios were calculated according to Forbes (1995) as follows:

Hunger ratio = meal kg DM / premeal interval ; and

Satiety ratio = meal kg DM / postmeal interval.

Ratios were calculated for individual meals and averaged for the 4 d of feeding behavior data collection.

Energy values were calculated as follows:

DE intake = GEI x GE digestibility [GE digestibility as reported in Chapter 4]

Selection was calculated as concentration of the component in the diet consumed divided by the concentration of the component in the diet fed.

All data were analyzed using the fit model procedure of JMP® (Version 5, SAS Institute, Cary, NC) according to the following model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk}$$

where

μ = overall mean,

C_i = random effect of cow ($i = 1$ to 16),

P_j = fixed effect of period ($j = 1$ to 4),

T_k = fixed effect of treatment ($k = 1$ to 4),

e_{ijk} = residual error.

Period by treatment interaction was evaluated, but was removed from the statistical model when not significant ($P > 0.10$). Period by treatment interaction was not significant for any variable of primary interest; variables with significant interactions are noted in the tables. Data points with Studentized Residuals greater than three were considered outliers and excluded from analysis. Few points were excluded in analysis and rarely more than one per response variable. Preplanned contrasts included the effect of addition of RPF (CON vs. SAT, INT and UNS), linear effect of increasing concentration of unsaturated FA [L (SAT vs. UNS)], and quadratic effect of increasing concentration of unsaturated FA [Q (INT vs. SAT and UNS)]. The preplanned contrasts did not allow individual comparison of each fat treatment to the control. Protected LSD and was used for mean separation when the model treatment effect was significant. Pearson correlation coefficients were determined between cow-period observations for some parameters. Treatment effects, linear and quadratic responses, and correlations were declared significant at $P < 0.05$, and tendencies were declared at $P < 0.10$.

Data from two cow-periods were excluded from statistical analysis. One cow developed clinical mastitis on d 19 of period 3, rumen samples, body weight and body condition score was not collected for this period. Data previously collected in this period

was included in our analysis. The cow did not fully recover and data from period 4 was not used.

RESULTS AND DISCUSSION

Intake

Addition of RPF decreased DMI compared to control ($P < 0.001$), and increasing UNS linearly decreased DMI ($P=0.02$; Table 2). Mean comparison showed that SAT and INT had no effect on intake but UNS decreased intake compared to CON. Intake of OM, starch, and FA were similarly decreased. Neutral detergent fiber intake was less affected because of differences in NDF concentration among diets from the inclusion of rice hulls as dietary fill in balancing diets. Digestible energy intake was decreased by RPF ($P = 0.04$), and tended to linearly decrease with increasing UNS ($P = 0.06$).

Allen (2000) reported that 11 out of 24 summarized studies feeding increasing amounts of calcium salts of palm oil showed a decrease in intake, while 22 of the 24 studies resulted in numerical decreases in intake. Calcium salts of palm oil has a lower concentration of unsaturated FA and a higher C16:C18 FA ratio than the calcium salts of blended oil used in this experiment. Also reported, hydrogenated FA or triglycerides resulted in decreased feed intake in only one study and increased feed intake in two out of 21 studies reported (Allen, 2000).

Abomasal infusion of unsaturated fat consistently decreases intake relative to no fat and saturated fat infusions (Benson and Reynolds, 2001). Drackley et al. (1992) and Christensen et al. (1994) showed abomasal infusions of increasing unsaturated FA with a

lower C16:C18 FA ratio decreased DM and energy intake. The RPF treatments in the current study differed in unsaturated fat concentration while maintaining similar C16:C18 ratios. Bremmer et al. (1998) demonstrated decreased intake with increasing FA unsaturation with abomasal infusions of FA with the same C16:C18 FA ratio. Lastly, protected oleamide FA compared to free oil that is readily biohydrogenated in the rumen consistently linearly decreased intake (Jenkins, 1998, Jenkins, 2000, and DeLuca and Jenkins, 2000).

Dietary PUFA were extensively biohydrogenated in the current study, but decreased biohydrogenation of trans-C18:1 increased duodenal flow of mono-unsaturated FA (Table 1). Duodenal FA flow of C18:2 did not differ between treatments, and C18:3 flow was linearly decreased with increasing UNS. Intake depression cannot be attributed to increased intestinal absorption of PUFA. Duodenal flow of both cis-C18:1 and trans-C18:1 increased with increasing UNS corresponding to the linear decrease in DMI.

Unsaturated FA treatment decreased DMI and tended to decrease energy intake, however a larger intake depression would be expected with the large reduction in energy required from milk fat production. Increased BW gain and the failure to regulate energy homeostasis may be because of metabolite balance. Intake is regulated by the type and temporal pattern of fuels available (Allen, 2000) and the interaction of available fuels and metabolic state. Mammary lipogenic enzyme concentration is decreased during milk fat depression (Peterson et al., 2003) leading to decreased *de novo* synthesis of milk fat and decreased metabolic use of acetate. Mammary nutrient use of other metabolites, especially gluconeogenic metabolites, is relatively unaffected.

Neither acetate nor glucose spared by utilization of the acetate as an energy source are directly oxidized in the ruminant liver to generate a satiety signal (Allen, 2000). Decreasing intake would only decrease availability of propionate and protein needed for milk production. Partitioning of excess acetate to adipose tissue without negative feedback allows maintenance of protein and gluconeogenic nutrient intake. Cows in this experiment were lower in body condition score (mean 1.9) and replenishment of body energy reserves may have created an acetate demand that limited accumulation of circulating acetate. We speculate that the extent to which milk fat depression results in decreased feed intake depends upon gluconeogenic nutrient absorption from the diet, gluconeogenic metabolite demand by tissues, and the extent to which acetate spares gluconeogenic metabolites. It is reasonable to speculate that UNS failed to regulate energy homeostasis because the positive energy balance is excess acetate, and not excess of a balance of metabolites.

We reported no effect of treatment on intake of eight non-cannulated cows fed the same diet (Chapter 3), although these cows differed greatly in body condition (~1 BCS heavier) and did not experience milk fat depression. Differences in biohydrogenation or metabolic or physiologic response to trans-FA and CLA related to greater body condition may explain variation in intake response to dietary FA reported in the literature and between cow groups.

Direct effects of FA saturation on intake are complicated to determine because of the multiple effects of FA on physiology and metabolism. Fatty acids are recognized as powerful physiologic modifiers affecting endocrine signaling and gene regulation (Drackley, 2000). These modifications result in production and nutrient partitioning

changes, as observed in CLA mediated milk fat depression (Bauman and Griinari, 2003). Intake is regulated by the effect of type and temporal variation of absorbed fuels (Allen, 2000) and animal metabolic state. The role of FA in regulation of intake is complicated by the dual roles of FA as fuels and metabolic modifiers.

Rumen pools

Increasing unsaturated FA concentration linearly decreased wet weight of rumen digesta and rumen digesta volume (Table 3). Addition of RPF decreased wet weight and volume of rumen contents because of a linear decrease by UNS. Treatments did not affect rumen DM percent but RPF decreased pool size of DM, OM and NDF; DM and OM pool sizes tended to decrease, and NDF pool size decreased with increasing UNS. Rumen pools decreased in proportion to intake resulting in no difference in rumen nutrient turnover of DM, OM or NDF (Chapter 4). The observation that UNS decreased DMI and rumen digesta OM pool without affecting turnover of OM provides strong evidence that the intake depression is not an intestinal brake that slows passage rate leading to increased digesta pool size and increased distension in the reticulorumen may have affected intake if physiological response to FA saturation altered the response threshold in the central satiety center.

Feeding and chewing behavior

Intake is determined by the number and size of meals consumed over a day. Treatment did not affect total time spent eating or the number of meals, but UNS linearly decreased meal size by 0.22 kg ($P < 0.03$; Table 4). There were no treatment effects

detected for meal length, meal frequency or intermeal interval. Unsaturated FA linearly increased eating rate. Hunger ratio (meal size over premeal interval) was quadratically affected by UNS but satiety ratio (meal size over postmeal interval) was not changed. Addition of RPF and FA saturation had no effect on chewing time per meal, or per kg of DMI, NDF or forage NDF. The reduction in feed intake by UNS treatment was because of decreased meal size, which implicates greater stimulation of satiety with UNS. Heirichs et al. (1982) observed smaller initial meal but increased number of spontaneous meals with increased FA concentration.

SAT increased time spent ruminating compared to CON and UNS. SAT linearly increased time spent ruminating up to 50 min per d ($P < 0.01$). This increase in rumination time was because SAT linearly increased rumination bout length because there were no treatment effects on number of rumination bouts or interval between bouts. SAT linearly increased rumination chewing time per bout, and quadratically affected rumination time per kg of DMI, NDF, and forage NDF. Allen (1997) reported that total chewing time per day was not related to DMI across treatment means reported in the literature. In this study, the increased rumination for SAT was not because of increased DMI because rumination per kg of DM and fiber intake was quadratically affected by SAT. Benson and Reynolds (2001) were unable to detect significant differences in rumination behavior with rapeseed infusion despite large decreases in time spent ruminating for some individual cows. We have previously observed that SAT increased time spent ruminating but did not change time spent eating compare to calcium salts of palm oil when observed by manual observation every five minutes (Chapter 2). In the current experiment, the linear increase in rumination with increasing SAT appears to be

because of increased duodenal flow of saturated FA because SAT increased rumination compared to CON but UNS did not.

Total time spent chewing linearly decreased with SAT, but was not affected by RPF. Similar to rumination, total time spent chewing per kg of DMI and NDF were quadratically affected by SAT. There were no effects of treatment on rumination or chewing rate, showing that changes in chewing time relate directly to number of chews. Number of eating and ruminating chews per day followed similar patterns to the time spent in these activities previously reported. There was no treatment effect on total eating chews per day but increasing SAT linearly increased total rumination chews per day.

The changes in rumination behavior may be mediated through feed intake differences, associative effects on digestion, or stimulation of gut peptides. As previously mentioned, intake differences cannot explain the changes in chewing behavior because time spent ruminating per kg of NDF was affected by treatment. In addition, treatment diets differed only in FA concentration and profile and contained the same base ration. Diets were not expected to differ in effectiveness of stimulating rumination; chewing activity is more highly related to forage NDF concentration than total NDF concentration (Allen, 1997), and forage NDF concentration was the same across treatments. It is unlikely that increased rumination for SAT was because of increased distention in the reticulorumen because digesta pool size and volume was numerically lower for SAT compared to CON.

We have previously proposed that time spent ruminating may be related to reticular-rumen motility (Chapter 2). Deswysen et al. (1987) reported a strong positive

relationship between the number of rumen contractions and rumination time. We speculate that less rumination for the UNS treatment compared to SAT is associated with reduced ruminal motility related to duodenal FA flow. Nicholson and Omer (1983) showed intestinal infusion of unsaturated FA decreased rumen motility of sheep, and Grovum (1984) reported almost total cessation of rumen motility after 13 h of intragastric infusion of unsaturated fat compared to intravenous infusion. UNS increased plasma concentrations of the gut peptides cholecystokinin (CCK; Choi and Palmquist, 1996), and glucagon-like-peptide-1 (GLP-1; Benson and Reynolds, 2001), and direct intravenous infusions of CCK depressed reticular-rumen motility and intake in sheep (Grovum, 1981). These gut peptide are normally secreted in response to FA ingestion and effect gut motility (Reidelberger, 1994; and Hellstöm and Näslund, 2001). Most experiments testing stimulation of gut peptide secretion employed PUFA treatments and no fat controls. The effect of saturated FA on gut peptide secretion and gut motility is not understood and the mechanism for the increase in rumination time for SAT compared to CON is unknown.

Nutrient Selection

CON treatment resulted in selection against NDF and total FA and for starch compared to RPF treatments. The CON diet contained lower total FA concentration and slightly higher NDF concentration due to inclusion of rice hulls in the space of RPF. Selection against both NDF and FA for the CON treatment might be explained by selection against cottonseeds, which are a source of both nutrients. UNS linearly increased selection against GE and FA. This might be because of palatability differences

in the fat sources, although it is not consistent with the linear increase in eating rate with increasing UNS. An alternative explanation is that selection against GE concentration was because of decreased energy requirements experienced with UNS because of milk fat depression. Differences in selection were very small but highly significant. Small differences may be expected because feeding to 15% orts limits selection in the amount of each nutrient available, and large difference in ort composition is needed to change the composition of diet consumed.

Ruminal pH

Daily mean, minimum and maximum pH were not changed by treatment, but pH range and variance were linearly increased by SAT (Table 5). UNS range and variance was not different from CON, but SAT increased range and variance. Increased range and variance for SAT compared to UNS may reflect the larger meal size observed with SAT; increasing meal size increases fermentable organic matter per meal, increasing VFA production and acid load after the meal. However, meal size was not different for SAT and CON and we are unable to explain the increased range and variance in ruminal pH for SAT compared to CON.

CONCLUSION

Increasing dietary energy density with rumen-protected fat may not increase digestible energy intake. Direct and indirect hypophagic effects of unsaturated FA are not separable as FA and fuel type modify short and long-term physiology. The reduction in feed intake from rumen-protected FA that are unsaturated is through decreased meal

size without an increase in meal frequency. Addition of saturated FA increases rumination bout length and rumination time per day, possibly through modification of gut peptide secretion.

Table 1. Effects of dietary rumen-protected fatty acids on duodenal fatty acid flow.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Duodenal Flow, g/d									
C16:0	290	440	430	350	30	<0.001	<0.001	0.007	0.29
C18:0	580	880	680	470	53	<0.0001	0.07	<0.0001	0.89
C18:1 trans	160	170	280	260	24	<0.001	0.003	0.001	0.02
C18:1 cis	75	84	100	100	6.3	<0.001	<0.001	0.003	0.15
C18:2	94	100	99	97	6.6	0.57	0.28	0.37	0.99
C18:3	7.4	7.8	7.6	6.6	0.4	0.16	0.87	0.04	0.41
Duodenal Composition, % Total FA									
C16:0	20.4	22.3	22.8	23.4	0.33	<0.0001	<0.0001	0.03	0.88
C18:0	41.1	44.6	36.7	30.9	1.4	<0.0001	0.03	<0.0001	0.53
C18:1 trans	11.1	8.5	15.0	18.2	1.4	0.0001	0.07	<0.0001	0.31
C18:1 cis	5.2	4.2	5.4	6.9	0.16	<0.0001	0.11	<0.0001	0.47
C18:2	6.7	5.1	5.3	6.4	0.28	<0.001	0.003	0.002	0.15
C18:3	0.53	0.39	0.41	0.47	0.02	0.001	<0.001	0.02	0.39

¹ Treatments were CON- control with no supplemental rumen-protected fat, SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

Table 2. Effects of dietary rumen-protected fatty acids on intake of nutrients.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Intake, kg/d									
DM	27.3	25.7	25.1	24.1	1.0	0.001	<0.001	0.02	0.60
OM	24.4	23.0	22.6	21.6	0.9	0.001	<0.001	0.02	0.61
NDF	7.3	6.7	6.6	6.3	0.25	<0.001	<0.001	0.09	0.52
Starch	8.2	7.4	7.3	7.0	0.3	<0.001	<0.001	0.04	0.64
FA	1.5	2.1	2.0	1.8	0.07	<0.0001	<0.0001	<0.0001	0.68
DE ³ , Mcal/d	78.0	75.3	74.0	70.1	3.1	0.07	0.04	0.06	0.59

¹ Treatments were CON- control with no supplemental rumen-protected fat, SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

Table 3. Effects dietary rumen-protected fatty acids on ruminal nutrient pool.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Ruminal wet									
Contents, kg	86.6	85.7	80.7	74.4	3.7	0.041	<0.10	0.02	0.87
Ruminal contents									
Volume, L	101	97	93	89	4	0.06	0.04	0.05	0.94
Ruminal contents,									
%DM	16.9	17.1	17.4	16.9	0.4	0.72	0.62	0.73	0.35
Ruminal pool, kg									
DM	14.7	13.7	14.0	12.5	0.6	0.02	0.02	0.09	0.12
OM	13.5	12.7	12.9	11.6	0.5	0.02	0.03	0.09	0.10
NDF	7.9	7.5	7.4	6.5	0.3	<0.01	0.02	0.01	0.16

¹ Treatments were CON- control with no supplemental rumen-protected fat, SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

Table 4. Effects of dietary rumen-protected fatty acids on meal patterns and water consumption.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Meals									
Bouts / d	10.1	10.2	10.0	10.6	0.6	0.585	0.76	0.37	0.32
Length, min/meal	30.5	29.5	30.1	26.9	2.0	0.30	0.41	0.19	0.27
Interval, min	104	111	109	106	7	0.75	0.51	0.80	0.91
Eating rate, kg DM/min	0.089	0.085	0.086	0.095	0.006	0.16	0.98	0.04	0.33
Meal size, kg									
DM	2.51	2.50	2.54	2.28	0.15	0.04	0.48	0.03	0.07
Total FA									
Hunger ratio ³ , kg/min	0.057	0.051	0.058	0.046	0.007	0.15	0.42	0.34	0.05
Satiety ratio ³ , kg/min	0.056	0.053	0.059	0.051	0.008	0.66	0.81	0.73	0.25
Rumination									
Bouts /d	13.3	13.0	13.6	13.5	0.5	0.55	0.88	0.25	0.41
Bout length, min	44.2	48.1	43.0	42.5	1.6	0.01	0.84	<0.01	0.10
Bout interval, min	63.9	59.8	59.2	62.2	3.6	0.55	0.30	0.47	0.52
Ruminating chew rate, chews/min	63.9	64.9	64.9	63.9	1.5	0.47	0.46	0.23	0.49
Water drunk, L/d	105.9	99.6	99.4	100.4	3.7	0.20	0.04	0.81	0.82
Drinking bouts /d	12.6	9.9	11.3	12.8	1.2	0.02	0.12	0.007	0.53

¹ Treatments were CON- control with no supplemental rumen-protected fat, SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

³ Hunger ratio = meal kg DM / premeal interval.

Satiety ratio = meal kg DM / postmeal interval.

Table 5. Effects of dietary rumen-protected fatty acids on chewing behavior.

	Treatment LS Means ¹					<i>P</i> ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Eating chewing Time, min									
/d	257	257	253	246	10.6	0.65	0.61	0.26	0.86
/bout	25.3	25.6	25.6	23.2	1.6	0.33	0.74	0.12	0.35
/kg of DMI	10.1	10.7	10.4	10.6	0.6	0.41	0.18	0.82	0.30
/kg of NDF intake	37	39	38	39	2.4	0.53	0.29	0.65	0.36
/kg of forage NDF intake	62	65	63	65	4.0	0.37	0.16	0.79	0.28
Ruminating chewing time, min									
/d	574	616	568	560	15	0.02	0.65	0.004	0.16
/bout	43.5	47.6	42.1	41.6	1.6	<0.01	0.84	<0.01	0.08
/kg of DMI	23	25	23	24	0.9	0.01	0.04	0.08	0.02
/kg of NDF intake	83	93	85	87	3.2	<0.01	0.04	0.03	0.02
/kg of forage NDF intake	138	154	141	147	5.5	0.01	0.03	0.08	0.02
Total chewing time, min									
/d	833	872	822	805	22	0.08	0.98	0.01	0.41
/kg of DMI	18.2	20.0	18.6	19.2	0.8	0.02	0.04	0.17	0.03
/kg of NDF intake	120	132	122	125	5.4	0.02	0.06	0.07	0.04
Chew Rate									
Eating	83.4	81.8	85.9	83.0	3.0	0.44	0.97	0.66	0.12
Ruminating	64.0	64.9	64.9	63.9	1.5	0.47	0.46	0.23	0.49
Chews, # / d									
Eating	21500	21200	22000	20600	1500	0.74	0.85	0.66	0.33
Ruminating	37100	40200	37500	36200	1500	0.04	0.53	<0.01	0.51
Total	58800	61200	59400	56700	2400	0.31	0.13	0.07	0.83

¹ Treatments were CON- control with no supplemental rumen-protected fat, SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

Table 6. Effects of dietary rumen-protected fatty acids on nutrient selection.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Cannulated									
NDF Selection ³	95.6	98.8	99.3	99.2	1.4	0.08	0.01	0.80	0.81
INDF Selection ³	99.2	100.9	101.2	99.0	1.5	0.37	0.37	0.23	0.34
Starch Selection ³	102.1	99.0	99.0	99.8	1.2	0.08	0.01	0.54	0.74
CP Selection ³	100.4	100.2	100.6	100.3	0.29	0.77	0.90	0.89	0.31
GE Selection ³	99.6	99.9	99.8	99.6	0.08	0.009	0.12	0.003	0.33
TFA Selection ³	99.4	101.3	100.9	101.7	0.68	0.10	0.02	0.63	0.43

¹ Treatments were CON- control with no supplemental rumen-protected fat, SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

³ Selection= (nutrient concentration consumed / nutrient concentration fed) x 100

Table 7. Effects of dietary rumen-protected fatty acids on ruminal pH.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Daily ruminal pH									
Mean	5.97	5.99	6.05	6.01	0.06	0.76	0.50	0.74	0.46
Minimum	5.42	5.34	5.42	5.42	0.05	0.61	0.58	0.29	0.50
Maximum	6.66	6.66	6.67	6.56	0.06	0.33	0.29	0.23	0.35
Range	1.13	1.31	1.25	1.15	0.05	0.02	0.03	<0.01	0.62
Variance	0.11	0.14	0.13	0.1	0.01	0.01	<0.1	0.005	0.46
Standard deviation	0.32	0.37	0.35	0.31	0.02	0.01	0.08	0.005	0.50
Time < 6.0, h	12.3	11.5	9.9	10.9	1.3	0.53	0.30	0.72	0.35
Area below 6.0, h	497	470	376	380	73	0.51	0.30	0.38	0.56

¹ Treatments were CON- control with no supplemental rumen-protected fat, SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

CHAPTER 7

CONCLUSIONS AND IMPLICATIONS

Fatty acids (FA) are bioactive compounds that have significant effects on animal physiology and metabolism. Commonly fed FA sources can be used to alter absorbed FA, feed intake, milk fat synthesis, and energy partitioning. This research showed that FA profile reaching the duodenum is important in determining intake response to fat supplementation.

The first experiment demonstrated that the decrease in feed intake from rumen-protected palm oil was not related to milk yield across cows. Calcium salts of palm oil did not induce milk fat depression and is expected to have increased duodenal flow of unsaturated FA. Saturation of FA is an important determinant of insulin secretion and plasma NEFA concentration. Saturated FA increased milk protein and the magnitude of the response appears to be related to production level, or insulin signaling.

Dietary unsaturated FA may have a large impact on milk fat yield and body weight gain, depending on production of trans-FA isomers in the rumen. Absorbed fatty acid profile may have a larger effect on energy balance than does the amount of FA absorbed because fatty acids can drastically modify production. Milk fat depression induced by biohydrogenation intermediates decreases milk fat production through decreased *de novo* synthesis, reducing the concentration of short and medium chain fatty acids. Increased body weight gain during milk fat depression may be due to increased

availability of acetate while intake is partially maintained to sustain protein and gluconeogenic metabolite homeostasis.

Prediction of the profile of duodenal FA flow requires a greater understanding of rumen FA metabolism. Calculation of individual FA biohydrogenation with a simplified model allows a more mechanistic description of rumen FA biohydrogenation. Future ability to predict duodenal FA profile depends on observation and analysis of individual steps of FA metabolism. Modeling biological systems requires simplification and assumption of unknown or undeterminable events. Assumptions for this model must be tested in the future. More complex models may be developed to model duodenal flow of individual trans isomers, although flux through such pathways may require isotope labeling. Calcium soaps provide limited protection of polyunsaturated FA in the rumen. Polyunsaturated FA are highly biohydrogenated, but trans-monounsaturated FA biohydrogenation is limited especially with increased levels of dietary polyunsaturated FA.

Increased rumination with saturated FA might alter rumen nutrient passage rate, increasing passage of more highly digestible particles. Increasing passage rate of highly digestible particle decreases ruminal OM and NDF digestibility due to selective retention of less digestible feed particle. The impact of rumination on digestion and passage kinetics has not been explored. Changing the amount of rumination and gut motility may be very applicable to lower fiber diets containing less effective fiber. Increasing FA intake and saturation together decrease FA digestibility, although the effects are confounded. Fatty acid digestibility may be modified by associative effects of other FA and duodenal OM composition and the amount of duodenal flow. Addition of rumen-

protected FA may not increase energy intake because of associative effects on ruminal digestion and decreased DM intake. More controlled experiments of FA digestibility are needed to titrate the effect of duodenal FA flow and associative effects of digesta on FA digestibility.

Increasing dietary energy density with rumen-protected fat may not increase digestible energy intake. Direct and indirect hypophagic effects of unsaturated FA are not separable because FA are an energy source and modify physiology. The reduction in feed intake from unsaturated rumen-protected FA is through decreased meal size without an increase in meal frequency. Addition of saturated FA increases rumination bout length and rumination time per day, possibly through modification of gut peptide secretion. Stimulation of gut peptide secretion may be tested with duodenal challenges of different FA to provide a clearer understanding of FA profile on gut peptides without confounding of FA intake, biohydrogenation and rumen FA activity.

Fatty acids play a much larger role in animal nutrition than simply an energy source. As Storlein (2000) observed “Oils ain’t oils”. Fatty acids provide an opportunity to modify ruminal digestion, rumination, milk component yield, body weight gain and intake through manipulation of a small portion of the diet. It is expected that many of these effects are mediated by very small concentrations of a FA or FA isomers. Future research should allow identification of effects of individual FA and an understanding of their mechanism of action. The experiments presented provide strong preliminary evidence for further research in a number of areas. Saturated FA have large effects on rumination that has not been previously observed. The effect of saturated FA on rumination and gut motility should be examined independently of intake with abomasal

infusion of different FA at the same intake. The effect of fat saturation on gut peptide secretion should also be examined using abomasal infusion challenges of different FA. The role of saturated FA in plasma insulin concentration also deserves further research to determine if plasma concentrations rise because of increased insulin secretion or decreased clearance. The biohydrogenation model proposed may also provide a framework to understand associative dietary and rumen environment effects on biohydrogenation. Lastly, the effect of C18:1 FA isomers on intake and feeding behavior should be examined.

APPENDIX

Table 1. Effects of dietary rumen-protected fatty acids on fatty acid intake of non-cannulated cows

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Intake, g/d									
C16:0	270	500	470	450	17	<0.001	<0.001	0.01	0.62
C18:0	40	390	220	60	11	<0.001	<0.001	<0.001	0.63
C18:1	240	290	340	400	12	<0.001	<0.001	<0.001	0.66
C18:2	680	690	710	770	26	0.004	0.02	0.002	0.47
C18:3	40	41	43	47	1.5	0.001	0.02	<0.001	0.56

¹ Treatments were CON- control with no supplemental rumen-inert fat, SAT- saturated fat from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

Table 2. Effects of dietary rumen protected fatty acids on ruminal pool variance.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Before v After Feeding Rumen Profile, Before / After									
Total FA	0.82	0.83	0.70	0.72	0.05	0.11	0.22	0.09	0.14
C16:0	0.83	0.83	0.69	0.71	0.05	0.06	0.13	0.06	0.14
C18:0	0.95	0.90	0.85	0.92	0.05	0.32	0.17	0.76	0.23
C18:1 trans	0.84	1.05	0.95	0.88	0.09	0.37	0.24	0.19	0.90
C18:1 cis	0.67	0.74	0.53	0.54	0.08	0.17	0.45	0.08	0.23
C18:2	0.66	0.73	0.51	0.54	0.10	0.30	0.52	0.16	0.24
C18:3	0.48	0.65	0.44	0.47	0.10	0.30	0.66	0.14	0.23

¹ Treatments were CON- control with no supplemental rumen-protected FA, SAT- saturated FA from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² TRT: treatment effect, RPF: fat supplement effect, L: linear effect, and Q: quadratic effect.

Table 3. Effects of dietary rumen-protected fatty acids on flux of ruminal fatty acid disappearance.

	Treatment LS Means ¹					P ²			
	CON	SAT	INT	UNS	SE	TRT	RPF	L	Q
Disappearance Flux (g/d)									
C18:1	680	630	600	570	29	0.02	0.009	0.07	0.99
C18:1 trans	740	660	590	540	38	0.002	0.001	0.01	0.87
C18:1 cis	160	180	230	240	8	<0.001	<0.001	<0.001	0.07
C18:2	560	540	620	620	25	0.03	0.005	0.02	0.01
C18:3	33	31	38	40	1.4	<0.001	0.007	<0.001	<0.001

¹ Treatments were CON- control with no supplemental rumen-protected FA, SAT- saturated FA from prilled FA, INT- intermediate saturation as a mix of prilled FA and calcium soaps of LCFA, and UNS- unsaturated fat fed as Ca soaps of LCFA.

² TRT: treatment effect, Fat: fat supplement effect, L: linear effect, and Q: quadratic effect.

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