# BIOHYDROGENATION, POSTRUMINAL FLOW, AND APPARENT DIGESTIBILITY OF DIETARY LIPIDS IN LACTATING HOLSTEIN COWS

by

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(ABSTRACT)

Lactating cows with cannulae in the rumen and proximal duodenum were used in two 4 X 4 Latin square experiments to evaluate biohydrogenation, flow rates, and digestibility of dietary fatty acids in the gastrointestinal tract. In the first experiment, four cows were fed diets with 0% supplemental fat, 3% tallow, 3% partially hydrogenated tallow, or 3% tallow coated with casein and corn syrup solids. Fatty acid intake and flow to the duodenum increased with fat supplementation. Total tract apparent fatty acid digestibility was reduced by partially hydrogenated tallow. Apparent digestibility of $C_{18:0}$ in the small intestine quadradically decreased ($R^2$=.86) as $C_{18:0}$ flow to the intestine increased. Fat supplementation increased milk production and decreased milk protein percent but did not affect milk fat percent or 3.5% FCM. Fat supplementation increased $C_{18:0}$ and $C_{18:1}$ and lowered $C_{12:0}$ and $C_{14:0}$ concentrations in milk fat. In the second experiment, four lactating Holstein cows were fed diets with 0% supplemental fat, 3% partially hydrogenated fatty acids, 1.5% fish oil plus 1.5% stearic acid, or 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil. Fish oil decreased DM intake. Fish oil and soybean oil reduced biohydrogenation of $C_{18:1}$ + $C_{18:2}$ + $C_{18:3}$ in the rumen from 65% (control) to 28% and 55%, respectively, and increased trans $C_{18:1}$ flow from the rumen 4-fold. Milk fat percent was decreased by fish oil and soybean oil. Milk fat percent across treatments linearly decreased with amount (g/d) of trans $C_{18:1}$ flowing to the duodenum ($R^2$=.92) and percent trans $C_{18:1}$ in milk fat ($R^2$=.94).

## DEDICATION

This thesis is dedicated to my wife, Kelly; my parents, Bob and Marie; and my relatives, Rich and Monica Cincera.

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# CHAPTER 1

## INTRODUCTION

Genetic selection of dairy cows for high milk production has increased the number of cows unable to consume enough energy to meet production requirements during early lactation. Cows frequently do not reach maximum dry matter intake until 3 to 6 wk following peak milk production. Body tissues are utilized as an energy source to compensate for inadequate dietary energy intake; thus, body weight decreases. Supplementing diets with large amounts of starch (cereal grains) increases energy density of the diet. However, excessive amounts of cereal grains at the expense of forages can adversely affect rumen function, dry matter intake, nutrient digestibilities, and milk composition.

Fat supplementation has been evaluated as an alternative for increasing energy density of dairy cattle diets. Fats contain approximately 2.25 times as much energy as equal portions of carbohydrates or protein. Therefore, fat supplements increase the energy content of a diet by replacing cereal grains without reducing amount of forage. Fat supplementation, however, is not without limitations or pitfalls. In order for fats to be considered a viable dietary energy source, they should not adversely affect rumen function, nutrient digestibilities, and milk production (volume or composition).

Fat supplements in dairy cattle diets commonly include oilseeds (raw soybeans, roasted soybeans, whole cottonseed), Ca-salts of palm oil, tallow, or tallow hydrogenated to reduce the amount of unsaturated fatty acids. Research has been conducted to evaluate the effects of different types and levels of fat supplementation on milk volume and composition. Further understanding of how various fat supplements are metabolized in conjunction with other components of the diet is needed.

Few experiments with high-producing, early-lactation cows consuming large quantities of DM with supplemental fat have been conducted to intensively evaluate digestion of fatty acids in the gastrointestinal tract. Cows with digestive tract cannulae are needed to obtain data for calculation of nutrient flows and digestibilities when fat supplements are added to diets.

The objectives of this study were to evaluate the effects of various fat supplements on:

1. Extent of unsaturated fatty acid biohydrogenation in the rumen.
2. DM, ADF, N, OM, and fatty acid (bacterial and dietary) flow to the small intestine.
3. Apparent digestibility of nutrients in the gastrointestinal tract.
4. Trans $C_{18:1}$ flow to the small intestine and incorporation into milk fat.
5. Milk production and composition.
6. Fatty acid concentrations in milk fat.

# CHAPTER 2

## REVIEW OF LITERATURE

### History

Research trials utilizing high-fat feeds for lactating dairy cow diets have been reported over the past 50 yr. Early researchers investigated effects of fat supplementation on feed intake, milk production, milk components, and nutrient digestibilities. Lucas and Loosli (79) reported that supplementing dairy cow diets with soybean meal increased milk production, but soybean oil and corn oil (2% of diet DM) decreased milk flow without drastically affecting milk fat percent. However, Maynard et al. (85) and Loosli et al. (77) observed increased milk production when various levels of fat were added to the concentrate portion of lactating dairy cow diets. Research of that era exposed the benefits and pitfalls of fat supplementation. Since then, numerous experiments have been conducted in an attempt to enhance our understanding of lipid metabolism so that fat supplements may be better utilized by ruminants.

### Lipid metabolism in the rumen

Forages and grains contain high proportions of unsaturated fatty acids, but ruminant adipose and milk fat contain appreciable amounts of saturated fatty acids. Research beginning in the mid-1950s set out to determine what effects the rumen and it's microbial populations could have on this unexplained phenomenon. Presently, it is known that the lipid fraction of forages contain large amounts of linolenic ($C_{18:3n3}$) and linoleic ($C_{18:2n6}$) acids, primarily in glycolipids and phospholipids. Supplementing diets with cereal grains and seed oils increases the

levels of linoleic ($C_{18:2n6}$) and oleic ($C_{18:1n9}$) acids, primarily in triglycerides.

Shorland et al. (113), in 1957, demonstrated the conversion of linolenic and linoleic acids to monounsaturated fatty acids when incubated with rumen contents. Garton et al. (36) were first to show that triglycerides were hydrolyzed by microbial enzymes when incubated in vitro with rumen contents. Critical work by Garton (33, 37) with triglycerides, and Dawson et al. (21, 22) with galactolipids have verified that rumen microbial lipases are responsible for hydrolyzing ester linkages of dietary acyl lipids. Kepler et al. (64) and Hazlewood et al (48) went on to show that a free carboxyl group is required for hydrogenation to take place.

Polan et al. (105) clarified some mechanisms for biohydrogenation in the rumen. They found that washed suspensions of mixed rumen bacteria could hydrogenate linoleic and linolenic acids. However, pure cultures of Butyrivibrio fibrisolvens (a common rumen bacterium) could biohydrogenate linoleic acid to octadecenoic acid but not to stearic acid. They theorized biohydrogenation of $C_{18:2}$ to $C_{18:0}$ involved two separate systems, one that converted $C_{18:2}$ to $C_{18:1}$ and the other that converted $C_{18:1}$ to $C_{18:0}$.

The number of biohydrogenating species of bacteria isolated is relatively small. For a complete listing of biohydrogenating bacteria and their principle investigators, see Harfoot and Hazlewood (44).

A striking feature of isolated biohydrogenating bacteria is the wide range of endproducts produced. Although biohydrogenating endproducts are diverse, Bickerstaffe et al. (12) found the predominant endproducts of biohydrogenation to be stearic (80%) and $\alpha^{11}$-trans octadecenoic (12%) acid. He also reported that 90% of dietary linolenate, linoleate, and oleate were biohydrogenated in the rumen of lactating goats. Mattos and Palmquist (84) found 68% of linoleic acid

biohydrogenated in lactating cows. Hazlewood et al. (48) and Kemp and Lander (61) grouped bacteria by biohydrogenation capabilities. The generally accepted biohydrogenation pathways for α-linolenic and linoleic acids can be found in Fig. 1 and Fig. 2 respectively. Kemp and Lander (60) also proposed a scheme for the biohydrogenation of α-linolenic acid (minor importance). Members of group A bacteria primarily hydrogenated α-linolenic and linoleic acids to trans-11-octadecenoic. Kepler et al. (63) showed that Butyrivibrio fibrisolvens (group A bacterium) isomerized cis-9, cis-12 linoleic acid to a cis-9, trans-11 conjugated diene. Subsequent hydrogenation then occurred at the cis-9 double bond. Members of group B bacteria hydrogenate a wide range of octadecenoic acids, including cis-9 and trans-11 acids, to stearic acid. Apparently, complete hydrogenation of α-linolenic and linoleic acids to stearic requires the presence of both group A and group B bacteria. This may explain why Polan et al. (105), using strains of Butyrivibrio fibrisolvens (group A bacterium), did not get complete hydrogenation of linoleic acid. Hydrogenation was complete when Kemp and Lander (61) mixed cultures containing pure strains of bacteria belonging to each group.

Recently, it has been suggested that Butyrivibrio fibrisolvens hydrogenate cis-9, trans-11 octadecadienoic acid (conjugated diene) to trans-11-octadecenoic in the presence of α-tocopherolquinol and a flavin-like compound (52, 140). It appears two molecules of α-tocopherolquinol are oxidized in order to provide electrons for the reduction of the cis bond of the conjugated diene with the flavin-like compound acting as an intermediary in the transfer of electrons. Hughes et al. (53) have since purified a cis-9, trans-11 octadecadienoate reductase (absolute requirement for $Fe^{3+}$) that is directly involved

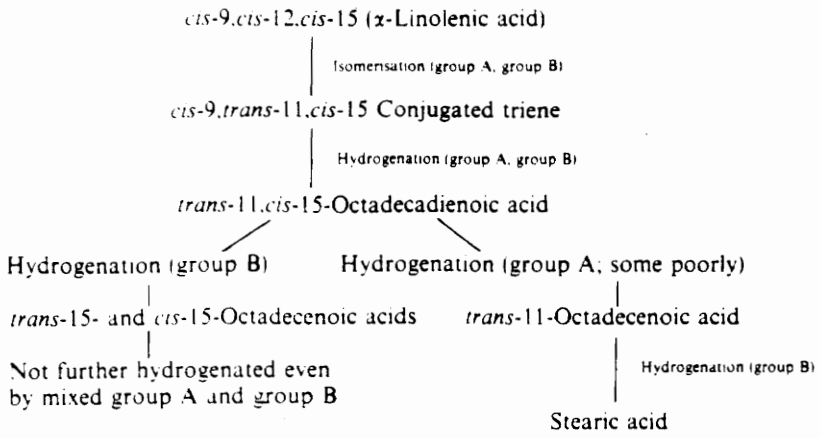


**Figure 1.** **Scheme for biohydrogenation of α-linolenic acid; group A and group B refer to two class of biohydrogenating bacteria. Adapted from (44).**

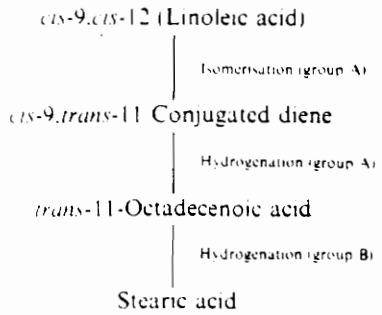


**Figure 2.** **Scheme for biohydrogenation of linoleic acid; group A and group B refer to two classes of biohydrogenating bacteria. Adapted from (44).**

with the oxidation-reduction reaction mentioned above. It has not been established whether or not this system is used by group B bacteria to biohydrogenate trans-11-octadecenoic to stearic acid.

The general consensus is that unsaturated fatty acids are absorbed by rumen microbes (primarily bacteria), hydrogenated, and then liberated as more saturated derivatives. The role of biohydrogenation, however, is not completely understood. Polan et al. (105) theorized that unsaturated fatty acids serve as terminal acceptors for metabolic hydrogen. They showed that hydrogenation of linoleic acid could be inhibited by hydrogen acceptors such as carbon dioxide, fumarate, crotonate and acrylate. Lennary (76) also suggested that the main function of biohydrogenation was disposal of reducing power (metabolic hydrogen) in the rumen. A different theory is that biohydrogenation serves to detoxify unsaturated fatty acids (61). Harfoot et al. (45) showed that fatty acids in rumen contents preferentially bind to food particles, competing with bacteria for adsorption. They proposed that such a mechanism would decrease inhibitory effects of LCFA on rumen microorganisms.

Another factor affecting in vivo biohydrogenation is whether or not dietary fat is introduced as a free acid or triglyceride. Moore et al. (89) and Noble et al. (94) found esterified linoleic acid added to the rumen of sheep was completely hydrogenated to stearic acid. When linoleic acid was added as a free acid, biohydrogenation was incomplete and larger amounts of trans-11-octadecenoic were recovered.
A possible explanation is that fat added as a free acid may saturate the biohydrogenation system leading to incomplete biohydrogenation of unsaturated fatty acids.

Lipid composition of rumen microorganisms

Viviani et al. (137) reported that lipids of mixed rumen bacteria contained 30% phospholipid and 70% nonphospholipid, in which over 40% of the fatty acids were unesterified. In contrast, mixed protozoa contain approximately 70% phospholipid and 30% neutral lipid (59). The fatty acids in bacterial and protozoal lipids can be branched or straight carbon chains synthesized (de novo) by metabolism of straight or branched-chain amino acids (34). Recent evidence (11) confirms that there may also be incorporation of preformed free fatty acids into bacterial lipids. Bauchart et al. (11) found that addition of oil or fat to experimental diets increased the free fatty acid content of solid- and liquid-associated bacteria by 150%. They found that additional free fatty acids were incorporated into the intracellular free fatty acid pool rather than associated with the cell envelope.

Distribution of bacteria in the rumen

Bacterial populations may be associated with the liquid phase, loosely attached to feed particles, or firmly attached to feed particles. Legay-Carmier and Bauchart (75) showed that lipid and fatty acid concentrations are twice as high in solid-adherent bacteria as in liquid-associated bacteria. Thus, harvesting mixed bacterial populations from rumen liquid may underestimate total bacterial contribution to duodenal fatty acid flow, because liquid-associated bacteria constitute only a small fraction of total microbial mass in the rumen (75). Cecava et al. (16) reported that liquid-associated bacteria may be unrepresentative of the overall rumen bacteria population with respect to nitrogen content. They found differences in composition of bacteria harvested from the liquid phase compared with particle-associated or mixed populations of ruminal bacteria. The differences

translated into different estimates of bacterial nitrogen supplied to the small intestine.

Effect of fat supplementation on DM intake and nutrient digestibilities

Fat supplementation can have a variety of effects on dry matter intake and nutrient digestibility. A reduction in dry matter intake could reflect diet palatability, reduced fiber digestibility in the rumen, or regulation of energy intake. Macleod et al. (80) found sheep diets supplemented at 3% of DM with soybean oil, saturated fatty acids, or hydrogenated tallow reduced crude fiber and ADF digestibility. Jenkins (54) showed (in vitro) that corn oil and tallow fatty acid supplementation reduced fiber digestibility and total volatile fatty acid production. Jenkins and Jenny (56) reported lactating cows fed diets containg 5% yellow grease or 3 or 5% hydrogenated yellow grease reduced total tract apparent fiber digestibility. Reduced fiber digestibility has been linked to physical coating of food particles and rumen microbes with fat and an apparent toxic effect of unsaturated fatty acids on rumen microorganisms. Reduction of fiber digestibility often leads to reduced rumen DM turnover and intake.

Oil seeds (seedcoat surrounds lipid), and physical or chemically altered fats supposedly provide "inert" or "protected" sources of supplemental fat for diets of lactating dairy cows. The purpose of feeding such fats is to minimize "contact" of fat supplements with rumen microorganisms and feed particles. A successful (but not commercially available) method to protect lipids from microbial attack is to envelope fats in formaldehyde treated protein. Tamminga et al. (129) found protecting sunflower oil in this manner did not affect DM intake or overall apparent digestion of the diet. Another method is complexing fatty acids with metal cations to form insoluble salts (57). This has led to development of Megalac$^{R}$ (Ca-salts of palm oil). Other commercial

products include partially hydrogenated tallow fatty acids (Alifet$^{R}$, Carolac$^{R}$) and partially hydrogenated nonesterified long chain fatty acids (Energy Booster 100$^{TM}$). Saturated fatty acids may be more insoluble in the rumen (less likely to associate with rumen microorganisms or feed particles).

Ca-salts of long chain fatty acids (29, 40, 62, 69) prilled fats (40, 58, 108), and cottonseed (115) did not affect nutrient digestibility. DM intake was not affected when diets contained Ca-salts of long chain fatty acids (40, 68, 108), prilled fat (40, 108), cottonseed (115), extruded soybeans (65), whole roasted soybeans (26, 69), or tallow (100). However, Palmquist et al. (103) observed decreased DM intake (across treatments) when cows were supplemented with commercial fat products (5.70% of diet DM), but total tract digestibilities of DM, ADF, and NDF were not affected. In the same study, DM intake was not affected when fat products were supplemented at only 2.85% of diet DM. Ferguson et al. (32) observed decreased DM intake when lactating cows were supplemented with prilled fatty acids (3, 6, and 9% of diet DM). Jenkins and Jenny (56) found yellow grease (5% of diet DM) decreased DM intake of lactating dairy cows compared to controls. Nitrogen digestibility may increase (115), or not be affected by fat supplementation (29, 68, 100).

Effect of fat supplements on rumen VFA production

Fat supplements may reduce digestibility of fiber in the rumen. Storry et al. (125) found that unprotected fat impaired fiber digestion and depressed appetite. Reduced fiber digestibility was associated with decreased milk fat percent due to a shift from acetate to propionate production in the rumen (102). Balmain et al. (8) indicated the importance of acetate as a precursor for milk fat synthesis. Rumen VFA

production, therefore, is used as an indication of whether or not fat supplements adversely affect fermentation of dietary fiber in the rumen.

Jenkins and Palmquist (57) found that formation of Ca-salts (in vitro) prevented growth inhibition of ruminal bacteria caused by free long chain fatty acids. Jenkins (54) added blended animal-vegetable fat (up to 10% of diet DM) to in vitro cultures and found only a decrease in butyric acid concentration. Corn oil and tallow fatty acids, however, increased propionic acid concentrations which significantly lowered the ratio of acetate to propionate. Chalupa et al. (17) found that VFA production in vitro was decreased by long chain fatty acids with less than 18 carbons and unsaturated long chain fatty acids with 18 carbons. However, stearic acid did not affect VFA production. In a follow-up study, Chalupa et al. (18) fed diets containing 10% added fat to lactating first-calf heifers. They found that stearic acid or Ca-salts of long chain fatty acids decreased the ratio of acetate to propionate by about 20%; whereas, tallow or oleic acid decreased the ratio by 50 to 60%. Storry et al. (126) fed tallow to lactating cows at 6% of diet DM and found it had minimal effects on ruminal VFA concentrations. Grummer (40) found no effect of prilled fats or Ca-salts of long chain fatty acids (.68 kg/d) on ruminal acetate production or the ratio of acetate to propionate. Schauff and Clark (109) reported increased acetate production and an increased acetate to propionate ratio when cows were fed Megalac$^{R}$ (6 and 9% of diet DM). Palmquist et al. (103) found that supplementing dairy cow diets with Megalac$^{R}$, Energy Booster 100$^{TM}$, Alifet$^{R}$, Booster Fat 95$^{TM}$ or animal-vegetable blend (all at 4.3% of diet DM) did not affect rumen VFA concentrations. Ferguson et al. (32) fed diets with 0, 3, 6, and 9% prilled hard fat (Dairy Fat Prills) to lactating cows and observed a dose-dependent, linear decrease in the ratio of acetate to propionate, because proportions of acetate decreased and propionate increased. Infusion of fish oil (50 ml/d) (119) and

feeding whole roasted soybeans up to 24% diet DM (69) did not affect rumen VFA molar percentages.

Long chain fatty acid recovery at the duodenum.

Evidence for long chain fatty acid absorption across the rumen mucosa appears to be limited. Wood et al. (138) recovered 85 to 96% of a dose of [1-$^{14}$C]-linoleic acid injected into the isolated rumen of sheep (ligation of the reticulo-omasum). Bickerstaffe et al. (12) dosed the rumen of lactating goats with a mixture of [1-$^{14}$C]-linolenic, [1-$^{14}$C]-linoleic, and [9,10-$^{3}$H]-oleic acids and found only .03% of the dose in blood plasma (labeled digesta was replaced with nonlabeled digesta prior to reaching the small intestine).

Opinions differ concerning the relative amount of lipid reaching the duodenum in comparison to that ingested in the diet. Sutton (128), quoting (43, 70, 127) reported long chain fatty acid flows to the duodenum of goats, sheep, and cattle ranged from -18 to >100%. Klusmeyer et al. (67) found 103 to 128% of dietary long chain fatty acids flowing to the duodenum when lactating dairy cows were fed Ca-salts of long chain fatty acids. However, Wu et al. (139) found an apparent 10% loss of fatty acids in the rumen of lactating dairy cows fed Ca-salts of long chain fatty acids and animal-vegetable blend fat. Bauchart et al. (10) reported an increase (17.3%) in duodenal long chain fatty acid flow when cows were fed a control diet (60% forage and 40% concentrate) but a net loss (-22.2%) when milk supplied 33% of the diet DM. Increased fatty acid flow (where found) was attributed to microbial fatty acid synthesis and endogenous sources. Knight et al. (71) used radiolabeled [1-C$^{14}$]-acetate to estimate that 35% of the 25% increase in fatty acids flowing to the duodenum of sheep fed hay and concentrate was accounted for by synthesis from acetate.

Some of the variation noted above may be due to methods used for estimating digesta flow (liquid or particulate digesta markers) and(or) lipid analysis procedures. Inherent errors in the various methods could affect the calculated amount of long chain fatty acids recovered at the duodenum. Generally, the above studies used a limited number of animals, which also could affect calculated fatty acid recovery, provided one or more of the animals was an outlier.

Digestion and absorption of lipids

Initial stages of dietary lipid digestion in ruminants begins before digesta reaches the small intestine. Within the rumen, dietary lipids present as mono- and di-galactolipids or triglycerides undergo extensive hydrolysis. Resultant free fatty acids may be hydrogenated incompletely to various dienoic and monoenoic intermediates or completely to stearic acid. If large amounts of lipid are ingested or if "protected" fat supplements are fed, amounts of unsaturated fatty acids and triglycerides reaching the small intestine may increase. Unlike monogastrics, ruminant digesta flow to the duodenum is relatively continuous. Triacylglycerols, free fatty acids, and rumen microbes preferentially bind to the particulate phase of digesta (78). As acidified digesta passes through the duodenum, it is augmented with bile and pancreatic secretions. However, unlike monogastrics, the neutralizing capacities of bile and pancreatic secretions are insufficient to rapidly raise pH of digesta. Therefore, absorption of fatty acids in the duodenum is limited. It appears the major site of lipid absorption in the ruminant is in the middle to lower distal jejunum (ph 5-6).

Bile and pancreatic secretions are a requirement for optimal fatty acid absorption in the acidic environment of the ruminant digestive tract. Whereas monogastrics rely on monoglycerides for micelle

formation, ruminants utilize biliary and bacterial phospholipids. Biliary lecithin, lysolecithin (via phospholipase $A_2$), and phosphotidylethanolamine (primarily microbial origin) enhance micellization and absorption of long chain fatty acids. Dispersion and solubilization of long chain fatty acids is a prerequisite for absorption. As previously mentioned, dietary and endogenous lipids predominantly bind to the particulate phase in digesta. Harrison et al. (46) estimated that in the acidic conditions (pH 2-3) of the upper jejunum of sheep, nearly 40% of bile salts were associated with particulate matter. As digesta moved down the jejunum, there was an increased transfer of nonesterified fatty acids from particulate matter into micelles.

When large amounts of triglyceride were presented for digestion and absorption within the small intestine of sheep, Moore et al. (88) found pancreatic lipase activity was not a limiting factor in lipolysis of triglyceride bypassing the rumen. However, infusing 760 ml of soy oil (but not 900 ml of soy lecithin) into the abomasum of lactating cows caused diarrhea (41).

Degree of fatty acid unsaturation, esterification, and physical form (ie. oil, flakes, or free flowing beadlets) may influence the ability of the small intestine to efficiently absorb long chain fatty acids. Steele et al. (124) reported an inverse relationship between fatty acid melting point and fatty acid digestibility in sheep. As degree of saturation increased (higher melting point), fatty acids became less digestible (114). Subsequent work by Steele (121) showed that saturated fatty acids could be effieciently absorbed by sheep if adaquate dispersion of saturated fatty acids into micelles occurred.

Macleod (80) found that sheep fed hydrogenated tallow (3% diet DM) had lower apparent fatty acid digestibilities (34%) compared to those fed equivalent amounts of soybean oil (98%). They also supplemented

diets (at 4.8% of diet DM) with hydrogenated free fatty acids, flaked hydrogenated tallow, or hydrogenated tallow melted and mixed into the concentrate. More of the saturated free fatty acids were digested than either form of hydrogenated tallow. They suggested that flaked hydrogenated tallow resisted dispersion and hydrolysis in the rumen, but also resisted solubilization in the intestine. Hydrogenated FFA and hydrogenated tallow reduced total tract apparent fatty acid digestibility compared to controls when lactating dairy cattle diets were supplemented at 2% and 5% of DM (25). Jenkins and Jenny (56) also observed reduced total apparent fatty acid digestibilities in lactating Holstein cows fed 5% yellow grease or 3 or 5% hydrogenated yellow grease. They also reported individual long chain fatty acids in yellow grease (except for $C_{18:0}$) had higher digestibilities than long chain fatty acids in hydrogenated yellow grease. Klusmeyer (66) fed lactating cows Ca-salts of long chain fatty acids (level unknown) and observed significantly decreased $C_{18:0}$ apparent intestinal digestibility.

A deficiency of phospholipid (lecithin) in the intestine has been suggested as a limiting factor in saturated long chain fatty acid absorption in ruminants. Feeding lecithin to rats increased the digestibility of hydrogenated cottenseed oil (6). Jenkins (55) however, found steers fed hydrogenated fat (79% saturated) and lecithin in weight ratios of 100:0, 84:14, and 72:28 did not improve apparent total tract digestibility of hydrogenated fat.

Triglyceride resynthesis within intestinal mucosal of non-ruminants occurs primarily via the monoglyceride pathway with the $\alpha$-glycerophosphate pathway playing only a minor role. Ruminants are more dependent on the $\alpha$-glycerophosphate pathway for triglyceride synthesis, because little monoglyceride is absorbed when "traditional" diets are fed. It appears, however, that the monoglyceride pathway can be used by

ruminants when triglycerides are protected from ruminal metabolism (20).

Effect of fat supplementation on plasma fatty acids

As previously discussed, biohydrogenation of unprotected polyunsaturated fatty acids in the rumen can lead to large amounts of saturated fatty acids reaching the small intestine. Garton and Duncan (35) reported that in spite of extensive absorption of saturated fatty acids relative to linoleic and other polyunsaturated fatty acids, sheep plasma contained large amounts of linoleic acid ($C_{18:2}$). It is now understood that $C_{18:2}$ and other polyunsaturated fatty acids are preferentially incorporated into cholesterol ester and phospholipid fractions of blood. Lascelles et al. (73) found triglycerides and major triglyceride-carrying lipoproteins have a rapid turnover compared to cholesterol esters and phospholipids. This mechanism allows conservation of essential and nonessential polyunsaturated fatty acids. However, Heathe et al. (49) found when large amounts of $C_{18:2}$ was absorbed, extensive incorporation of linoleic acid into triglycerides occurred. Linoleic acid can be incorporated into milk fat and adipose tissue when part of the triglyceride or free fatty acid pool of blood plasma.

Moore et al. (90) found that supplementing the diet of lactating cows with stearic acid ($C_{18:0}$) raised the total fatty acid content of plasma. They also found the relative proportion of stearic acid in each of the lipid classes (except cholesterol esters) increased while polyunsaturated components decreased. Herbein (50, and personal communication) reported plasma from cows supplemented with $C_{18:0}$ (2% of diet DM) had decreased concentrations of $C_{16:0}$ and $C_{18:2}$ (no effect on $C_{18:0}$ or $C_{18:1}$). In the same study, cows supplemented with $C_{18:1}$ had decreased plasma $C_{16:0}$ and $C_{18:2}$ but increased $C_{18:1}$ concentrations. Spain

et al. (119) found fish oil infusions into the duodenum of lactating cows increased plasma $C_{20:5n3}$ and $C_{22:6n3}$ concentrations.

Effect of fat supplementation on milk production

Reports by Maynard et al. (85) and Loosli et al. (77) in the 1940s indicated that concentrates containing 3 to 4% fat, increased milk production from 2 to 10%. Mattias et al. (83) fed concentrates containing 5% tallow and found that second-lactation cows produced more milk. Tomlinson et al. (134) reported increased milk yield when cows were fed Megalac$^R$, raw soybeans or whole cottonseed (2.2% of diet DM). Schingoethe and Casper (110) found higher persistency of milk production throughout lactation when cows were fed extruded soybeans or rolled sunflower seeds during early lactation.

There also have been reports that supplemental fat did not increase milk production. Driver et al. (23) found no benefit from heat treated soybeans over heat treated soybean meal during the first 15 weeks of lactation. Schneider et al. (111) did not get increased milk production from Ca-salts of long chain fatty acids (.77 kd/d) unless cows were supplemented with bST. In agreement, Ca-salts of long chain fatty acids (40, 68, 108) protected tallow (24), tallow (100), or prilled fat (40, 108) did not improve milk production. Lack of response to dietary fat supplementation may be due to stage of lactation (early versus mid to late lactation) or use of cows with lower genetic potential for milk production. Palmquist (98) offered an explanation for the delayed response to fat supplementation in early lactation. He stated that if excess dietary fat is provided during rapid mobilization of endogenous fatty acids in early lactation, the cow must decrease feed intake to regulate plasma fatty acid concentration.

Effect of fat supplementation on milk fat production.

Palmquist et al. (102) used [1-$^{14}$C]-linoleic acid to estimate that approximately 50% of milk fatty acids are synthesized de novo in the gland from acetate and B-hydroxybutyrate, 40-45% from dietary fatty acids, and less than 10% from adipose. Storry et al. (126) reported that fatty acid uptake by the mammary gland inhibits de novo synthesis of short chain fatty acids. It was proposed that acetyl-CoA carboxylase was inhibited by increased concentrations of long chain acyl CoA. He noted increased butyric acid concentrations in milk fat (synthesis independent of malonyl CoA formation) during milk fat depression.

Fat supplementation, especially during early lactation, also can increase milk fat concentration and fat corrected milk production. Protected lipid supplements (i.e. Ca-salts of long chain fatty acids, formaldehyde treated oil, and hydrogenated fats) consistently increased milk fat percent (97). Palmquist (97) theorized that uptake of long chain fatty acids by mammary gland exceeded the compensatory reduction in short chain acid synthesis resulting in higher milk fat secretion. Recently, Knapp et al. (69) found that dietary supplemention with whole roasted soybeans (18% of diet DM) significantly increased milk fat %. Mohamed et al. (87) found that soybean oil but not raw or roasted soybeans (4% of diet DM) decreased milk fat percent. Horner et al. (51) found increased milk fat percent when whole cottonseeds were fed. Supplemental fat did not improve milk fat percent when tallow (7, 99), Ca-salts of long chain fatty acids (13, 40, 106, 108), or prilled fat (40, 108) were fed. In spite of no change or a decrease in milk fat percent, milk fat yield may increase if milk volume increases. It is apparent that stage of lactation, energy status of the animal, DM intake, and "health" of rumen environment could influence milk fat production.

Dietary fats also can alter fatty acid composition of milk fat. For example, a given fatty acid may be ingested, absorbed (without change), and transported to the mammary gland where it is esterified and incorporated into milk fat in a higher proportion than is found in control animals. Alternatively, fatty acids may undergo biohydrogenation in the rumen and be esterified in hydrogenated form by the mammary gland and incorporated into milk fat. Fatty acids reaching the mammary gland may also be desaturated before incorporation into milk fat. This may be a compensatory response of the mammary gland to maintain milk fat globule fluidity when receiving large amounts of saturated fatty acids. In general, increased uptake and secretion of dietary fatty acids appears to decrease de novo production of $C_{4:0}$-$C_{14:0}$ fatty acids by the mammary gland.

Kim et al. (65) found supplementing cows with extruded soybeans and Megalac$^{R}$ increased milk fat production and increased the unsaturated fatty acid content of milk fat. Herbein et al. (50) reported dietary oleic acid supplementation increased milk fat $C_{18:1}$ concentration and decreased concentrations of short and medium chain fatty acids. Canale et al. (14) found cows fed animal-vegetable blend and Ca-salts of long chain fatty acid supplements increased the percentage and yield of $C_{16:0}$-$C_{18:2}$ in milk fat while reducing $C_{4:0}$-$C_{14:0}$ concentrations. Steele (122) reported cows fed tallow and ground nut oil had higher yields of $C_{18:0}$-$C_{18:2}$ and reduced yields of $C_{4:0}$-$C_{14:0}$ compared to cows fed an unsupplemented diet. Similarly, Schneider et al. (111) also observed reduced $C_{4:0}$-$C_{14:0}$ milk fatty acids when Ca-salts of long chain fatty acids were fed or if bST was administered.

Varman et al. (136) indicated the potential for polyunsaturated fatty acids (>18 carbons) to escape biohydrogenation and affect post-absorptive lipid metabolism. Emery (28) theorized that C20 and C22 polyunsaturates in cod liver oil may have an inhibitory affect on

mammary lipoprotein lipase. Pennington et al. (104) found that cod liver oil infusions into the rumen and abomasum decreased milk fat percent without affecting rumen VFA patterns. However, Spain et al. (119) did not see this effect when fish oil was infused (50 ml/d). Spain et al. (117) found no affect of eicosapentaenoic acid ($C_{20:5n3}$) on mammary slice utilization of acetate for de novo fatty acid synthesis.

Effects of trans fatty acids on milk fat synthesis

Astrup et al. (5) demonstrated partially hydrogenated soybean oil (protected from rumen biohydrogenation) depressed milk fat percent. Selner and Schultz (112) verified that hydrogenated vegetable oil (49% trans $C_{18:1}$) significantly depressed milk fat % with minimal effects on rumen VFA production. Teter et al. (131) found cows that consumed 80% concentrate and exhibited milk fat depression had significantly elevated concentrations of trans $C_{18:1}$ in their milk fat. They attributed the high level of trans $C_{18:1}$ to incomplete biohydrogenation of polyunsaturated fatty acids in the diet. Teter et al. (133) also found that cows consuming fishmeal (14% of the concentrate) exhibited milk fat depression and increased concentrations of trans fatty acids in rumen bacterial lipids and milk fat. In an attempt to better understand the effects of trans fatty acids on milk fat production, Teter et al. (132) used lactating C57Bl/6J mice to evaluate diets containing mixtures of fats that provided similar amounts of fatty acids, but the ratio of cis to trans fatty acids was varied. They observed a significant depression in percent milk fat when mice consumed trans $C_{18:1}$ provided by partially hydrogenated fat sources. When trans $C_{18:1}$ was removed from the diet, trans fatty acid concentrations in milk decreased and milk fat percent increased to levels prior to trans fatty acid supplementation. The mechanism by which trans fatty acids inhibit milk fat synthesis is not completely understood, but Askew et al. (3) demonstrated that trans $C_{18:1}$

inhibited $^{14}C$-labelled $C_{16:0}$ esterification by mammary homogenates to a greater extent than cis $C_{18:1}$.

Effects of fat supplementation on milk protein production.

Dietary fat supplementation has frequently influenced milk protein production by lactating dairy cows. Unfortunately, supplemental fat often depresses milk protein concentration, regardless of the type of fat supplement. Heat treated soybeans have decreased milk protein content (86, 107, 125). Steele (123) also found that crushed soybeans decreased milk protein percent. Whole cottonseed supplementation decreased milk protein percentage (51, 87, 115). Klusmeyer et al. (67) found that tallow and Ca-salts of long chain fatty acids decreased milk protein percent. In addition, Grummer et al. (40), Kent and Arambel (62), and Burgess et al. (13) found that Ca-salts of long chain fatty acids depressed milk protein. In contrast, milk protein was not depressed in other studies when cows were fed Ca-salts of long chain fatty acids (26, 109), roasted soybeans (26), tallow (100), or prilled fat (40, 108).

The mechanism for milk protein depression is not completely understood, but a number of explanations have been documented. Inadequate amino acids reaching the small intestine can limit yield of milk and milk protein (19). Canale et al. (14) found that ruminally protected amino acids added to diets during early lactation increased milk protein percent. Smith et al. (116) speculated that reduced protein percent was due to altered glucose metabolism. Moser and Palmquist (91) implicated insulin resistance (i.e., inability of insulin to stimulate tissue glucose utilization) as an inhibitory affect on protein production. Horner et al. (51) reported that niacin supplementation alleviated milk protein depression caused by fat

supplementation. Driver et al. (23) suggested that niacin supplementation corrected milk protein depression induced by dietary heat-treated soybeans. However, Eastridge et al. (26) found no beneficial effects of niacin supplementation when cows were fed roasted soybeans or Ca-salts of long chain fatty acids. Finally, Casper et al. (15) proposed that fat supplementation inhibits somatotropin release from the anterior pituitary; thus, reducing the stimulus for uptake of amino acids by mammary gland. They felt that administration of exogenous somatotropin could alleviate milk protein depression.

Digesta flow markers

Liquid and particulate digesta markers are used as an alternative to total collection of digesta and(or) feces for calculating rates of digesta passage and nutrient digestibilities. Faichney (30) described the ideal digesta marker as one that is nonabsorbable, indigestible, physically similar to the test feed, and sensitive to analytical methods.

Particulate markers include $Cr_2O_3$, Cr, Yb, La, and Sm. $Cr_2O_3$ can be administered as a powder, an oil suspension in gelatin capsules, or impregnated onto paper. MacRae and Armstrong (81) reported nearly complete recovery of $Cr_2O_3$ in sheep feces collected over a 7-day period. This was in agreement with Ortigues et al. (95) who recovered 98.5% in sheep feces. In 1980, Uden et al. (135) reported a method for mordanting feedstuffs with Cr. A limitation of this system, proposed by Ellis et al. (27), was that Cr-mordanting decreased DM digestibility of mordanted feedstuffs. Mader et al. (82) reported lower rumen turnover rates with Cr-labeled sorghum-sudan hay fed to steers and found that estimated fecal output using Cr-mordants was only 77.3% of actual fecal output. Rare-earth elements (Yb, La, Sm) also have a strong attraction for particulate matter. Hartnell and Satter (47) reported that rare-

earth markers migrated from originally-labeled material to unmarked material. Ledoux et al. (74) and Mader et al. (82) immersed Yb-labeled feeds in distilled water, causing loosely bound Yb to wash off. The residual marker after washing was less susceptable to migration in the digestive tract.

Common liquid markers include polyethylene glycol, Co-EDTA and Cr-EDTA. All were found suitable for the estimation of liquid passage rate (130). A commonly used procedure for preparing Co-EDTA crystals was described by Uden et al. (135).

It has been shown that particulate and liquid phases have different rates of flow out of the rumen and abomasum (39). Faichney (31) suggested that a single marker may not be used for estimation of digesta flow at the duodenum, because samples obtained from simple T-type cannulae may not be typical of normally distributed liquid and particulate phases.

Armentano and Russell (2) suggested using multiple markers to correct for nonhomogeneity of samples (i.e. samples in which phases are present in proportions other than normal). The system involves individually "marking" the particulate and liquid phases of digesta (ie. Cr for particulate, and Co-EDTA for liquid) and then analyzing content of each marker in both phases. An algorithm is used to calculate individual phase flow, because markers are not completely separated (via centrifugation) into particulate and liquid phases by any practical procedure. A description of the algorithm can be found in Chapter 3 page 35.

## CHAPTER 3

# BIOHYDROGENATION, POSTRUMINAL FLOW AND APPARENT DIGESTIBILITY OF DIETARY LIPIDS IN LACTATING DAIRY COWS 1. TALLOW, PARTIALLY HYDROGENATED TALLOW, AND COATED TALLOW.


## ABSTRACT

Lactating Holstein cows with cannulae in their rumen and proximal duodenum were used in a 4 X 4 Latin square experiment to evaluate biohydrogenation, flow rates, and digestibility of dietary fatty acids in the gastrointestinal tract. Cows were fed diets with 0% supplemental fat, 3% tallow, 3% partially hydrogenated tallow, or 3% tallow coated with casein and corn syrup solids. Basal diets containing 38% alfalfa haylage, 14% corn silage, and 48% concentrate (DM basis) were fed ad libitum. Specified fat supplements replaced a corresponding amount of corn grain in the concentrate mixtures. Rumen VFA concentrations and ruminal and total tract apparent digestibilities (%) of dietary DM, ADF, N, and OM were not affected by fat supplements. Fatty acid intake and flow to the duodenum increased with fat supplementation. Apparent digestibility of total dietary fatty acids was reduced by partially hydrogenated tallow. Apparent digestibility of $C_{18:0}$ in the small intestine quadradically decreased ($R^2$=.86) as $C_{18:0}$ flow to the intestine increased. Fat supplementation increased milk production and decreased milk protein percent, but did not affect milk fat percent or 3.5% FCM. Fat supplementation increased $C_{18:0}$ and $C_{18:1}$ and lowered $C_{12:0}$ and $C_{14:0}$ concentrations in milk fat. Results indicated tallow-based products supplemented at 3% of diet DM effectively increase milk production, without affecting rumen fermentation, but adversely affected $C_{18:0}$ digestibility and protein percent in milk.

# INTRODUCTION

Many dairy cows produce large quantities of milk during early lactation, but often cannot consume enough dietary energy to meet production demands. The problem intensifies when peak DM intake lags behind peak milk production. Increasing the proportion of concentrate boosts energy content of a diet, but excess soluble carbohydrate intake can lead to rumen acidosis and milk fat depression (42).

Fat supplements provide an alternative source of dietary energy for cows in negative energy balance. Fat contains approximately 2.25 times the energy of carbohydrates and protein. Fat, therefore, can increase energy density of diets without reducing forage content. Research has been conducted to investigate effects of different types and levels of fat supplementation on milk production and milk components (23, 40, 68, 83, 100, 110, 111). Variable production responses have been attributed to stage of lactation (108), number of lactations (83), genetic potential to produce milk, altered rumen fermentation (101), and fatty acid digestibility (56, 80).

Few experiments with high-producing, early-lactation cows consuming large quantities of DM with supplemental fat have been conducted to intensively evaluate digestion of fatty acids. Cannulated (rumen and duodenum) cows have been used to calculate nutrient flow and digestibility when fat supplements were added to dairy diets (66, 92, 139). This study was conducted to determine how various forms of tallow affected ruminal biohydrogenation, postruminal nutrient flow, apparent digestibility of fatty acids, and lactation performance of cannulated cows during early lactation.

# MATERIALS AND METHODS

## Surgical procedures

Four multiparous Holstein cows were chosen for duodenal and ruminal cannulation. Major criteria for selection included physical soundness, calving dates within a 30 d period, and projected milk production over 8,900 kg. Approximately two months prior to parturition, cows were taken to the Virginia-Maryland College of Veterinary Medicine. A full-circle, T-shaped cannula was surgically inserted in the duodenum approximately 10 cm posterior the pyloric sphincter. A paravertebral (T13, L1, L2) nerve block provided local anesthesia, so the surgical procedure could be conducted while cows were standing. Ten ml of 2% lidocaine was injected into each spinal nerve tract. Cows were monitored for a day after surgery then returned to the dairy farm. Approximately 2 weeks later, cows underwent surgery for a 2-stage rumen cannulation. Local anesthesia was induced by an inverted L-block using 2% lidocaine in the lumbar fossa. Post-operative care for each surgery included penicillin injections for 10 days, daily incision cleaning, and analgesics as prescribed by the attending veterinarian.

## Animals

In addition to the four cows described above, two cows cannulated during their previous lactation also were available. All cannulated cows were housed in comfort stalls prior to parturition and fed diets containing chopped orchardgrass hay, corn silage, and soybean meal. Approximately 2 wk before parturition cows were moved to box stalls. A control diet (Table 1) supplemented the dry-cow diet in increasing amounts until parturition. After parturition, cows were fed only the control diet. Four cows (53, 53, 66, and 88 d postpartum) were selected for the first experiment.

## Experimental design

A 4 x 4 Latin square design with 21 d periods was used to investigate responses of cows fed either a control diet or one of three fat supplemented diets. Randomization gave each possible configuration of treatment sequences equal chance of selection (Appendix Table 31). Cows were then randomly assigned to a sequence of treatments. Period length was set at 21 d to minimize carry over effects from preceeding treatments.

## Diets

Experimental diets included a control (0% supplemental fat) or one of three fat-supplemented (3% lipid in total diet DM) diets. All diets contained 38% alfalfa haylage, 14% corn silage, and 48% concentrate (DM basis). Cows were fed 75% of their daily ration at 1400 h and 25% at 0600 h. Ingredient and chemical composition of diets are given in Table 1. Fat supplements included tallow fatty acids (Bleachable Fancy Tallow; Valley Proteins Inc., Winchester, VA), hydrogenated tallow fatty acids (Alifet$^{R}$; Alifet USA INC., Cincinnati, OH), and tallow fatty acids coated with casein and corn syrup solids (Energy Pack 4-80$^{TM}$; Merrick's INC., Middleton, WI). Fat supplements were kept in a cool, dry area until mixed into respective diets. Ethoxyquin (1.7 ml/gal) was added to the Bleachable Fancy Tallow to reduce fatty acid oxidation. Fat supplements were substituted for corn grain at 6.0% (tallow fatty acids), 6.5% (hydrogenated tallow fatty acids), and 7.9% (coated tallow fatty acids) of the total concentrate to obtain diets with 3% supplemental fat. All diets were formulated to meet or exceed NRC requirements (93). Fatty acid composition of fat supplements, forages, and concentrates (wt % data) are given in Table 2.

Table 1. Ingredients and chemical composition of diets.[1]

| Item | C | T | HT | CT |
|---|---|---|---|---|
| **Ingredients**[2] | ----------% | | | |
| Alfalfa haylage | 38.0 | 38.0 | 38.0 | 38.0 |
| Corn silage | 14.0 | 14.0 | 14.0 | 14.0 |
| Corn grain | 35.5 | 32.5 | 32.0 | 31.5 |
| Supplemental fat | 0.0 | 3.0 | 3.5 | 4.0 |
| Soybean meal 44% CP | 8.0 | 8.0 | 8.0 | 8.0 |
| Dried brewer's grains | 4.0 | 4.0 | 4.0 | 4.0 |
| Trace mineral salt | .3 | .3 | .3 | .3 |
| Monocalcium phosphate | .2 | .2 | .2 | .2 |
| Vitamins[3] | * | * | * | * |
| **Chemical composition**[2] | | | | |
| DM | 61.0 | 61.5 | 61.5 | 61.5 |
| CP | 18.0 | 18.0 | 18.0 | 18.0 |
| ADF | 17.0 | 17.0 | 17.0 | 17.0 |
| Crude Fat | 4.2 | 7.2 | 7.2 | 7.2 |

[1]C = control (no supplemental fat), T = tallow, HT = hydrogenated tallow, and CT = coated tallow supplemented diets.
[2]DM basis.
[3]Diets contained 2309 IU Vit A, 228 IU Vit $D_3$, and 21 IU Vit E/kg DM.

Table 2. Major long chain fatty acids in diet components.

| | 16:0 | 18:0 | 18:1 | 18:2 | 18:3n3 |
|---|---|---|---|---|---|
| **Forages** | ----------%[1]---------- | | | | |
| Alfalfa haylage | 17.3 | 3.6 | 2.3 | 15.1 | 30.1 |
| Corn silage | 16.2 | 4.4 | 16.8 | 45.8 | 4.5 |
| **Fat supplements** | | | | | |
| Tallow | 25.0 | 14.4 | 48.5 | 3.8 | <1.0 |
| Hydrogenated tallow | 27.8 | 41.9 | 24.2 | 2.2 | <1.0 |
| Coated tallow | 25.4 | 18.8 | 45.5 | 2.9 | <1.0 |
| **Concentrates**[2] | | | | | |
| C | 16.7 | 2.7 | 21.0 | 53.6 | 2.5 |
| T | 22.1 | 13.9 | 33.1 | 21.3 | 1.2 |
| HT | 22.1 | 26.2 | 25.2 | 20.8 | 1.1 |
| CT | 21.8 | 11.1 | 34.5 | 22.6 | 1.3 |

[1]Percent of total ($C_{4:0}$-$C_{22:6n3}$) fatty acids.
[2]C = control, T = tallow, HT = hydrogenated tallow, and CT = coated tallow.

Liquid and particulate markers

Cobalt ethylenediaminetetraacetic acid (Co-EDTA) was used as a liquid-phase digesta marker and prepared according to Uden et al. (135). Sixteen grams of Co-EDTA (2.64 g Co) were dosed daily into the rumen from d 11 through d 21 of each period. Co-EDTA crystals were dissolved in distilled $H_2O$ (16 g Co-EDTA/240 ml $H_2O$) and administered at the rate of 180 ml prior to the 1400 h feeding and 60 ml prior to the 0600 h feeding. The solution was delivered via syringe through the rumen cannula and rumen contents were mixed to disperse the marker.

Chromium (Cr) was mordanted to fecal fibers for use as a particulate-phase digesta marker. Mordanted fibers were prepared using an unpublished procedure obtained from Michael S. Allen (Dept. of Dairy Sci., Michigan State University, personal communication). Feces were collected for 2 wk from five cows fed orchardgrass hay diets, then washed over a screen to harvest undigested fibers. Fibers were washed with mild detergent solution, rinsed, and dried seven days at 60°C in a forced-air oven.

Mordanting began by mixing 300 g of fibers with 2.25 L of distilled $H_2O$ containing 77.1 g of $K_2CrO_7$ in a 4 L Pyrex beaker. Fibers were mordanted by heating at 100°C for 24 h. Fibers then were washed in a 10 L Nalgene tank for 24 h to remove loosely attached Cr. A spigot at the bottom of the tank allowed water to spray through the fibers and spill over the top. Cheese cloth covered the tank to retain fibers. After excess $H_2O$ was drained, fibers were soaked for 1 h in 500 ml of distilled $H_2O$ containing 150 g ascorbic acid. Finally, fibers were washed in the 10 L Nalgene tank for 6 to 8 h with distilled $H_2O$ and dried at 90°C in a forced-air oven. Mordanted fibers contained 58.75 mg Cr/g fecal fibers (approximately 6% binding of Cr to fibers).

Twenty four g of Cr-mordanted fecal fibers (1.41 g Cr) were dosed daily into the rumen from 7 d through 21 d in each period. Doses were

18g at 1400 h and 6 g at 0600 h. Rumen contents were mixed to disperse the fibers.

Measurements and sampling

Forages and concentrates were sampled on Monday of alternate wk throughout the experiment. Samples were freeze-dried and stored in sealed containers until determination of chemical composition.

Feed refusals were weighed Tuesday through Friday each week. Daily intakes were used to calculate a weekly average. Feed refusals were sampled the last week of each period, composited by cow, freeze-dried, ground, and stored in sealed containers until analyzed.

Milk production was measured daily (0030 h, 1230 h) to establish a weekly average. Duplicate milk samples were taken from two consecutive milkings on d 20 of each period for component analysis. Milk was analyzed by Virginia Dairy Herd Improvement Association with a 4-channel spectrophotometer for milk fat, protein, SNF, and SCC. A 500 ml milk sample, collected during the 1230 h milking, was centrifuged at 10,000 x g for 1 h to separate milk fat from other components. Harvested milk fat was stored at -20°C until analyzed for fatty acid composition.

Cows were weighed on the first and last day each period. Blood samples were obtained by jugular venipuncture at 0500 h on d 18 and d 20. Blood (22 ml) was transferred from syringe to tubes containing .3 ml heparin and centrifuged at 3,200 x g for 15 min. Plasma was harvested and frozen at -20°C until fatty acid analysis.

Six duodenal and fecal samples were collected from each cow over 72 h the last 4 d of each period. Samples were collected at 0500 h and 2100 h (d 18), 1300 h (d 19), 0100 h and 1700 h (d 20), and 0900 h (d 21). An inflatable urethra catheter was placed in the distal end of each full circle cannula (to prevent back flow from the intestine) prior to collecting 1500 ml of digesta. Each sample was mixed and four

aliquots (227 ml) were stored at -20°C. At the end of the period, two of the aliquots from each collection (12/cow) were thawed and composited by cow. A sample of whole digesta was retrieved from each composite. The remainder of the composite was separated into particulate and liquid phases by centrifugation at 3000 x g for 10 min. Whole digesta and separated phases were placed in previously weighed plastic cups, freeze-dried, ground, and placed in sealed containers. Liquid phase samples were ground by mortar to conserve sample DM. Individual fecal grab-samples (6/cow/period) were thawed, composited, and freeze-dried. Dried fecal samples were ground and stored in sealed containers.

Rumen fluid (2 L) was collected through the rumen cannula on d 21 (0800 h). A plastic pipe (1 m) with 4 mm holes facilitated collection of fluid for removal by suction. Two 5 ml aliquots of rumen fluid from each cow were placed in tubes containing 1 ml of 25% meta-phosphoric acid and 30.2 umol internal standard (isocaproic acid). Aliquots were frozen at -20°C until analysis for VFA.

Liquid-associated bacteria were harvested from each 2 L rumen fluid sample. Formaldehyde was not added to the sample following collection. Within 30 min of collection, rumen fluid was strained through 6 layers of cheese cloth and centrifuged at 200 x g for 10 min to remove feed particles and protozoa. The supernatant was centrifuged at 35,000 x g for 20 min to precipitate bacteria. Bacterial residue was washed with double-distilled, deionized $H_2O$ and recentrifuged three times to remove contaminants. Washed microbial residue was frozen, freeze-dried, and stored in sealed containers.

Chemical analysis and calculations

DM concentrations of forages, concentrates, feed refusals, microbial residue, whole duodenal digesta, particulate and liquid phase digesta, and feces were determined by freeze-drying to a constant

weight. All samples were ground through a 1 mm mesh screen (Thomas-Wiley Laboratory Mill) prior to analyses. Unless otherwise noted, all analyses were run in duplicate.

ADF was determined using the method of Goering et al. (38) for forages, concentrates, whole duodenal digesta, feed refusals, and feces. To avoid overestimating ADF content, forages and feed refusal analyses were conducted with NDF residues. Ash was determined by placing forages, concentrates, whole duodenal digesta, or feces in a 600°C muffle furnace for 5 h. Organic matter was calculated by subtracting ash from DM. Nitrogen was determined using the Kjeldahl procedure (4).

Cobalt (Co) and chromium (Cr) were extracted from particulate phase, liquid phase, and feces by wet ashing. Approximately 400 mg were placed in 100 ml digestion tubes, 4 ml concentrated nitric acid added and allowed to react overnight. The next day, 3 ml 70% (vol/vol) perchloric acid and 3 drops 20% hydrogen peroxide were added to each tube. Tubes were slowly heated to 400°C over approximately 4 d, allowing complete digestion of samples. Samples of Cr-mordanted fecal fibers and Co-EDTA crystals were also digested. Digested samples were diluted to 50 ml and filtered (Whatman #1) prior to elemental analysis.

Concentrations of Cr and Co were determined using a Varian AA-475 atomic absorption spectrophotometer. The conditions for analysis are given in Appendix Table 32. Six standard solutions (.5, 1, 2, 3, 4, 5 ppm Cr and Co) were prepared and analyzed in the same manner as physiological samples.

Cr and Co marker concentrations were used to calculate particulate and liquid phase DM flow, as described by Armentano et al (2) using simultaneous equations with two unknown variables:

$$I_1 = F_P[Cr]_S + F_L[Cr]_L$$
$$I_2 = F_P[Co]_S + F_L[Co]_L$$

where $I_1$ and $I_2$ = infusion rates of Cr and Co (g/d),

$F_P$ and $F_L$ = nutrient DM flow of particulate and liquid phases (g/d),

$[Cr]_P$ and $[Cr]_L$ = Cr concentration in particulate and liquid phases (ppm),

$[Co]_P$ and $[Co]_L$ = Co concentrations in particulate and liquid phases (ppm).

Cytosine content of rumen bacteria, particulate phase, and liquid phase samples was determined by the method described by Kwak et al. (72). Approximately .175 g rumen bacteria or liquid-phase DM or .250 g particulate-phase DM were placed into 15 ml, screw-cap, glass extraction tubes. To each tube, 2.5 ml of 70% (vol/vol) perchloric acid was added, mixed, and allowed to react at least 16 h. Samples were then hydrolyzed in a dry-block at 90°C for 1 h. After cooling, contents were diluted to 10 ml with double-distilled, deionized water.

A quantitative transfer of hydrolized rumen bacteria, particulate phase, or liquid phase was made into 100 ml, 100 ml, or 50 ml volumetric flasks respectively. Ten ml of 1.97 M $NH_4H_2PO_4$ buffer and 2.3 ml of concentrated $NH_4OH$ were added to samples to raise pH to 3.5. Flasks were brought to volume with double-distilled, deionized water and thoroughly mixed. Approximately 20 ml were filtered through 45 um Millipore filters and stored at room temperature in glass, screw-cap, tubes until analyzed.

Cytosine was analyzed by high performance liquid chromatography using a Varian 2510 pump in conjunction with a Linear 200 UV/VIS detector. Cytosine was separated by a 25 cm Partisil-10 SCX L column at room temperature. The mobile phase was .15 M $NH_4H_2PO_4$ (pH 3.5) flowing at .6 ml/min. Injection volumes for rumen microbes, particulate phase,

and liquid phase were 10 ul, 25 ul, and 25 ul, respectively. Cytosine detection was at 254 nm and normally eluted at 10 minutes.

Duodenal particulate and liquid phase microbial nitrogen (N) as percent of total N in each phase was calculated using the following equation:

$$\%PMN = (MN/MC * PC/PN)*100$$

where %PMN = individual phase microbial nitrogen, %

MN = microbial total N, g/g DM

MC = microbial cytosine, umoles/g DM

PC = phase microbial cytosine, umoles/g DM

PN = individual phase total nitrogen, g/g DM

Nonmicrobial N flow (g/d) was determined by subtracting particulate and liquid phase microbial N flow (g/d) from N intake (g/d). N recovery (%) was calculated as total N flow (g/d) to the duodenum as a percent of N intake (g/d). Apparent digestibility of N in the small intestine and total gastrointestinal tract were not corrected for endogenous N contributions.

Aliquots of rumen fluid containing 30.2 umol internal standard (isocaproic acid) were thawed and centrifuged (1000 x g for 10 min) prior to VFA analysis. Supernatant was filtered through .45 um Millipore filters and .5 ml injected into a Varian 6000 gas chromatograph.

Acetic, propionic, butyric, valeric, isobutyric and isovaleric acids were separated on a glass column packed with 10% SP-1200/1% $H_3PO_4$ liquid phase on 80/100 Chromasorb WAW packing (Supelco Inc.) Column temperature was 125°C, inlet temperature was 180°C, and detector temperature was 175°C. A VFA standard containing acetic (51.66 umol/ml), propionic (30.63 umol/ml), valeric (5.18 umol/ml), isobutyric

(4.96 umol/ml), and isovaleric (4.95 umol/ml) acids was used to determine sample VFA concentrations by integration.

Fatty acids in forages, concentrates, feed refusals, digesta, milk fat, plasma, and feces were methylated by direct transesterification, as described by Outen et al (96). Samples were mixed with 291.6 ug internal standard (undecenoate, $C_{11:1}$) prior to direct methylation. Three ul of sample was injected by auto-sampler into a Hewlett Packard 5890A gas chromatograph equipped with a flame ionization detector (Hewlett Packard Co., Sunnyvale, CA). Samples were split onto a 30 m DB-23 GLC glass capillary column with .25 mm i.d. and .25 um film thickness (J & W Scientific, Folsom, CA). Injector and detector temperatures were set at 225°C. A temperature program initiated runs at 60°C, warmed to 190°C at 4°C/min, held for 6 minutes, and then warmed to 214°C at a rate of 1°C/min and held for 3 minutes.

Identification of peaks was based on relative retention times of a commercial standard (Nu-Check-Prep Inc., Elysian, MN). A linear, multi-level calibration table was constructed (from known fatty acid concentrations in the standard) to correct sample peak areas by appropriate response factors prior to individual fatty acid quantification.

Fatty acid composition of individual fat supplements, forages, concentrates, rumen fluid, rumen bacteria, duodenal digesta, feces, and milk fat was based on wt % calculations. Fatty acid intake and flow was calculated by multiplying wt % concentrations by DM intake and DM flow respectively.

## Statistical analysis

All data were analyzed using General Linear Model of SAS (120). The model was:

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

where $Y_{ijk}$ = dependent variable,

$u$ = overall population mean,

$C_i$ = average effect of $cow_i$,

$P_j$ = average effect of $period_j$,

$T_k$ = average effect of $treatment_k$,

$E_{(ijk)}$ = residual error.

Tukey's multiple-comparison procedure was used to test differences between treatment means. Means were accepted as significantly different at ($p<.05$). Treatment means were compared using data with equal sample sizes. The SE of treatment means was calculated as (mean square error)$^{1/2}$/(sample size)$^{1/2}$.

Additionally, simple regression analysis was performed with data using $C_{18:0}$ flow to the duodenum as the independent variable and $C_{18:0}$ digestibility as the response (120). A regression model was selected if statistical significance ($p<.05$) of linear or quadratic responses was observed.

## RESULTS AND DISCUSSION

Average daily DM intakes of control and fat-supplemented diets were similar and averaged 24.2 kg/d (Table 3). Fat supplements apparently did not affect the palatability of diets. Visual observation and chemical analysis of feed refusals verified minimal sorting of concentrates and fat supplements. Similar responses have been observed by others when Ca-salts of long chain fatty acids (26, 40, 68), tallow (100), animal-vegetable blend (99), partially hydrogenated tallow (103),

Table 3. Intake, flow, and digestibility of DM in the digestive tract of Holstein cows fed diets with 0% supplemental fat (C), 3% tallow (T), 3% partially hydrogenated tallow (HT), or 3% tallow coated with casein and corn syrup solids (CT).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | T | HT | CT | SE |
| DM intake, kg/d | 24.1 | 23.8 | 24.8 | 24.1 | .6 |
| DM intake, %BW | 4.1 | 4.1 | 4.2 | 4.0 | .1 |
| Flow to duodenum | | | | | |
| Particulate, kg/d[1] | 9.6 | 9.5 | 10.3 | 9.5 | .4 |
| Liquid, kg/d[2] | 6.4 | 6.8 | 7.0 | 6.2 | .3 |
| Total, kg/d | 16.0 | 17.3 | 15.7 | 16.3 | .6 |
| Digestibility in rumen | | | | | |
| Apparent, % | 33.6 | 31.4 | 30.3 | 35.0 | 2.4 |
| Corrected, %[3] | 44.6 | 42.7 | 40.7 | 45.9 | 2.6 |
| Flow to feces, kg/d | 7.2 | 6.9 | 7.5 | 7.2 | .3 |
| Apparent digestibility in total tract, % | 70.1 | 71.1 | 69.7 | 70.1 | 1.2 |

[1]Pellet from centrifugation (3000 x g for 10 min) of whole digesta.
[2]Supernatant from centrifugation (3000 x g for 10 min) of whole digesta.
[3]100 - [(DM flow at duodenum - microbial DM flow)/DM intake x 100].

or partially hydrogenated yellow grease (56) were fed to lactating cows at approximately 3% of diet DM.

Palmquist et al. (103) however, observed decreased DM intake when lactating Jersey cows were fed diets supplemented with animal-vegetable blend, Megalac$^{R}$, Alifet$^{R}$, Energy Booster 100$^{TM}$, and Booster Fat 95$^{TM}$ at 5.70% of the diet DM. Herbein (unpublished data) observed reduced DM intake when Holstein cows were fed diets supplemented with 4% tallow during early lactation. Neither study clarified whether reduction in DM intake was a consequence of energy regulation, apparent nutrient digestibility, or diet palatability.

Fat supplementation did not affect DM, ADF, N, OM, or digesta flow to the small intestine (Tables 3, 4, 5, 6, and Appendix Table 33). Apparent digestibilities of DM, ADF, N, and OM in the rumen and total digestive tract were not affected by type or level of fat supplementation. Palmquist postulated that esterified and hydrogenated dietary fats reduced negative affects of fatty acids on rumen fermentation (101). Chalupa (17) found that Ca-salts of long chain fatty acids and hydrogenated fatty acids did not affect fermentation in vitro. Fiber digestibility in this study was not affected by the dietary form of tallow fatty acids, perhaps due to the moderate level of fat supplementation. Moderate fat supplementation did not adversely affect nutrient digestibility in other studies (40, 100, 103, 108). However, DM digestibility in rumen and total tract was decreased in cows fed 1 or 2 kg/d full-fat rapeseed (92). In agreement, 5% yellow grease or 3 or 5% hydrogenated yellow grease decreased ADF and N apparent digestibilities (56).

Ruminal VFA concentrations were used to evaluate the effects of fat supplements on microbial fermentation, because acetate is an important precursor for milk fat synthesis. A decrease in acetate production or ratio of acetate to propionate may indicate that fats

Table 4. Intake, flow, and digestibility of ADF in the digestive tract of Holstein cows fed diets with 0% supplemental fat (C), 3% tallow (T), 3% partially hydrogenated tallow (HT), or 3% tallow coated with casein and corn syrup solids (CT).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | T | HT | CT | SE |
| ADF intake, kg/d | 4.0 | 4.0 | 4.1 | 4.0 | .1 |
| Flow to duodenum, kg/d[1] | 2.7 | 2.7 | 2.7 | 2.6 | .1 |
| Apparent digestibility in rumen, % | 32.5 | 32.0 | 34.5 | 35.8 | 2.5 |
| Flow to feces, kg/d | 2.1 | 2.0 | 2.1 | 2.2 | .1 |
| Apparent digestibility in total tract, % | 46.8 | 49.1 | 47.8 | 46.2 | 2.3 |

[1]Sum of particulate and liquid phase acid detergent fiber.

Table 5. Intake, flow and digestibility of N in the digestive tract of Holstein cows fed diets with 0% supplemental fat (C), 3% tallow (T), 3% partially hydrogenated tallow (HT), 3% tallow coated with casein and corn syrup solids (CT).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | T | HT | CT | SE |
| N intake, g/d | 696 | 685 | 716 | 696 | 16 |
| Flow to duodenum[1] | | | | | |
| Particulate N flow | | | | | |
| Total, g/d | 283 | 274 | 279 | 269 | 14 |
| Microbial, g/d | 118 | 122 | 118 | 124 | 10 |
| Nonmicrobial, g/d | 165 | 152 | 161 | 145 | 9 |
| Liquid N flow | | | | | |
| Total, g/d | 319[b] | 305[c] | 337[a] | 318[b] | 2 |
| Microbial, g/d | 36 | 33 | 31 | 33 | 2 |
| Nonmicrobial, g/d | 204 | 204 | 232 | 213 | 8 |
| Total microbial, g/d | 233 | 223 | 224 | 229 | 15 |
| Total nonmicrobial, g/d | 369 | 356 | 393 | 358 | 12 |
| Total, g/d | 602 | 579 | 617 | 587 | 15 |
| N recovery, %[2] | 86.5 | 84.5 | 86.2 | 84.5 | 1.8 |
| Flow to feces, g/d | 203 | 188 | 205 | 200 | 10 |
| Apparent digestibility in total tract, %[1] | 70.8 | 72.5 | 71.3 | 71.1 | 1.5 |

[a,b]Means within same row with different superscripts differ ($p<.05$).
[1]Data not corrected for endogenous protein contribution.
[2]Total nitrogen flow at the duodenum as a percent of nitrogen intake.

Table 6. Intake, flow, and digestibility of OM in the digestive tract of Holstein cows fed diets with 0% supplemental fat (C), 3% tallow (T), 3% partially hydrogenated tallow (HT), or 3% tallow coated with casein and corn syrup solids (CT).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | T | HT | CT | SE |
| OM intake, kg/d | 22.9 | 22.6 | 23.6 | 22.9 | .5 |
| Flow to duodenum, kg/d[1] | 15.5 | 15.7 | 16.8 | 15.2 | .5 |
| Apparent digestibility in rumen, % | 32.4 | 30.2 | 28.6 | 33.7 | 2.4 |
| Flow to feces, kg/d | 6.5 | 6.2 | 6.8 | 6.5 | .2 |
| Apparent digestibility in total tract, % | 71.5 | 72.5 | 71.2 | 71.7 | 1.1 |

[1]Sum of particulate and liquid phase organic matter.

impared fiber digestion (18). Results indicate fat supplements did not affect total or individual VFA concentrations or ratio of acetate to propionate (Table 7). These findings agree with other studies (40, 103, 109, 126) when lactating cows were fed up to 6% tallow or commercial fat supplements. Chalupa (18), however, found that tallow, oleic acid, stearic acid, and Ca-salts of long chain fatty acids (10% diet DM) significantly reduced the ratio of acetate to propionate. It appears that there is minimal risk of reducing fiber digestibility and acetate production when lactating cows are fed supplemental fat at 3% of the diet DM.

Fat supplementation, in general, did not affect fatty acid concentrations of mixed rumen bacteria (Table 8). The only exception was lower concentration of $C_{15:0}$, but there was minimal contribution of this fatty acid to total bacterial lipids and fatty acid flow to the duodenum. Total bacterial lipid (mg/g bacterial DM) was significantly increased by all fat supplements. Klusmeyer and Clark (66) observed a similar response when lactating cows were fed Ca-salts of long chain fatty acids. Bauchart et al. (11) observed that adding vegetable oils or tallow to diets fed to lactating Holstein cows increased the FFA content of solid- and liquid-associated bacteria by 150%. It also has been observed that fatty acid concentrations in solid-adherent bacteria are twice as high as in liquid-associated bacteria (75). Primarily liquid-associated bacteria were isolated in the present study. Therefore, total bacterial lipid contributions to duodenal fatty acid flow may have been underestimated.

Fatty acid intake increased due to fat supplementation (Table 9). DM intake was not affected by treatments, so fatty acids supplied by forages and concentrates (excluding fat supplements) did not contribute to increased intake of fatty acids. Cows receiving HT had higher

Table 7. VFA concentrations in rumen fluid from Holstein cows fed diets with 0% supplemental fat (C), 3% tallow (T), 3% partially hydrogenated tallow (HT), or 3% tallow coated with casein and corn syrup solids (CT).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | T | HT | CT | SE |
| VFA concentration | ----------umol/ml---------- | | | | |
| Acetate | 77.7 | 74.1 | 77.9 | 71.8 | 3.3 |
| Propionate | 29.1 | 24.1 | 28.0 | 27.1 | 2.4 |
| Isobutyrate | 1.7 | 1.4 | 1.6 | 1.5 | .1 |
| Butyrate | 15.7 | 15.2 | 15.3 | 14.5 | 1.0 |
| Isovalerate | 2.7 | 2.6 | 2.6 | 2.4 | .2 |
| Valerate | 2.6 | 2.2 | 2.5 | 2.4 | .2 |
| Total | 129 | 120 | 128 | 120 | 6 |
| Acetate:Propionate | 2.9 | 3.2 | 3.0 | 2.7 | .1 |

Table 8. Fatty acid concentration in mixed rumen bacteria from Holstein cows fed diets with 0% supplemental fat (C), 3% tallow (T), 3% partially hydrogenated tallow (HT), or 3% tallow coated with casein and corn syrup solids (CT).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | T | HT | CT | SE |
| Fatty acid[1] | ----------%---------- | | | | |
| $C_{14:0}$ | 4.8 | 3.9 | 3.4 | 4.1 | .4 |
| $C_{15:0}$ | 4.2[a] | 2.7[b] | 2.7[b] | 3.0[b] | .2 |
| $C_{16:0}$ | 30.3 | 29.5 | 27.5 | 29.0 | 1.0 |
| $C_{18:0}$ | 44.1 | 47.1 | 50.3 | 47.0 | 2.1 |
| $C_{18:1}$ | 8.7 | 9.8 | 10.4 | 10.5 | 1.4 |
| $C_{18:2}$ | 4.4 | 2.9 | 2.7 | 3.2 | .5 |
| Total, mg/g DM | 53.3[b] | 77.5[ab] | 82.8[a] | 74.3[ab] | 5.4 |

[a,b]Means within same row with different superscripts differ ($p<.05$).
[1]Percent of total ($C_{4:0}$-$C_{22:6n3}$) fatty acids.

Table 9. Fatty acid intake and flow to the duodenum of Holstein cows fed diets with 0% supplemental fat (C), 3% tallow (T), 3% partially hydrogenated tallow (HT), or 3% tallow coated with casein and corn syrup solids (CT).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | T | HT | CT | SE |
| Fatty acid intake | ----------g/d---------- | | | | |
| Total[1] | 703[c] | 1224[ab] | 1264[a] | 1122[b] | 28 |
| $C_{16:0}$ | 114[c] | 266[a] | 282[a] | 234[b] | 6 |
| $C_{18:0}$ | 21[c] | 132[b] | 284[a] | 105[b] | 6 |
| $C_{18:1}$ | 100[c] | 336[a] | 220[b] | 310[a] | 6 |
| $C_{18:2}$ | 272 | 272 | 284 | 256 | 6 |
| $C_{18:3n3}$ | 78 | 78 | 80 | 80 | 2 |
| Fatty acid flow | | | | | |
| Total[1] | 603[b] | 1003[a] | 1110[a] | 1026[a] | 37 |
| Dietary | 462[b] | 797[a] | 895[a] | 835[a] | 37 |
| Bacterial | 142 | 207 | 219 | 193 | 22 |
| $C_{16:0}$ | 113[b] | 233[a] | 254[a] | 244[a] | 9 |
| $C_{18:0}$ | 303[b] | 481[a] | 559[a] | 492[a] | 18 |
| $C_{18:1}$ | 68[b] | 144[a] | 152[a] | 146[a] | 10 |
| $C_{18:2}$ | 69 | 67 | 71 | 63 | 2 |
| $C_{18:3n3}$ | 12 | 13 | 13 | 12 | 1 |
| Fatty acid recovery | ----------% | | | | |
| $C_{18:0}$-$C_{18:3n3}$ | 96.1[a] | 87.0[b] | 91.3[ab] | 94.7[a] | 1.7 |
| $C_{18:1}$-$C_{18:3n3}$ | 33.2 | 32.8 | 40.2 | 34.2 | 1.6 |

[a,b]Means within same row with different superscripts differ ($p<.05$).
[1]Total ($C_{4:0}$-$C_{22:6n3}$) fatty acid analysis.

$C_{18:0}$ intakes than CT and T, which reflected higher $C_{18:0}$ concentration in the fat supplement of that diet (Table 2).

Fat supplementation increased total fatty acid ($C_{4:0}$-$C_{22:6n3}$) flow to the duodenum, because more dietary and similar amounts of bacterial fatty acids reached the duodenum (Table 9). Klusmeyer and Clark (66) observed increased bacterial fatty acid flow to the duodenum of cows fed Ca-salts of long chain fatty acids. Rumen bacteria have been shown to synthesize fatty acids from acetate (71) or branched and straight chain amino acids (34). Bacteria also incorporate long chain fatty acids into their bacterial lipids (11).

Total $C_{18:0}$-$C_{18:3n3}$ recovery also is listed in Table 9. Limited degradation of long chain fatty acids is thought to ocour in the rumen (12, 138). Approximately 8% of the intake of total fatty acids was not accounted for at the duodenum. This is in agreement with (139) but not (67, 71). Many variables could influence the calculated recovery of fatty acids reaching the duodenum including: species of animal, type of markers used to estimate fatty acid flow, procedures for lipid analysis, or number of cannulated animals.

Rumen microorganisms biohydrogenated dietary $C_{18:1}$-$C_{18:3n3}$ with equal efficiency across treatments (65%), as indicated by <u>recovery</u> of these fatty acids in the duodenum. Unsaturated fatty acids in "protected" fat supplements (HT and CT) did not resist biohydrogenation any better than the "nonprotected" fat supplement in T.

The "dual" marker system used in this experiment allowed differentiation between particulate- and liquid-associated fatty acids flowing to the duodenum (Table 10). It has been reported that rumen bacteria and nonesterified fatty acids preferentially bind to particulate matter in digesta (78). We found higher amounts of fatty acids associated with particulate phase flow for all fat-supplemented diets. However, there also was a higher amount of fatty acids

Table 10. Fatty acid flow in duodenal particulate and liquid digesta of Holstein cows fed diets with 0% supplemental fat (C), 3% tallow (T), 3% partially hydrogenated tallow (HT), or 3% tallow coated with casein and corn syrup solids (CT).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | T | HT | CT | SE |
| | ----------g/d---------- | | | | |
| Particulate | | | | | |
| Total[1] | 489[b] | 822[a] | 780[a] | 833[a] | 50 |
| $C_{16:0}$ | 89[b] | 189[a] | 173[a] | 194[a] | 13 |
| $C_{18:0}$ | 239[b] | 384[a] | 387[a] | 398[a] | 27 |
| $C_{18:1}$ | 56[b] | 122[a] | 102[a] | 118[a] | 7 |
| $C_{18:2}$ | 63 | 61 | 61 | 57 | 2 |
| $C_{18:3n3}$ | 12 | 13 | 13 | 12 | 1 |
| Liquid digesta | | | | | |
| Total[1] | 115[b] | 181[ab] | 330[a] | 193[ab] | 35 |
| $C_{16:0}$ | 24[b] | 44[ab] | 81[a] | 50[ab] | 8 |
| $C_{18:0}$ | 64[b] | 97[ab] | 171[a] | 94[ab] | 19 |
| $C_{18:1}$ | 12[b] | 22[ab] | 49[a] | 28[ab] | 7 |
| $C_{18:2}$ | 6[b] | 6[b] | 10[a] | 6[b] | 1 |

[a,b]Means within same row with different superscripts differ ($p<.05$).
[1]Total ($C_{4:0}$-$C_{22:6n3}$) fatty acid analysis.

associated with liquid phase flow when cows were fed HT. When expressed as a percent of total $C_{16:0}$-$C_{18:3n3}$ flow, cows fed HT had 30% of the fatty acids associated with the liquid phase versus 18% for C, T, and CT. It appears the fat supplement in HT (nearly 75% saturated) may have been less soluble in the rumen; thus, providing less opportunity for binding to particulate matter in the digesta. Similarly, hydrogenated tallow has been shown to resist dispersion and hydrolysis in the rumen (80).

Fat supplementation affected the apparent digestibility of long chain fatty acids in the intestine (Table 11). Digestibility of $C_{16:0}$ was lower for HT than for C, T, and CT. All fat supplements decreased $C_{18:0}$ digestibility, but the extent of decrease was greatest for HT. Regardless of treatment, apparent digestibility of $C_{18:0}$ quadradically decreased ($R^2$=.86) as $C_{18:0}$ flow to the intestine increased ($p<.006$). Although not different from C, HT had lower $C_{18:1}$ digestibility than T and CT. Digestibility of $C_{18:2}$ and $C_{18:3}$ were not affected by treatment, but this should be expected because fat supplements contained small amounts of $C_{18:2}$ and $C_{18:3}$. Klusmeyer and Clark (66) observed increased digestibility of $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$, but decreased digestibility of $C_{18:0}$ when lactating cows were fed Ca-salts of long chain fatty acids.

The present study indicates a limitation in the ability of the small intestine to absorb large amounts of $C_{18:0}$. However, data from this and other studies have not accounted for hindgut biohydrogenation. Digestibility of $C_{18:0}$ may have been underestimated, and $C_{18:1}$-$C_{18:3}$ overestimated if extensive biohydrogenation of undigested $C_{18:1}$-$C_{18:3}$ occurred in the hindgut. Endogenous fatty acids from bile and pancreatic secreations also contributed to fatty acid content of digesta beyond the duodenal cannula.

Fat supplementation increased the amounts of $C_{16:0}$ and $C_{18:1}$ apparently absorbed from the small intestine. More $C_{18:0}$ was absorbed from HT, despite the lower apparent (%) digestibility. Therefore,

Table 11. Apparent fatty acid digestion in the gastrointestinal tract of Holstein cows fed diets with 0% supplemental fat (C), 3% tallow (T), 3% partially hydrogenated tallow (HT), or 3% tallow coated with casein and corn syrup solids (CT).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | T | HT | CT | SE |
| Intestinal digestibility[1] | ----------%---------- | | | | |
| $C_{16:0}$ | 70.8[a] | 75.4[a] | 62.5[b] | 75.7[a] | 2.9 |
| $C_{18:0}$ | 80.0[a] | 67.8[b] | 57.8[c] | 65.7[b] | 2.5 |
| $C_{18:1}$ | 73.3[ab] | 86.8[a] | 66.5[b] | 85.0[a] | 3.2 |
| $C_{18:2}$ | 66.0 | 68.7 | 66.6 | 70.8 | 2.9 |
| $C_{18:3n3}$ | 70.5 | 72.2 | 72.0 | 80.1 | 7.7 |
| Intestinal digestion | ----------g/d---------- | | | | |
| $C_{16:0}$ | 80[b] | 176[a] | 162[a] | 186[a] | 11 |
| $C_{18:0}$ | 241[b] | 318[ab] | 330[a] | 324[ab] | 17 |
| $C_{18:1}$ | 50[b] | 125[a] | 104[a] | 125[a] | 10 |
| $C_{18:2}$ | 46 | 46 | 47 | 45 | 3 |
| $C_{18:3n3}$ | 8 | 9 | 9 | 10 | 1 |
| Fecal fatty acids | 184[c] | 313[b] | 456[a] | 319[b] | 22 |
| Apparent digestibility in total tract, %[2] | 73.8[a] | 74.4[a] | 63.6[b] | 71.5[ab] | 1.8 |

[a,b]Means within same row with different superscripts differ ($p<.05$).
[1]Percent of total ($C_{4:0}$-$C_{22:6n3}$) fatty acids.
[2]Apparent digestibility of all ($C_{4:0}$-$C_{22:6n3}$) fatty acids.

higher amounts of fatty acids were apparently absorbed when cows were fed T, HT, and CT versus C.

Higher intakes and lower digestibilities of fatty acids contributed to the higher amount of fatty acids in the feces of cows when fed fat-supplemented diets. It has been suggested that hydrogenated tallow resists dispersion and solubilization in the small intestine because saturated fatty acids have a high melting point (114). Others have shown that hydrogenated fatty acids have lower apparent digestibilities (25, 56, 80). Supplementing ruminant diets with lecithin (important for micelle formation in ruminants) did not improve apparent digestibilities of hydrogenated fat (55).

Fat supplementation did not affect plasma $C_{16:0}$, $C_{18:0}$, and $C_{18:3n3}$ concentrations (Table 12). Compared to C and HT, T and CT increased plasma $C_{18:1}$ and decreased $C_{18:2}$ concentrations. Similar amounts of $C_{18:2}$ were apparently absorbed in all treatments, so additional $C_{18:1}$ supplied by T and CT may have diluted $C_{18:2}$ fractions in triglyceride, phospholipid, and cholesterol ester classes. Herbein (50 and personal communication) observed similar results when lactating cows were supplemented with $C_{18:1}$ at 2% of diet DM. Moore et al. (90) observed that dairy cattle diets supplemented with $C_{16:0}$ and $C_{18:0}$ at 10% of diet DM reduced the concentration of $C_{18:2}$ in phospholipid and cholesterol ester fractions.

Milk production, composition, and component yield data are listed in Table 13. Cows had higher milk production when fed HT and CT compared to C. Positive milk production responses have been reported by other investigators when fat supplements were fed during early lactation (83, 110, 134). There also have been reports that fat supplementation in early lactation does <u>not</u> improve milk production (23, 58, 62, 111).

Milk fat percent and fat yield were not affected by treatment. This response has been observed by other investigators when cows were fed similar fat supplements (7, 56, 99).

Table 12. Fatty acid concentration in plasma from Holstein cows fed diets with 0% supplemental fat (C), 3% tallow (T), 3% partially hydrogenated tallow (HT), or 3% tallow coated with casein and corn syrup solids (CT).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | T | HT | CT | SE |
| Fatty acid[1] | ----------%---------- | | | | |
| $C_{16:0}$ | 11.3 | 11.6 | 11.6 | 12.4 | .4 |
| $C_{18:0}$ | 15.8 | 15.7 | 16.1 | 17.4 | .8 |
| $C_{18:1}$ | 5.8[b] | 8.3[a] | 6.1[b] | 8.2[a] | .2 |
| $C_{18:2}$ | 46.2[ab] | 42.5[b] | 47.2[a] | 40.8[b] | 1.2 |
| $C_{18:3n3}$ | 7.5 | 8.0 | 7.6 | 7.9 | .3 |

[a,b]Means within same row with different superscripts differ ($p<.05$).
[1]Percent of total ($C_{4:0}$-$C_{22:6n3}$) fatty acids.

Table 13. Milk production, composition, and component yields of Holstein cows fed diets with 0% supplemental fat (C), 3% tallow (T), 3% partially hydrogenated tallow (HT), or 3% tallow coated with casein and corn syrup solids (CT).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | T | HT | CT | SE |
| Milk, kg/d | 35.1[b] | 37.4[ab] | 38.6[a] | 38.5[a] | .5 |
| 3.5% FCM, kg/d[1] | 33.7 | 37.9 | 39.0 | 37.3 | 1.6 |
| Milk composition | ----------% | | | | |
| Fat | 3.24 | 3.60 | 3.54 | 3.32 | .15 |
| Protein | 3.20[a] | 3.17[ab] | 3.17[ab] | 3.09[b] | .02 |
| Lactose | 4.80 | 4.76 | 4.71 | 4.77 | .03 |
| Solids-not-fat | 8.55 | 8.53 | 8.52 | 8.58 | .05 |
| Milk component yields | ----------kg/d | | | | |
| Fat | 1.14 | 1.44 | 1.37 | 1.27 | .10 |
| Protein | 1.12 | 1.18 | 1.22 | 1.19 | .02 |
| Lactose | 1.67 | 1.75 | 1.81 | 1.70 | .08 |
| Solids-not-fat | 2.89 | 3.15 | 3.28 | 3.07 | .10 |

[a,b]Means within same row with different superscripts differ ($p<.05$).
[1]3.5% fat corrected milk = (.432)(kg milk)+(16.23)(kg fat).

Milk protein percent tended to decrease when cows were fed T and HT, but decreased ($p<.05$) when cows were fed CT compared to C. There were no differences in milk protein yield (kg/d), because fat supplementation simulated milk production. Fat supplementation has been shown to decrease (40, 67) or not affect milk protein percent (100, 108). Decreased protein concentrations in milk have been linked to inadequate amino acids reaching the mammary gland (14, 19), altered glucose metabolism (91, 116), and inadequate somatotropin release from the anterior pituitary to maintain uptake of amino acids by the mammary gland (15).

In general, increased uptake and secretion of dietary fatty acids into milk fat appears to decrease de novo synthesis of $C_{4:0}$-$C_{14:0}$ by the mammary gland (14, 50, 122). This is in agreement with our findings (Table 14). Fat supplements decreased $C_{12:0}$ and $C_{14:0}$ and increased $C_{18:0}$ and $C_{18:1}$ concentrations in milk fat. Treatments T and CT decreased $C_{18:2}$ concentrations in milk fat, which may be a reflection of lower $C_{18:2}$ concentrations in plasma (see Table 12). Plasma $C_{18:2}$ is preferentially placed in phospholipid and cholesterol ester fractions of plasma and highly conserved. Higher concentrations of $C_{18:0}$ and $C_{18:1}$ in milk fat when cows were fed T led to increased $C_{18:0}$ and $C_{18:1}$ yield (g/d) (Table 15). There was a similar tendency when cows were fed HT and CT.

## CONCLUSION

The results of this experiment indicated that supplementing diets with various types of tallow at 3% of diet DM will not adversly affect DM intake, ADF, N, and OM digestibilities, or VFA production in the rumen. Digestibility of $C_{18:0}$ and total long chain fatty acids was lower

Table 14. Fatty acid concentration in milk fat from Holstein cows fed diets with 0% supplemental fat (C), 3% tallow (T), 3% partially hydrogenated tallow (HT), or 3% tallow coated with casein and corn syrup solids (CT).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | T | HT | CT | SE |
| Fatty acid[1] | ----------%---------- | | | | |
| $C_{4:0}$ | 4.4 | 4.8 | 4.6 | 4.4 | .2 |
| $C_{6:0}$ | 2.7 | 2.5 | 2.6 | 2.2 | .1 |
| $C_{10:0}$ | 3.5[a] | 2.8[b] | 3.0[ab] | 2.4[b] | .1 |
| $C_{12:0}$ | 5.0[a] | 3.5[b] | 4.0[b] | 3.2[b] | .2 |
| $C_{14:0}$ | 13.6[a] | 11.3[b] | 11.5[b] | 10.9[b] | .3 |
| $C_{16:0}$ | 32.5 | 30.1 | 29.6 | 29.7 | .7 |
| $C_{18:0}$ | 14.0[b] | 17.8[a] | 17.3[a] | 17.6[a] | .6 |
| $C_{18:1}$ | 16.0[b] | 20.4[a] | 20.0[a] | 21.8[a] | .6 |
| $C_{18:2}$ | 2.9[a] | 2.3[b] | 2.7[a] | 2.3[b] | .1 |

[a,b]Means within same row with different superscripts differ ($p<.05$).
[1]Percent of total ($C_{4:0}$-$C_{22:6n3}$) fatty acids.

Table 15. Fatty acid production in milk fat by Holstein cows fed diets with 0% supplemental fat (C), 3% tallow (T), 3% partially hydrogenated tallow (HT), or 3% tallow coated with casein and corn syrup solids (CT).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | T | HT | CT | SE |
| Fatty acid production | ----------g/d---------- | | | | |
| $C_{4:0}$ | 69[a] | 50[b] | 64[ab] | 55[ab] | 4 |
| $C_{6:0}$ | 30 | 37 | 36 | 28 | 3 |
| $C_{10:0}$ | 40 | 40 | 42 | 31 | 4 |
| $C_{12:0}$ | 57 | 52 | 55 | 41 | 6 |
| $C_{14:0}$ | 156 | 164 | 158 | 138 | 14 |
| $C_{16:0}$ | 370 | 436 | 407 | 378 | 32 |
| $C_{18:0}$ | 161[b] | 254[a] | 243[ab] | 223[ab] | 17 |
| $C_{18:1}$ | 182[b] | 291[a] | 275[ab] | 277[ab] | 20 |
| $C_{18:2}$ | 33 | 34 | 37 | 29 | 3 |

[a,b]Means within same row with different superscripts differ ($p<.05$).

when cows were fed hydrogenated tallow compared to other treatments, but fatty acids apparently absorbed in the small intestine increased with fat supplementation. In general, tallow-based fat supplements tended to increase milk production and decrease milk protein percent, but output (kg/d) of milk components was not significantly altered.

# CHAPTER 4

## BIOHYDROGENATION, POSTRUMINAL FLOW, AND APPARENT DIGESTIBILITY OF DIETARY LIPIDS IN LACTATING DAIRY COWS. 2. PARTIALLY HYDROGENATED FREE FATTY ACIDS, FISH OIL, AND SOYBEAN OIL.


### ABSTRACT

Four lactating Holstein cows with cannulae in the rumen and proximal duodenum were fed diets with 0% supplemental fat, 3% partially hydrogenated fatty acids, 1.5% fish oil plus 1.5% stearic acid, or 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil. Basal diets containing 40% alfalfa haylage, 14.5% corn silage, and 45.5% concentrate (DM basis) were fed ad libitum. Specified fat supplements replaced a corresponding amount of corn grain in the concentrate mixtures. Fish oil decreased DM intake. Rumen VFA concentrations and ruminal and total tract apparent digestibilities (%) of DM, ADF, N, and OM were not affected by dietary fat supplements. Fish oil and soybean oil reduced biohydrogenation of $C_{18:1}$ + $C_{18:2}$ + $C_{18:3}$ in the rumen from 65% (control) to 28% and 55%, respectively, and increased trans $C_{18:1}$ flow from the rumen 4-fold. Fish oil increased apparent digestibility of total fatty acids in the small intestine. Milk fat percent was decreased by fish oil and soybean oil. Milk fat percent across treatments linearly decreased with amount (g/d) of trans $C_{18:1}$ flowing to the duodenum ($R^2$=.92) and percent trans $C_{18:1}$ in milk fat ($R^2$=.94). Dairy cows consuming fish by-product feeds or fats containing large amounts of trans $C_{18:1}$ may be at increased risk of developing milk fat depression.

# INTRODUCTION

Type and amount of fatty acids in dietary lipids can influence ruminal and post-ruminal digestion of dietary lipids by ruminants. Polyunsaturated fatty acids in plant oils inhibited fermentation in the rumen to a greater extent than saturated fatty acids (17, 54). Saturated fatty acids, however, were less digestible than unsaturated fatty acids in the small intestine (25, 56, 66, 80).
Few reports (66), however, provided an intensive evaluation of the manner in which dairy cows consuming large quantities of DM during peak lactation biohydrogenate, digest, and utilize long chain fatty acids for milk synthesis.

Recent reports suggested an adverse effect of dietary fishmeal (118, 133), partially hydrogenated vegetable oils (5, 112), and high concentrate feeding, (131) on milk fat production by cows. Lactating mice responded in a similar manner (132). Apparently trans $C_{18:1}$, resulting from incomplete biohydrogenation of unsaturated fatty acids in the rumen or feeding large amounts of trans $C_{18:1}$, reduced concentration of fat in milk (112, 131, 132, 133).

The objective of this study was to determine how partially hydrogenated fatty acids and polyunsaturated fatty acids affect ruminal biohydrogenation, postruminal fatty acid flow, apparent digestibility of fatty acids, and lactation performance of dairy cows. Relationships between trans $C_{18:1}$ flow to the small intestine and trans $C_{18:1}$ incorporation into fat were evaluated by selecting dietary fats that contained a substantial amount of trans $C_{18:1}$ or enhanced the production of trans $C_{18:1}$ in the rumen.

# Materials and Methods

## Animals and experimental design

Four cannulated cows (68, 104, 116, and 129 d postpartum) were used in this experiment. Two of the cows had completed the first experiment (Chapter 3). Replacement cows provided milk production and feed intake averages for the group that were similar to those of the first experiment. A 4 X 4 Latin square design (Appendix Table 34) with 21 d periods was used to investigate responses to 0% supplemental dietary fat or 3% supplemental fat from one of three fat sources .

## Diets

All diets contained 40.0% alfalfa haylage, 14.5% corn silage, and 45.5% concentrate DM. Ingredient and chemical composition of diets are listed in Table 16. Fat supplements included prilled fatty acids (Energy Booster 100™; Milk Specialties CO., Dundee, Il), crude Menhaden fish oil (Zapata Haynie Co., Reedville, VA), stearic acid (Hystrene 9718; Humko Chemical Div, Memphis, TN), soybean oil (Imperial oil; Bunge Edible Oil Corp., Chattanoga, TN), and hydrogenated soybean oil (Cube Fan Fry; Bunge Edible Oil Corp., Chattanoga, TN). Ethoxyquin (1.7 ml/gal) was added to fish oil to reduce fatty acid oxidation. Fat supplements were substituted for corn grain at 6% (prilled fatty acids), 6% (one part fish oil and one part stearic acid), and 6% (one part soybean oil and one part hydrogenated soybean oil) of the total concentrate to obtain diets with approximately 3% supplemental fat. Stearic acid (major endproduct of fatty acid biohydrogenation in the rumen) was paired with fish oil to minimize the effects of polyunsaturated fatty acids on fiber digestion in the rumen. All diets were formulated to meet NRC requirements (93). Fatty acid composition

Table 16. Ingredients and chemical composition of diets.[1]

| Item | C | PF | F | S |
|---|---|---|---|---|
| **Ingredients**[2] | ----------% | | | |
| Alfalfa haylage | 40.0 | 40.0 | 40.0 | 40.0 |
| Corn silage | 14.5 | 14.5 | 14.5 | 14.5 |
| Corn grain | 34.0 | 31.0 | 31.0 | 31.0 |
| Supplemental fat | 0.0 | 3.0 | 3.0 | 3.0 |
| Soybean meal 44% CP | 7.4 | 7.4 | 7.4 | 7.4 |
| Dried brewer's grains | 3.6 | 3.6 | 3.6 | 3.6 |
| Trace mineral salt | .3 | .3 | .3 | .3 |
| Monocalcium phosphate | .2 | .2 | .2 | .2 |
| Vitamins[3] | * | * | * | * |
| **Chemical composition**[2] | | | | |
| DM | 62.5 | 63.0 | 63.0 | 63.0 |
| CP | 18.0 | 18.0 | 18.0 | 18.0 |
| ADF | 17.0 | 17.0 | 17.0 | 17.0 |
| Crude Fat | 4.1 | 7.1 | 7.1 | 7.1 |

[1]C = control (no supplemental fat), PF = prilled fat, F = 1:1 of fish oil and stearic acid, and S = 1:1 of soybean oil and hydrogenated soybean oil supplemented diets.
[2]DM basis.
[3]Diets contained 2194 IU Vit A, 217 IU Vit $D_3$, and 20 IU Vit E/kg DM.

of fat supplements, forages, and concentrates are in Table 17. Cows were fed 75% of their daily ration at 1400 h and 25% at 0600 h.

Liquid and particulate markers

Cobalt ethylenediaminetetraacetic acid (Co-EDTA) was used as a liquid-phase digesta marker. Cr-mordanted fecal fibers were used as a particulate-phase marker. Markers were prepared and dosed as described previously (see page 30).

Measurements and sampling

All measurements and sampling procedures were described previously (see page 32).

Chemical analyses and calculations

Chemical analyses and subsequent calculations were described previously (see page 34). In addition, separation of trans and cis $C_{18:1}$ isomers in duodenal digesta, milk fat, forages, fat supplements, and concentrates was achieved by argentation TLC and subsequent gas-liquid chromatography as described by Al-Athari and Watkins (1). Silver nitrate-impregnated TLC plates were prepared by dipping silica-coated plates into a saturated methanolic solution of silver nitrate. Previously methylated samples of duodenal digesta, milk fat, forages, fat supplements, and concentrates were applied to the plates and developed twice in hexane:diethylether:glacial acetic acid (94:4:2, by vol). Methyl nonadecanoate ($C_{19:0}$) was added to the recovered fractions for use as an internal standard during subsequent GLC. The ratio of cis to trans $C_{18:1}$ determined by the above procedure was used to calculate the amount of each isomer in the "total" $C_{18:1}$ peak of the fatty acid profile chromatograms.

Table 17. Major long chain fatty acids in diet components.

| | 16:0 | 18:0 | 18:1 | 18:2 | 18:3n3 | 20:5n3 | 22:6n3 |
|---|---|---|---|---|---|---|---|
| **Forages** | ---------- | ---------- | ---------- | %[1] | ---------- | ---------- | ---------- |
| Alfalfa haylage | 21.1 | 4.7 | 2.8 | 17.3 | 35.6 | 0.0 | 3.9 |
| Corn silage | 16.3 | 4.0 | 17.9 | 50.2 | 4.1 | 0.0 | 0.0 |
| **Fat Supplements** | | | | | | | |
| Fish oil | 25.7 | 4.9 | 12.2 | 1.4 | 2.0 | 15.7 | 13.0 |
| Stearic acid | 5.0 | 94.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Soybean oil | 10.4 | 4.5 | 24.5 | 53.5 | 7.1 | 0.0 | 0.0 |
| Hydrogenated soybean oil | 10.7 | 15.5 | 72.5 | 1.2 | 0.0 | 0.0 | 0.0 |
| Prilled fat | 46.2 | 31.3 | 15.4 | 1.4 | 0.0 | 0.0 | 0.0 |
| **Concentrates[2]** | | | | | | | |
| C | 16.5 | 2.8 | 20.0 | 54.4 | 2.8 | 0.0 | 0.0 |
| PF | 36.0 | 20.9 | 16.4 | 21.2 | 1.2 | 0.0 | 0.0 |
| F | 15.7 | 31.6 | 13.6 | 24.4 | 1.6 | 3.2 | 3.1 |
| S | 13.5 | 7.6 | 35.1 | 39.0 | 3.3 | 0.0 | 0.0 |

[1]Percent of total ($C_{4:0}$-$C_{22:6n3}$) fatty acids.
[2]C = control, PF = prilled fat, F = 1:1 of fish oil and stearic acid, and S = 1:1 of soybean oil and hydrogenated soybean oil.

Statistical Analysis

Statistical analysis was described previously (see page 38). Additionally, simple regression analysis was performed with data using trans-$C_{18:1}$ flow to the duodenum (g/d) and trans-$C_{18:1}$ incorporation into milk fat (rel %) as the independent variables and milk fat % as the response (120).

## RESULTS AND DISCUSSION

Average daily DM intakes are given in Table 18. When cows were fed diet F, intake was approximately 12% lower than intake of C or S. Visual observation and chemical analysis of feed refusals indicated that cows avoided consuming concentrates containing fish oil. Cows may have adapted to diet F if periods were longer than 21 d, because intake was depressed to the greatest extent during the first 5 d of each period, then increased through the remainder of the period. Spain et al. (118) observed an 8% decrease in DM intake when lactating cows were fed diets supplemented with fish meal at 7.5% of diet DM (cows consumed appproximately .15 kg fish oil/d).

Intake of diets PF and S was similar to that of the control, despite the higher caloric density. This response has been observed by others when similar levels and types of fat were fed to lactating cows (40, 108). Ferguson et al. (32) however, observed decreased DM intake when diets contained 3, 6, and 9 % partially hydrogenated fatty acids. Mohamad et al. (87) observed decreased DM intake when lactating cows were fed soybean oil at 4% of diet DM. The major difference between studies reporting reduced DM intake (32, 87) and this study was that alfalfa silage, rather than corn silage, provided the majority of the forage DM. Also, cows in this study had higher milk production.

Table 18. Intake, flow, and digestibility of DM in the digestive tract of Holstein cows fed diets with 0% supplemental fat (C), 3% partially hydrogenated fatty acids (PF), 1.5% fish oil plus 1.5% stearic acid (F), or 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil (S).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | PF | F | S | SE |
| DM intake, kg/d | 24.6[a] | 22.9[ab] | 21.4[b] | 24.1[a] | .5 |
| DM intake, %BW | 4.3 | 4.0 | 3.9 | 4.1 | .1 |
| Flow to duodenum | | | | | |
| Particulate, kg/d[1] | 9.8 | 8.9 | 7.6 | 10.3 | .6 |
| Liquid, kg/d[2] | 7.2[a] | 6.5[a] | 4.9[b] | 6.4[a] | .3 |
| Total, kg/d | 17.0[a] | 15.3[ab] | 12.5[b] | 16.7[a] | .8 |
| Digestibility in rumen | | | | | |
| Apparent, % | 30.7 | 33.0 | 41.6 | 30.7 | 2.4 |
| Corrected, %[3] | 42.3[ab] | 43.3[ab] | 51.5[a] | 40.9[b] | 2.0 |
| Flow to feces, kg/d | 7.0 | 7.0 | 5.3 | 6.7 | .4 |
| Apparent digestibility in total tract, % | 71.9 | 69.7 | 75.5 | 72.5 | 1.5 |

[a,b]Means within same row with different superscripts differ ($p<.05$).
[1]Pellet from centrifugation (3000 x g for 10 min) of whole digesta.
[2]Supernatant from centrifugation (3000 x g for 10 min) of whole digesta.
[3]100 - [(DM flow at duodenum - microbial DM flow)/DM intake x 100].

Reduced DM intake when cows were fed diet F resulted in decreased DM, N, OM, and digesta flow to the duodenum (Tables 18, 19, 20, 21, and Appendix Table 33). Ruminal DM digestibility (corrected for bacterial DM) was highest for diet F. Otherwise, apparent digestibilities of DM, ADF, N and OM in the rumen and total tract were not affected by treatment. Lucas and Loosli (79) observed that 7% soybean oil reduced apparent crude fiber digestibility in the digetive tract of lactating cows. Hydrogenation of fatty acids has been shown to reduce the negative effects ("toxicity") of unsaturated fatty acids on rumen fermentation (17, 101). Apparently, the levels and types of fat supplements in this experiment were not toxic to rumen microorganisms nor did they physically coat feed particles to the extent that fermentation or nutrient digestibility was decreased in the rumen.

Concentrations of VFA in the rumen were measured to evaluate the effect of fat supplements on microbial fermentation for the reasons stated previously (page 37). Results indicate that the types and amounts of fat in the diets fed in this study did not affect total or individual VFA concentrations or the ratio of acetate to propionate (Table 22). These findings are in agreement with (40, 87, 108, 119) but not (9, 32) when lactating cows were fed fat supplements (up to 9% of diet DM).

Unlike the first experiment (Chapter 3), long chain fatty acid composition of mixed rumen bacteria was altered by diets fed in this experiment (Table 23). Rumen bacteria had similar concentrations of $C_{14:0}$ through $C_{18:2}$ when cows were fed C and PF. Fatty acids in PF were approximately 80% saturated and appeared to have little interaction with the rumen microorganisms. Diets F and S decreased $C_{14:0}$ through $C_{16:0}$ and increased $C_{18:1}$ concentrations in bacterial lipids. Rumen bacteria had higher $C_{18:1}$ and lower $C_{18:0}$ concentrations compared to other treatments when cows were fed diet F.

Table 19. Intake, flow, and digestibility of ADF in the digestive tract of Holstein cows fed diets with 0% supplemental fat (C), 3% partially hydrogenated fatty acids (PF), 1.5% fish oil plus 1.5% stearic acid (F), or 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil (S).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | PF | F | S | SE |
| ADF intake, kg/d | 3.7[a] | 3.5[ab] | 3.2[b] | 3.6[a] | .1 |
| Flow to duodenum, kg/d[1] | 2.7 | 2.5 | 2.2 | 2.6 | .1 |
| Apparent digestibility in rumen, % | 27.8 | 26.9 | 31.0 | 26.1 | 3.2 |
| Flow to feces, kg/d | 2.1 | 2.0 | 1.6 | 1.9 | .1 |
| Apparent digestibility in total tract, % | 43.8 | 42.0 | 49.4 | 46.0 | 3.0 |

[a,b]Means within same row with different superscripts differ ($p<.05$).
[1]Sum of particulate and liquid phase acid detergent fiber.

Table 20. Intake, flow, and digestibility of N in the digestive tract of Holstein cows fed diets with 0% supplemental fat (C), 3% partially hydrogenated fatty acids (PF), 1.5% fish oil plus 1.5% stearic acid (F), or 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil (S).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | PF | F | S | SE |
| N intake, g/d | 707[a] | 637[bc] | 596[c] | 670[ab] | 13 |
| At proximal duodenum[1] | | | | | |
| Particulate N flow | | | | | |
| Total, g/d | 302 | 266 | 235 | 288 | 17 |
| Microbial, g/d | 126 | 103 | 95 | 114 | 8 |
| Nonmicrobial, g/d | 176 | 163 | 139 | 175 | 10 |
| Liquid N flow | | | | | |
| Total, g/d | 382[a] | 328[a] | 242[b] | 326[a] | 15 |
| Microbial, g/d | 130 | 102 | 87 | 104 | 12 |
| Nonmicrobial, g/d | 252[a] | 226[ab] | 155[c] | 223[b] | 6 |
| Total microbial, g/d | 256 | 205 | 182 | 217 | 16 |
| Total nonmicrobial, g/d | 428[a] | 389[a] | 294[b] | 397[a] | 13 |
| Total, g/d | 684[a] | 594[ab] | 476[b] | 615[a] | 26 |
| N recovery, %[2] | 96.8 | 93.4 | 80.0 | 92.1 | 4.3 |
| Flow to feces, g/d | 216[a] | 208[a] | 159[b] | 194[ab] | 10 |
| Apparent digestibility in total tract, %[1] | 69.1 | 67.3 | 73.1 | 70.8 | 1.6 |

[a,b,c]Means within same row with different superscripts differ ($p<.05$).
[1]Data not corrected for endogenous protein contribution.
[2]Total nitrogen flow at the duodenum as a percent of nitrogen intake.

Table 21. Intake, flow, and digestibility of OM in the digestive tract of Holstein cows fed diets with 0% supplemental fat (C), 3% partially hydrogenated fatty acids (PF), 1.5% fish oil plus 1.5% stearic acid (F), or 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil (S).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | PF | F | S | SE |
| OM intake, kg/d | 23.1[a] | 21.5[ab] | 20.1[b] | 22.7[a] | .4 |
| Flow to duodenum, kg/d[1] | 15.4[a] | 13.8[ab] | 11.2[b] | 15.1[a] | .7 |
| Apparent digestibility in rumen, % | 33.0 | 35.6 | 44.0 | 33.2 | 2.4 |
| Flow to feces, kg/d | 6.3 | 6.3 | 4.7 | 6.0 | .4 |
| Apparent digestibility in total tract, % | 73.0 | 70.9 | 76.7 | 73.4 | 1.5 |

[a,b]Means within same row with different superscripts differ ($p<.05$).
[1]Sum of particulate and liquid phase organic matter.

Table 22. VFA concentrations in rumen fluid of Holstein cows fed diets with 0% supplemental fat (C), 3% partially hydrogenated fatty acids (PF), 1.5% fish oil plus 1.5% stearic acid (F), or 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil (S).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | PF | F | S | SE |
| VFA concentration | ----------umol/ml---------- | | | | |
| Acetate | 69.8 | 83.5 | 70.0 | 78.3 | 3.9 |
| Propionate | 22.3 | 27.0 | 25.2 | 25.0 | 1.5 |
| Isobutyrate | 1.4 | 1.6 | 1.6 | 1.8 | .2 |
| Butyrate | 13.2 | 14.6 | 14.9 | 14.8 | 1.3 |
| Isovalerate | 2.2 | 3.2 | 2.5 | 2.4 | .4 |
| Valerate | 2.2 | 2.5 | 2.4 | 1.9 | .2 |
| Total | 110 | 132 | 116 | 124 | 7 |
| Acetate:Propionate | 3.2 | 3.3 | 2.9 | 3.2 | .1 |

Table 23. Fatty acid concentration in mixed rumen bacteria from Holstein cows fed diets with 0% supplemental fat (C), 3% partially hydrogenated fatty acids (PF), 1.5% fish oil plus 1.5% stearic acid (F), or 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil (S).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | PF | F | S | SE |
| Fatty acid[1] | ----------%---------- | | | | |
| $C_{14:0}$ | 4.4[a] | 4.0[a] | 3.4[b] | 2.8[b] | .2 |
| $C_{15:0}$ | 4.7[a] | 3.7[ab] | 2.6[b] | 3.1[b] | .3 |
| $C_{16:0}$ | 30.6[a] | 34.8[a] | 25.8[b] | 24.3[b] | 1.0 |
| $C_{18:0}$ | 44.2[a] | 42.5[a] | 29.9[b] | 44.7[a] | 1.2 |
| $C_{18:1}$ | 7.4[c] | 7.5[c] | 28.7[a] | 18.6[b] | 1.3 |
| $C_{18:2}$ | 6.8 | 5.2 | 4.6 | 4.7 | .5 |
| Total, mg/g DM | 47.1[c] | 52.9[bc] | 76.5[a] | 65.6[ab] | 3.6 |

[a,b,c]Means within same row with different superscripts differ ($p<.05$).
[1]Percent of total ($C_{4:0}$-$C_{22:6n3}$) fatty acids.

Apparently, $C_{18:1}$ was preferentially incorporated into bacterial lipid when available. It has not been elucidated, however, if the presence of unsaturated fatty acids in microbial lipids directly relates to biohydrogenation in the rumen. Although intake of $C_{18:2}$ increased when diet S was fed, there were no differences in concentration of $C_{18:2}$ in bacterial lipids. Total bacterial lipid (mg/g bacterial DM) was significantly increased ($p<.05$) when cows were fed F and S. Klusmeyer and Clark (66) observed a similar response when lactating cows were fed Ca-salts of long chain fatty acids. Bauchart et al. (11) observed that addition of vegetable oils or tallow to diets fed to lactating Holstein cows increased FFA content of solid- and liquid-associated bacteria by 150% over controls. It has also been observed that fatty acid concentrations in solid-adherent bacteria are twice as high as those in liquid-associated bacteria (75). Bacterial fatty acid concentrations in this study were derived from liquid-associated bacteria; therefore, bacterial lipid concentrations may have been slightly underestimated.

Total fatty acid intake increased due to fat supplementation (Table 24). Individual fatty acid ($C_{16:0}$ through $C_{18:3n3}$) intakes varied with type of fat supplement fed and DM intake. The fat supplement in PF contained approximately 46% $C_{16:0}$ and resulted in increased $C_{16:0}$ intake when cows were fed PF. Cows fed PF and F had higher $C_{18:0}$ intakes than S and reflected the higher $C_{18:0}$ concentration in the fat supplements of those diets (Table 17). When cows were fed diet S, $C_{18:1}$ intake was higher compared to other treatments, which reflected the higher concentration of $C_{18:1}$ in the fat supplement (Table 17).

TLC was used to find the proportion of cis and trans $C_{18:1}$ isomers present in dietary supplements. Cows fed C and F did not consume detectible amounts of trans $C_{18:1}$. Trans-$C_{18:1}$ intake, however, increased when diets PF and S were fed (Table 24). Catalytic hydrogenation

Table 24. Fatty acid intake and flow to the duodenum of Holstein cows fed diets with 0% supplemental fat (C), 3% partially hydrogenated fatty acids (PF), 1.5% fish oil plus 1.5% stearic acid (F), or 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil (S).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | PF | F | S | SE |
| **Fatty acid intake** | ----------g/d---------- | | | | |
| Total[1] | 562[c] | 898[a] | 734[b] | 837[a] | 34 |
| $C_{16:0}$ | 86[b] | 311[a] | 122[b] | 135[b] | 15 |
| $C_{18:0}$ | 18[b] | 155[a] | 174[a] | 47[b] | 10 |
| total-$C_{18:1}$ | 89[b] | 131[b] | 102[b] | 242[a] | 11 |
| cis-$C_{18:1}$ | 89[b] | 119[b] | 102[b] | 173[a] | 10 |
| trans-$C_{18:1}$ | 0[c] | 12[b] | 0[c] | 69[a] | 2 |
| $C_{18:2}$ | 239[b] | 204[b] | 188[b] | 314[a] | 11 |
| $C_{18:3n3}$ | 73[ab] | 75[ab] | 64[b] | 80[a] | 2 |
| **Fatty acid flow** | | | | | |
| Total[1] | 519[c] | 805[a] | 663[b] | 851[a] | 29 |
| Dietary | 380[c] | 679[a] | 500[b] | 687[a] | 18 |
| Bacterial | 139 | 126 | 163 | 164 | 16 |
| $C_{16:0}$ | 95[b] | 253[a] | 130[b] | 129[b] | 12 |
| $C_{18:0}$ | 247[b] | 359[a] | 213[b] | 395[a] | 10 |
| total-$C_{18:1}$ | 68[b] | 76[b] | 192[a] | 194[a] | 17 |
| cis-$C_{18:1}$ | 32 | 38 | 30 | 42 | 5 |
| trans-$C_{18:1}$ | 37[b] | 38[b] | 163[a] | 152[a] | 16 |
| $C_{18:2}$ | 69 | 67 | 71 | 63 | 2 |
| $C_{18:3n3}$ | 12 | 13 | 13 | 12 | 1 |
| **Fatty acid recovery** | ----------% | | | | |
| $C_{18:0}$-$C_{18:3n3}$ | 93.5 | 89.2 | 89.7 | 101.0 | 3.0 |
| $C_{18:1}$-$C_{18:3n3}$ | 35.5[c] | 34.7[c] | 71.6[a] | 45.0[b] | 3.7 |

[a,b,c]Means within same row with different superscripts differ ($p<.05$).
[1]Total ($C_{4:0}$-$C_{22:6n3}$) fatty acid analysis.

(commercial process) of unsaturated fatty acids in these fats resulted in formation of trans $C_{18:1}$.

Fat supplementation increased total fatty acid flow to the duodenum because more dietary and similar amounts of bacterial fatty acids reached the duodenum (Table 24). Klusmeyer and Clark (66) observed increased bacterial fatty acid flow to the duodenum of cows fed Ca-salts of long chain fatty acids. Rumen bacteria have been shown to synthesize fatty acids de novo from acetate (71) or branched and straight chain amino acids (34), but can also incorporate FFA into their bacterial lipids (11).

Amounts of individual fatty acids flowing to the duodenum, in general, were dependent on diet fatty acid profiles and DM intake. When cows were fed PF, flow of $C_{16:0}$ increased, reflecting dietary $C_{16:0}$ intake. Flow of $C_{18:0}$ to the duodenum, however, was similar for diets C and F, in spite of the fact that 1.5% of diet F was stearic acid ($C_{18:0}$). Diets F and S caused increased total $C_{18:1}$ and trans $C_{18:1}$ flow to the duodenum. This was of particular interest with diet F, because cows did not consume any dietary trans $C_{18:1}$. It is concluded that trans $C_{18:1}$ accumulated due to incomplete biohydrogenation of unsaturated fatty acids in the rumen. This also would explain the small lack of an increase in $C_{18:0}$ flow to the duodenum when cows were fed F.

Recovery of $C_{18:0}$-$C_{18:3n3}$ (% of intake) also is listed in Table 24. Degradation of long chain fatty acids in the rumen is thought to be minimal (12, 138). In general, approximately 6.5% of $C_{18:0}$-$C_{18:3}$ intake did not reach the duodenum. This is in agreement with (139) but not (67, 71).

Apparently, some factor in fish oil was responsible for reducing biohydrogenation in the rumen. It is not known if fish oil was toxic to group B hydrogenators (bacteria responsible for converting trans $C_{18:1}$ to $C_{18:0}$), altered a feed-back regulation in the biohydrogenation pathway,

or favored production of intermediates ($C_{18:1}$ positional isomers) resistant to complete biohydrogenation (44). The inability of cows to completely biohydrogenate unsaturated fatty acids (primarily trans $C_{18:1}$) was reflected in the highest recovery of $C_{18:1}$-$C_{18:3}$ at the duodenum when cows were fed diet F.

The "dual" marker system used in this experiment allowed differentiation between particulate- and liquid-associated fatty acids flowing to the duodenum (Table 25). Rumen bacteria and nonesterified fatty acids preferentially bind to particulate matter in digesta (78). In this study, amount of fatty acids associated with the particulate phase for all fat-supplemented diets was increased, but a nonproportional increase in the amount of fatty acids associated with the liquid phase resulted when cows were fed PF. When expressed as a percent of total $C_{16:0}$-$C_{18:3n3}$ flow, approximately 24% of the fatty acids were associated with the liquid phase when cows were fed PF versus approximately 14% for C, T, and CT. Fat supplement in PF (approximately 80% saturated) may have been less soluble in the rumen and bound less frequently to particulate matter in the digesta. Similarly, hydrogenated tallow has been shown to resist dispersion and hydrolysis in the rumen (80).

The effects of fat supplementation on apparent digestion of fatty acids in the intestine is given in Table 26. Digestibility of $C_{16:0}$ was similar for C, PF, and S, but increased when cows were fed diet F. Digestibility of $C_{18:0}$ was similar for all treatments. Digestibility of $C_{18:1}$ increased when diets F and S were fed, compared to C and PF. Digestibility of $C_{18:2}$ and $C_{18:3}$ were not affected by treatment. Klusmeyer and Clark (66) observed increased $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$, but decreased $C_{18:0}$ digestibility when lactating cows were fed Ca-salts of long chain fatty acids.

Table 25. Fatty acid flow in particulate and liquid digesta of Holstein cows fed diets with 0% supplemental fat (C), 3% partially hydrogenated fatty acids (PF), 1.5% fish oil plus 1.5% stearic acid (F), or 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil (S).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | PF | F | S | SE |
| | ----------g/d---------- | | | | |
| Particulate | | | | | |
| Total[1] | 441[c] | 615[ab] | 578[b] | 740[a] | 26 |
| $C_{16:0}$ | 78[b] | 178[a] | 110[b] | 108[b] | 8 |
| $C_{18:0}$ | 214[c] | 277[b] | 180[c] | 341[a] | 12 |
| $C_{18:1}$ | 49[c] | 60[c] | 146[ab] | 169[a] | 18 |
| $C_{18:2}$ | 58[ab] | 48[b] | 44[b] | 71[a] | 3 |
| $C_{18:3n3}$ | 12[a] | 12[a] | 9[b] | 14[a] | 1 |
| Liquid | | | | | |
| Total[1] | 79[b] | 191[a] | 85[b] | 111[b] | 18 |
| $C_{16:0}$ | 17[b] | 75[a] | 20[b] | 21[b] | 8 |
| $C_{18:0}$ | 33[b] | 82[a] | 33[b] | 54[ab] | 7 |
| $C_{18:1}$ | 20 | 16 | 22 | 25 | 6 |
| $C_{18:2}$ | 5[ab] | 6[a] | 4[b] | 6[a] | 1 |

[a,b,c]Means within same row with different superscripts differ ($p<.05$).
[1]Total ($C_{4:0}$-$C_{22:6n3}$) fatty acid analysis.

Table 26. Apparent fatty acid digestion in the gastrointestinal tract of Holstein cows fed diets with 0% supplemental fat (C), 3% partially hydrogenated fatty acids (PF), 1.5% fish oil plus 1.5% stearic acid (F), or 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil (S).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | PF | F | S | SE |
| Intestinal digestibility[1] | ----------% | | | | |
| $C_{16:0}$ | 71.1[b] | 68.3[b] | 80.6[a] | 74.2[ab] | 1.4 |
| $C_{18:0}$ | 79.0 | 71.1 | 77.3 | 68.5 | 2.6 |
| $C_{18:1}$ | 81.1[b] | 79.4[b] | 94.2[a] | 92.0[a] | 2.1 |
| $C_{18:2}$ | 71.3 | 65.5 | 73.4 | 76.6 | 2.5 |
| $C_{18:3n3}$ | 100 | 100 | 100 | 100 | 0 |
| Intestinal digestion | ----------g/d | | | | |
| $C_{16:0}$ | 67[c] | 170[a] | 105[b] | 95[b] | 5 |
| $C_{18:0}$ | 194[bc] | 223[b] | 165[c] | 267[a] | 9 |
| $C_{18:1}$ | 57[b] | 60[b] | 181[a] | 178[a] | 17 |
| $C_{18:2}$ | 45[ab] | 35[b] | 37[b] | 59[a] | 4 |
| $C_{18:3n3}$ | 12[a] | 12[a] | 9[b] | 14[a] | 1 |
| Fecal fatty acids | 159[b] | 296[a] | 125[b] | 231[ab] | 25 |
| Apparent digestibility in total tract, %[2] | 71.0[b] | 68.2[b] | 82.0[a] | 72.2[b] | 1.5 |

[a,b,c]Means within same row with different superscripts differ ($p<.05$).
[1]Percent of total ($C_{4:0}$-$C_{22:6n3}$) fatty acids.
[2]Apparent digestibility of all ($C_{4:0}$-$C_{22:6n3}$) fatty acids.

Data from the first experiment (Chapter 3) suggested a limitation of the small intestine to absorb large amounts of $C_{18:0}$. The amounts of $C_{18:0}$ (from supplemented diets) flowing to the duodenum in this experiment were lower than in the first experiment. This may explain the similar $C_{18:0}$ digestibilities across treatments in this experiment. In both studies, amount of $C_{18:0}$ entering the duodenum was proportional to apparent digestibility of $C_{18:0}$ (regardless of source). Digestibility calculations did not account for hindgut biohydrogenation. Digestibility of $C_{18:0}$ may have been underestimated and $C_{18:1}+C_{18:2}+C_{18:3}$ overestimated if extensive biohydrogenation of undigested $C_{18:1}$-$C_{18:3}$ occurred in the hindgut. Endogenous fatty acids from bile and pancreatic secretions also would contribute to the fatty acid profile of digesta presented for absorption in the small intestine.

When cows were fed diet F digestibility of fatty acids in the gastrointestinal tract was higher compared to C, PF, and S. The increase probably was the result of the large proportion of unsaturated fatty acids in the digesta (approximately 46%), reduced DM intake, and reduced digesta flow to the intestine.

Individual plasma fatty acid concentrations are listed in Table 27. Plasma fatty acid concentrations were similar when cows were fed C and PF. Diet F reduced $C_{18:0}$ and $C_{18:2}$, but increased $C_{18:1}$, $C_{20:n6}$, $C_{20:5n3}$, and $C_{22:6n3}$ concentrations compared to controls. Spain et al. (119) found that fish oil infusion into the duodenum of lactating dairy cows increased concentrations of $C_{20:5n3}$ and $C_{22:6n3}$ in plasma.

The large increase in concentrations of $C_{20:5n3}$ and $C_{22:6n3}$ in plasma when cows were fed F is direct evidence that biohydrogenation of polyunsaturated fatty acids was inhibited when cows were fed diet F or rumen bacteria are unable to biohydrogenate $C_{20:5n3}$ and $C_{22:6n3}$. Low levels of $C_{20:5n3}$ when cows were fed C, PF, and S indicate that dietary $C_{20:5n3}$

Table 27. Fatty acid concentration in plasma from Holstein cows fed diets with 0% supplemental fat (C), 3% partially hydrogenated fatty acids (PF), 1.5% fish oil plus 1.5% stearic acid (F), or 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil (S).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | PF | F | S | SE |
| Fatty acid[1] | ----------% | | | | |
| $C_{16:0}$ | 10.8$^{ab}$ | 12.0$^{a}$ | 10.9$^{ab}$ | 10.0$^{b}$ | .3 |
| $C_{18:0}$ | 14.9$^{a}$ | 14.8$^{a}$ | 11.7$^{b}$ | 15.6$^{a}$ | .5 |
| $C_{18:1}$ | 4.7$^{c}$ | 6.0$^{bc}$ | 8.1$^{a}$ | 6.7$^{ab}$ | .3 |
| $C_{18:2}$ | 47.7$^{a}$ | 44.2$^{a}$ | 38.1$^{b}$ | 47.7$^{a}$ | .8 |
| $C_{18:3n3}$ | 8.3$^{ab}$ | 8.7$^{a}$ | 7.1$^{b}$ | 7.8$^{ab}$ | .3 |
| $C_{20:4n6}$ | 3.2$^{b}$ | 3.1$^{b}$ | 4.5$^{a}$ | 3.1$^{b}$ | .1 |
| $C_{20:5n3}$ | .7$^{b}$ | 1.4$^{b}$ | 7.2$^{a}$ | .9$^{b}$ | 1.0 |
| $C_{22:6n3}$ | 0.0$^{b}$ | 0.0$^{b}$ | 4.3$^{a}$ | 0.0$^{b}$ | .2 |

$^{a,b,c}$Means within same row with different superscripts differ ($p<.05$).
[1]Percent of total ($C_{4:0}$-$C_{22:6n3}$) fatty acids.

was probably placed into phospholipid and cholesterol ester fractions of blood when cows were fed diet F. It is known that phospholipid and cholesterol ester pools in blood turn over slowly (73). Therefore, it is likely that residual $C_{20:5n3}$ from the fish oil in diet F was detectable in later feeding periods. In the previous experiment, $C_{20:5n3}$ was not detected in plasma of cows fed control or fat-supplemented diets (see page 53).

Milk production, composition, and component yield data are listed in Table 28. Cows produced less milk when fed diet F than they did when fed diet S. Production responses to C and PF were intermediate. The decline in milk volume may be a consequence of depressed intake of diet F (less dietary energy). Fat supplements, in general, did not improve milk production compared to control. This is in agreement with others who fed supplemental fats similar to those fed in this study (40, 108, 118). In contrast, Fersuson et al. (32) reported that cows fed 3% prilled fatty acids had improved milk yield, but higher supplementation (6 and 9%) was ineffective due to reductions in DM intake.

Milk protein concentration and yield were not affected by treatment (Table 28). This in agreement with other investigators who fed diets containing hydrogenated fatty acids up to 9% of diet DM (32, 40, 108), and fishmeal at 6.5% of the diet DM (118). However, soybean oil at 4% of the diet DM decreased milk protein percent (87).

Milk fat percent and yield were similar for diets C and PF, but were lower when cows were fed F and S (Table 28). Ruminal VFA production and nutrient digestibilities were not altered by any treatments; thus, the response was most likely due to a post-ruminal effect. It has been reported that fishmeal and partially hydrogenated vegetable oils depress milk fat percent in mammals (5, 112, 118, 132). Trans $C_{18:1}$ in partially hydrogenated fats may (directly or indirectly) adversely affect the mammary gland , resulting in lower rates of

Table 28. Milk production, composition, and component yields of Holstein cows fed diets with 0% supplemental fat (C), 3% partially hydrogenated fatty acids (PF), 1.5% fish oil plus 1.5% stearic acid (F), or 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil (S).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | PF | F | S | SE |
| Milk, kg/d | 38.0[ab] | 37.5[ab] | 33.5[b] | 39.7[a] | 1.0 |
| 3.5% FCM, kg/d[1] | 36.2[a] | 35.2[a] | 29.1[b] | 35.6[a] | .7 |
| Milk composition | ----------%---------- | | | | |
| Fat | 3.26[a] | 3.18[a] | 2.78[b] | 2.95[b] | .05 |
| Protein | 3.01 | 2.97 | 2.95 | 2.98 | .04 |
| Lactose | 4.76 | 4.74 | 4.70 | 4.62 | .06 |
| Solids-not-fat | 8.43 | 8.35 | 8.27 | 8.22 | .10 |
| Milk component yields | ----------kg/d---------- | | | | |
| Fat | 1.22[a] | 1.17[a] | .90[b] | 1.14[a] | .02 |
| Protein | 1.14[a] | 1.11[ab] | .99[b] | 1.18[a] | .03 |
| Lactose | 1.80[a] | 1.77[a] | 1.57[b] | 1.82[a] | .04 |
| Solids-not-fat | 3.19[a] | 3.12[ab] | 2.76[b] | 3.24[a] | .07 |

[a,b]Means within same row with different superscripts differ ($p<.05$).
[1]3.5% fat corrected milk = (.432)(kg milk)+(16.23)(kg fat).

triacylglycerol synthesis (112, 132). Elevated concentrations of trans $C_{18:1}$ were observed in rumen contents and milk fat when cows were fed fishmeal at 14% of diet DM (133).

In general, increased uptake and secretion of dietary fatty acids into milk fat appears to decrease de novo synthesis of $C_{4:0}$ through $C_{14:0}$ by the mammary gland (14, 50, 122). Data in Table 29 support this concept. Fat supplements decreased $C_{12:0}$ and $C_{14:0}$ concentrations in milk fat. In addition, diets F and S elevated concentrations of total $C_{18:1}$ and trans $C_{18:1}$ in milk fat. Milk fat percent across treatments linearly decreased ($R^2$=.94) with percent trans $C_{18:1}$ in milk fat ($p<.0001$) and ($R^2$=.92) flow of trans $C_{18:1}$ (g/d) to the duodenum ($p<.001$). The presence of trans $C_{18:1}$ in milk fat has previously been linked to milk fat depression (112, 132). Askew et al. (3) demonstrated that trans $C_{18:1}$ inhibited $^{14}C$-$C_{16:0}$ esterification by mammary homogenates to a greater extent than the cis $C_{18:1}$ isomer.

Milk fatty acid yields (g/d) are listed in Table 30. Yields of medium chain ($C_{10:0}$, $C_{12:0}$, and $C_{14:0}$) fatty acids were reduced by fat supplementation. Diets F and S decreased $C_{16:0}$ yield, but increased trans $C_{18:1}$ output. When cows were fed diet F, compared to diet S, lower milk production and incomplete biohydrogenation of unsaturated fatty acids apparently decreased $C_{18:0}$, total-$C_{18:1}$, cis-$C_{18:1}$, and $C_{18:2}$ yields in milk fat.

## CONCLUSIONS

Results indicate diets supplemented with partially hydrogenated fatty acids or soybean oil at 3% of diet DM will not adversely affect DM intake, nutrient digestibility, rumen VFA production, or fatty acid digestibility. It does appear, however, that trans $C_{18:1}$

Table 29. Fatty acid concentration in milk fat from Holstein cows fed diets with 0% supplemental fat (C), 3% partially hydrogenated fatty acids (PF), 1.5% fish oil plus 1.5% stearic acid (F), or 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil (S).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | PF | F | S | SE |
| Fatty acid[1] | ----------%---------- | | | | |
| $C_{4:0}$ | 2.4 | 2.7 | 2.9 | 2.5 | .2 |
| $C_{6:0}$ | 1.8 | 1.7 | 1.8 | 1.7 | .1 |
| $C_{10:0}$ | $2.8^{a}$ | $2.0^{b}$ | $2.3^{ab}$ | $1.9^{b}$ | .1 |
| $C_{12:0}$ | $4.2^{a}$ | $2.8^{b}$ | $3.1^{b}$ | $2.6^{b}$ | .2 |
| $C_{14:0}$ | $13.8^{a}$ | $10.8^{bc}$ | $11.3^{b}$ | $9.6^{c}$ | .3 |
| $C_{16:0}$ | $34.6^{a}$ | $35.2^{a}$ | $28.0^{b}$ | $24.3^{c}$ | .5 |
| $C_{18:0}$ | $13.4^{b}$ | $16.7^{ab}$ | $14.1^{ab}$ | $18.5^{a}$ | .9 |
| total-$C_{18:1}$ | $19.1^{c}$ | $21.2^{c}$ | $27.3^{b}$ | $31.1^{a}$ | .6 |
| cis-$C_{18:1}$ | $18.1^{b}$ | $19.4^{ab}$ | $13.9^{c}$ | $22.7^{b}$ | .8 |
| trans-$C_{18:1}$ | $1.0^{c}$ | $1.8^{c}$ | $13.4^{a}$ | $8.4^{b}$ | .9 |
| $C_{18:2}$ | $3.3^{ab}$ | $2.8^{b}$ | $3.3^{ab}$ | $3.9^{a}$ | .1 |

[a,b,c]Means within same row with different superscripts differ ($p<.05$).
[1]Percent of total ($C_{4:0}$-$C_{22:6n3}$) fatty acids.

Table 30. Fatty acid production in milk fat by Holstein cows fed diets with 0% supplemental fat (C), 3% partially hydrogenated fatty acids (PF), 1.5% fish oil plus 1.5% stearic acid (F), or 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil (S).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | PF | F | S | SE |
| Fatty acid production | ----------g/d---------- | | | | |
| $C_{4:0}$ | 30 | 31 | 27 | 29 | 2 |
| $C_{6:0}$ | 21[a] | 19[ab] | 16[b] | 20[ab] | 1 |
| $C_{10:0}$ | 34[a] | 24[b] | 21[b] | 22[b] | 2 |
| $C_{12:0}$ | 52[a] | 33[b] | 29[b] | 29[b] | 3 |
| $C_{14:0}$ | 168[a] | 126[b] | 102[c] | 109[bc] | 5 |
| $C_{16:0}$ | 423[ab] | 410[a] | 253[b] | 277[b] | 8 |
| $C_{18:0}$ | 162[b] | 193[a] | 127[b] | 205[a] | 10 |
| total-$C_{18:1}$ | 232[b] | 248[b] | 246[b] | 353[a] | 11 |
| cis-$C_{18:1}$ | 220[a] | 226[a] | 127[b] | 257[a] | 8 |
| trans-$C_{18:1}$ | 12[b] | 21[b] | 119[a] | 97[a] | 13 |
| $C_{18:2}$ | 40[ab] | 32[bc] | 30[c] | 45[a] | 2 |

[a,b,c]Means within same row with different superscripts differ ($p<.05$).

(originating from either diet or incomplete biohydrogenation of unsaturated fatty acids in the rumen) may be involved in milk fat depression. Dairy cows consuming fish by-product feeds or fats containing large amounts of trans $C_{18:1}$ may be at increased risk of developing milk fat depression.

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## APPENDIX

Table 31. Latin square design used in experiment 1.

| | Period 1 | Period 2 | Period 3 | Period 4 |
|---|---|---|---|---|
| Cow 1 | CT | T | C | HT |
| Cow 2 | C | CT | HT | T |
| Cow 3 | HT | C | T | CT |
| Cow 4 | T | HT | CT | C |

C= control (0% supplemental fat)

T= 3% tallow

HT= 3% partially hydrogenated tallow

CT= 3% tallow coated with casein and corn syrup solids

"ANOVA TABLE"

| Source | DF |
|---|---|
| Model | 9 |
| Period | 3 |
| Treatment | 3 |
| Cow | 3 |
| Error | 6 |
| Total | 15 |

Table 32. Atomic absorption spectrophotometer conditions for elemental analysis of chromium (Cr) and cobalt (Co).

| Item | Cr | Co |
|---|---|---|
| measurement | atomic absorption | atomic absorption |
| flame | acetylene-nitrous oxide | air-acetylene |
| slit width | .5 nm | .2 nm |
| wavelength | 357.9 nm | 240.7 nm |
| current | 6 mA | 7 mA |

Table 33. Particulate and liquid digesta flow through the duodenum of Holstein cows fed diets with 0% supplemental fat (C), 3% tallow (T), 3% partially hydrogenated tallow (HT), or 3% tallow coated with casein and corn syrup solids (CT).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | T | HT | CT | SE |
| Digesta flow | ----------kg/d---------- | | | | |
| Particulate[1] | 45 | 45 | 44 | 45 | 2 |
| Liquid[2] | 412 | 392 | 409 | 384 | 19 |
| Total | 457 | 437 | 453 | 429 | 22 |
| Digesta flow | ----------kg/hr---------- | | | | |
| Particulate[1] | 2 | 2 | 2 | 2 | 1 |
| Liquid[2] | 17 | 16 | 17 | 16 | 1 |
| Total | 19 | 18 | 19 | 18 | 1 |

[1]Pellet from centrifugation (3000 x g for 10 min) of whole digesta.
[2]Supernantant from centrifugation (3000 x g for 10 min) of whole digesta.

Table 34. Latin square design used in experiment 2.

| | Period 1 | Period 2 | Period 3 | Period 4 |
|---|---|---|---|---|
| Cow 1 | PF | F | S | C |
| Cow 2 | C | S | F | PF |
| Cow 3 | S | PF | C | F |
| Cow 4 | F | C | PF | S |

C= control (0% supplemental fat)

PF= 3% partially hydrogenated nonesterified fatty acids

F= 1.5% fish oil plus 1.5% stearic acid

S= 1.5% soybean oil plus 1.5% partially hydrogenated soybean oil

"ANOVA TABLE"

| Source | DF |
|---|---|
| Model | 9 |
| Period | 3 |
| Treatment | 3 |
| Cow | 3 |
| Error | 6 |
| Total | 15 |

Table 35. Particulate and liquid digesta flow through the duodenum of Holstein cows fed diets with 0% supplemental fat (C), 3% partially hydrogenated fatty acids (PF), 1.5% fish oil plus 1.5% stearic acid (F), or 1.5% soybean oil plus 1.5% hydrogenated soybean oil (S).

| | Diets | | | | |
|---|---|---|---|---|---|
| | C | PF | F | S | SE |
| Digesta flow | ----------kg/d---------- | | | | |
| Particulate[1] | 43 | 40 | 34 | 44 | 2 |
| Liquid[2] | 467[a] | 399[ab] | 292[b] | 398[ab] | 24 |
| Total | 510[a] | 439[ab] | 326[b] | 443[ab] | 25 |
| Digesta flow | ----------kg/hr---------- | | | | |
| Particulate[1] | 2 | 2 | 2 | 2 | 1 |
| Liquid[2] | 20[a] | 17[ab] | 12[b] | 17[ab] | 1 |
| Total | 22[a] | 19[ab] | 14[b] | 19[ab] | 1 |

[1]Pellet from centrifugation (3000 x g for 10 min) of whole digesta.
[2]Supernantant from centrifugation (3000 x g for 10 min) of whole digesta.

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