DIETARY FACTORS AFFECTING FATTY ACID DIGESTION AND METABOLISM IN LACTATING DAIRY COWS

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ABSTRACT

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The five research chapters presented provide insight on the effects of different dietary factors on fatty acid (FA) digestion and metabolism in lactating dairy cattle. Together these experiments increase our understanding of factors that impact the amount of FA available for incorporation in milk fat and partitioning of energy into adipose tissue. In the first research chapter, digestibility estimates of individual FA from previous studies were analyzed using meta-analysis and meta-regression. Results from the meta-analysis indicated that minor differences in the digestibility of individual FA exist. However, when analyzing the data using meta-regression to generate best-fit equations, stearic acid (C18:0) digestibility was significantly reduced as the amount of stearic acid reaching the duodenum increased.

In the second research chapter, responses to diets differing in starch concentration were evaluated by lactating dairy cows across a wide range of milk production. The high-starch diet increased DMI, DM digestibility, yields of milk, fat and protein compared with the low-starch diet. Higher producing cows responded favorably for many production parameters to higher starch concentration in the diet whereas lower producing cows were able to maintain production on a diet containing only 12% starch.

The third research chapter investigated the effects of two diets similar in energy concentration yet differing in starch, NDF, and FA on milk production and energy partitioning. Intake was similar between treatments with the higher fiber and fat diet reducing milk yield, milk protein concentration, and milk protein yield. The higher fiber and fat diet increased milk fat

yield and concentration and milk energy output. The higher fiber and fat diet partitioned fewer nutrients towards body reserves as evident by reduced body weight gain, body condition score, and subcutaneous fat thickness. Our results indicate that although diets can be similar in energy content, the composition of the diet impacts production and body composition changes.

Research chapter four investigated the effects of additional fat supplemented to the diet as either free FA or esterified FA on production parameters and the FA composition of milk. Supplemental fat reduced DMI and increased milk yield with no changes in milk fat or protein yield. Although there was a reduction in the proportion of both de novo and 16-carbon milk FA, there was compensation in the form of an increase in preformed FA. Fat supplementation changed the FA profile of milk, however, there was no significant difference between free FA and esterified FA for common FA associated with milk fat depression (MFD).

In the final research chapter increasing doses of stearic acid were supplemented to lactating dairy cows to determine the effects on production parameters, digestibility, and incorporation of FA into milk fat. Stearic acid was supplemented at 0.8, 1.5, and 2.3% of diet DM compared to a non-fat supplemented diet. Supplementation of stearic acid resulted in no effect on yields of milk or milk components. Stearic acid inclusion resulted in reduced FA digestibility with the largest reduction in digestibility being 18-carbon FA. Increasing stearic acid to the greatest inclusion rate resulted in no effect on preformed milk FA yield and only a small increase in stearic and oleic acid. Results from all trials provide insight on FA digestibility, ways to utilize FA to repartition energy, and incorporation of FA into milk fat; however, further work is required to understand mechanisms of action for differences in digestibility and partitioning of energy and to determine if results are consistent across stages of lactation.

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KEY TO ABBREVIATIONS

ACC	acetyl-CoA carboxylase
AGPAT	acyl glycerol phosphate acyl transferase
AOAC	Association of Official Agricultural Chemists
ATP	adenosine triphosphate
AVF	animal-vegetable fat
BCS	body condition score
BW	body weight
Ca	calcium
CCK	cholecystokinin
CG	corn grain treatment
CLA	conjugated linoleic acid
CON	control treatment
CP	crude protein
CV	coefficient of variation
d	day(s)
DE	digestible energy
DIM	days in milk
DM	dry matter
DMD	dry matter digestibility
DMI	dry matter intake
ECM	energy-corrected milk

FA	fatty acid(s)
FAME	fatty acid methyl ester(s)
FAS	fatty acid synthase
FAYR	fatty acid yield response
FCM	fat-corrected milk
FFA	free fatty acid treatment
g	gram(s)
h	hour(s)
HCl	hydrochloric acid
HFF	high fiber and fat treatment
HS	high starch treatment
H-SA	2.3% C18:0-enriched fat supplement treatment
i.d.	internal diameter
kg	kilogram(s)
LPL	lipoprotein lipase
L-SA	0.8% C18:0-enriched fat supplement treatment
Max	maximum
MBW	metabolic body weight
Mcal	megacalories
ME	metabolizable energy
mEq	milliequivalent(s)
MFD	milk fat depression
min	minute(s)

Min	minimum
mL	milliliter(s)
μm	micrometer(s)
mm	millimiter(s)
M-SA	1.5% C18:0-enriched fat supplement treatment
n	number
N_2	nitrogen gas
NADP	nicotinamide adenine dineucleotide phosphate
NDF	neutral detergent fiber
NE	net energy
NEFA	non-esterified fatty acid
NE_L	net energy for lactation
NFFS	non-forage fiber sources
NRC	National Research Council
pMY	preliminary milk yield
PPARγ	peroxisome proliferator-activated receptor gamma
PUFA	poly-unsaturated fatty acid
pt(s)	point(s)
R^2	coefficient of determination
RMSE	root mean square error
RUFAL	rumen unsaturated fatty acid load
SA	C18:0-enriched fat supplement
SAS	Statistical Analysis System

SCD	stearoyl-CoA desaturase
SD	standard deviation
SE	standard error
SEM	standard error of the mean
SH	soyhulls treatment
SPE	solid phase extraction
SREBP1	sterol response element binding protein-1
TAG	triglyceride
TCA	tricarboxylic acid
TDN	total digestible nutrients
TMR	total mixed ration
TRT	*P*-value associated with the treatment effect
UFA	unsaturated fatty acid treatment
VFA	volatile fatty acid(s)
VIF	variance inflation factor
VLDL	very low density lipoprotein(s)

CHAPTER 1

INTRODUCTION

Background Information

The Agricultural Marketing Service in the United States currently uses a multiple component pricing system for Federal Milk Marketing Orders in which both milk fat and protein contribute to the price that dairy producers receive for their milk. It is well established that milk fat is a more variable component than milk protein and easier to manipulate with nutritional strategies. In order to alter the amount and composition of milk fat, one has to consider the metabolism and digestion of fatty acids (FA) in the rumen, small intestine, adipose tissue, and mammary gland. In the rumen, hydrolysis and biohydrogenation are two processes that convert unsaturated triglycerides to mostly saturated free FA. Large amounts of unsaturated fat coupled with an altered rumen environment leads to the production of specific biohydrogenation intermediates that causes a phenomenon known as milk fat depression (MFD) where milk fat is reduced up to 50% while other components in milk remain unchanged (Bauman et al., 2011).

The mostly saturated long-chain FA reaching the duodenum are available for absorption and ultimately enter circulation. Depending on stage of lactation, energy status, and hormonal regulation, FA can be partitioned to adipose tissue or taken up by the mammary gland. In addition to attempting to increase milk fat yield; another potential issue related to fat metabolism in dairy cattle is the consequence of established lactation cows depositing excessive adipose tissue. Dry matter intake (DMI) is typically maximized after peak milk production, therefore for a normal lactation curve the energy required for milk production is already decreasing before DMI and most likely energy intake reaches its maximum. As a consequence, mid- and late-lactation dairy cows can have an energy intake that exceeds energy demand, leading to storage of

energy as adipose tissue. Excessive body condition in late-lactation increases a cow's risk of developing metabolic diseases, culling, and death in the subsequent lactation (NRC, 2001; Roche et al., 2009). The problem of excessive body condition may be exacerbated in animals that have low milk fat yield, due to decreased energy output. Some of our research goals have been to understand nutritional strategies that will alter energy partitioning toward milk production and as a consequence away from adipose tissue.

Milk fatty acids (FA) originate from two distinct sources; de novo synthesized and preformed FA. De novo synthesized milk FA are produced in the mammary gland from acetate and β-hydroybutyrate that are taken up from circulation and produce milk FA ≤ 16 carbons in length whereas, preformed milk FA are taken up from circulation from either FA from the diet or adipose tissue and are ≥ 16 carbons in length (Lock and Bauman, 2004). Part of the difficulty involved in increasing milk fat yield is the coordinated regulation between preformed milk FA and de novo synthesized milk FA. Increasing preformed milk FA past a certain threshold results in a reduction in de novo synthesized milk FA (Glasser et al., 2008). Potentially this negates or at least diminishes the increase in milk fat from preformed sources. Therefore, there is the need for research on ways to effectively increase milk fat yield through increasing preformed milk FA from increased dietary FA.

Research Purpose

In order to understand the metabolism and digestion of FA in the lactating dairy cow, we utilized a series of studies that focused on the digestion and metabolism of FA in specific organs. The first research chapter used meta-analysis and meta-regression to determine if individual FA digestibility estimates differ and variables impacting digestibility of individual FA. The main focus of this chapter is the amount of individual FA absorbed in the small intestine. However,

individual FA digestibility estimates are dependent on the amount of FA reaching the duodenum, with the profile altered by rumen biohydrogenation. The amount of FA absorbed has a direct effect on the amount of FA available for use by the animal, of particular interest to us is the amount of FA available for milk fat synthesis.

The second research chapter focused on the effect of a high corn grain diet to alter the rumen environment and reduce the amount of milk fat synthesis due to the production of MFD-inducing biohydrogenation intermediates. The high corn grain diet is compared to a diet high in non-forage fiber across a wide range of milk production. Compared to a non-forage fiber source, the high corn grain would potentially increase the amount of propionate produced in the rumen and reduce rumen pH. Both of these factors may contribute to reduced milk fat yield through the production of MFD-inducing intermediates and/or increasing insulin concentrations partitioning more nutrients to body tissue gain and less to milk fat. We hypothesized that the large differences in starch concentration would have an impact in the rumen, which in turn would impact allocation of nutrients to adipose tissue and the mammary gland.

The third research chapter utilized diets similar in energy content to determine if partitioning of nutrients could be altered in lactating dairy cattle. A high fiber and fat diet was compared to a high starch diet with production parameters, metabolites and hormones, and changes in body weight and condition evaluated. We hypothesized that the high starch diet would increase plasma insulin concentrations and produce MFD-inducing biohydrogenation intermediates. Both of these factors may contribute to partitioning more energy towards body tissue gain, specifically subcutaneous fat and less energy towards milk energy output through a reduction in milk fat yield. Findings from this trial increase our understanding of interactions that occur between diet, rumen and portioning between adipose and mammary gland. In mid- and

late-lactation dairy cattle, finding ways to reduce the amount of body condition gain while increasing milk fat output has both economical and animal health implications.

The fourth research chapter analyzes whether FA fed as a triglyceride or free FA impacted rumen biohydrogenation and milk FA. Because of the increased availability of free FA to interact with rumen microbes, compared to triglycerides, we hypothesized the free FA would be more detrimental in the rumen and produce more MFD-inducing intermediates. Production of MFD-inducing intermediates would reduce milk fat synthesis in the mammary gland. If differences exist between free FA and triglycerides on rates and pathways of biohydrogenation, this information will improve our understanding of risk factors for MFD.

In the last research chapter increasing doses of a stearic acid-enriched fat supplement were fed to analyze the effect on absorption in the small intestine and incorporation into milk fat in the mammary gland. A stearic acid-enriched fat supplement was used because stearic acid is considered rumen inert and is the most common FA available for absorption by the small intestine. Therefore, digestibility of this FA as well as incorporation into milk fat in the mammary gland could be evaluated. We hypothesized that there would be an optimal dose of dietary stearic acid that will be incorporated into milk fat. The five research chapters use different dietary conditions to provide insight on FA digestion and metabolism of lactating dairy cattle.

REFERENCES

REFERENCES

Bauman, D. E., K. J. Harvatine, and A. L. Lock. 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Ann. Rev. Nutr. 31: 299-319.

Glasser, F., A. Ferlay, M. Doreau, P. Schmidely, D. Sauvant, and Y. Chilliard. 2008. Long-chain fatty acid yield in relation to duodenal flows and de novo synthesis. J. Dairy Sci. 91: 2771-2785.

Lock, A. L., and D. E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids 39:1197–1206.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Acad. Sci., Washington D.C.

Roche, J. R., N. C. Figgens, J. K. Kay, M. W. Fisher, K. J. Stafford, and D. P. Berry. 2009. Invited review: body condition score and its association with dairy cow productivity, health, and welfare. J. Dairy Sci. 92:5769-5801.

CHAPTER 2

LITERATURE REVIEW

Milk Pricing and Milk Fat

The current Federal Milk Order Program uses multiple component pricing to value milk produced on farm. Milk fat and protein content are the major contributors to the price that producers receive for milk. In an economic analysis assessing the value of milk components for the past ten years, a 5% increase in fat yield, protein yield, and milk yield increased net farm income by 13%, 15%, and 3%, respectively (St-Pierre, 2011). This underlies the importance of focusing on increasing the yield of milk fat and protein and not milk yield per se in order to maximize milk price and income. Fat is typically the most variable component in milk and is easier to influence (both positively and negatively) through diet alterations than milk protein. The price of milk fat is included in calculating all four classes of dairy products therefore increasing milk fat allows producers to improve milk income.

Dietary Fatty Acids

Dietary fatty acids (FA) are derived from forages, grains, byproducts, and any additional fat supplements fed to lactating dairy cattle. The form of dietary FA is mostly esterified FA, as triglycerides, glycolipids, or phospholipids; and to a lesser extent non-esterified FA. Dietary FA are a source of energy, which may be limited in high producing dairy cattle. Additionally, individual FA have been shown to have specific bioactive properties; however, most dietary FA are fed to improve milk production or improve energy balance (Weiss and Wyatt, 2004).

In forages, FA are primarily found in glycolipids, which contain two FA and one or two sugar molecules linked to a glycerol backbone. The FA content of forages is impacted by maturity of forages, genetics of species and cultivars, amount of N fertilization, time of harvest,

and post-harvest oxidation (Khan et al., 2012). For temperate grasses, FA content is highest during early leaf growth and is reduced during reproductive growth, therefore as a grass matures the FA content is reduced (Bauchart et al., 1984). In a study analyzing 101 samples of grass silage, Khan et al. (2012) reported the variation in FA content from 0.81 to 3.25 % DM with over 50% of the FA being linolenic acid. While there are several factors that impact FA content of forages, using redundancy analysis to determine which factors explain variation in FA content, Khan et al. (2012) estimated that 82% of the variation in FA content in grass silages is explained by plant maturity compared with 4% of the variation being explained by ensiling quality. Therefore stage of maturity at harvest is by far the most important factor determining FA content of forages. However, wilting of grass silages leads to a further reduction in FA in the form of oxidative loss with a reduction in the relative proportion of PUFA and an increase in the proportion of C16:0 (Khan et al., 2011).

Similarly corn silage FA content is impacted to the greatest extent by stage of maturity at harvest. As a plant matures, there is a reduction in linolenic acid contribution from the leaves as the leaf to stem ratio is reduced. However, a substantial amount of linoleic acid is found in the kernel of corn as the ear of corn fills, shifting the FA content from linolenic to linoleic acid as corn matures (Khan et al., 2011). Analysis of 96 corn silage samples indicated that substantial variation in FA content exists (1.24 to 3.53 % DM) with linoleic acid being the dominant FA present (Khan et al., 2012). Our lab group recently analyzed corn silage samples containing high concentrations of linoleic acid; differences existed between the FA profile of esterified and free FA fractions (Klein et al., 2013). This would suggest that within the same corn silage sample, the FA profile of esterified and free FA differs possibly due to individual FA having different affinities for hydrolysis. Free FA that enter the rumen are able to interact directly with rumen

microbes (Jenkins, 1993) and increasing the proportion of free to esterified FA has been shown to influence biohydrogenation rates and pathways (Moore et al., 1969; Noble et al., 1974) and reduce milk fat synthesis (Cooke et al., 2007).

The FA in cereal grains and oilseeds are primarily in triglycerides composed of three medium- to long-chain FA linked to a glycerol backbone. The FA profile of corn and soybean are similar with approximately 85% unsaturated FA, greater than 50% linoleic acid, and greater than 20% oleic acid (NRC, 2001). Cottonseed, an oil seed commonly fed to dairy cattle, contains approximately 50% linoleic acid and 19% linolenic acid (Rouse, 2003). The high concentrations of unsaturated FA found in commonly fed cereal grains and oilseeds requires extensive biohydrogenation in the rumen.

Several byproducts commonly fed to dairy cattle contain high contents of PUFA. For example, dry milling of corn grain or other starch sources for the production of ethanol results in production of distillers grains with substantial variation in FA content dependent on the amount of solubles added back to distillers grains (Stein and Shurson, 2009). The solubles fraction has a similar FA composition as that found in grains from which the distillers were fermented. The FA content of distiller grains is dependent on practices of the processing plant with a wide range of FA content products available on the market (Spiehs et al., 2002).

There is a wide range of commercially available fat supplements utilized in lactating dairy cow diets. Those described in this review of literature is limited to fat supplements utilized in research chapters and include calcium salts of FA, saturated FA prills, unsaturated supplements from soybean oil, and tallow. Due to the toxic effects of unsaturated FA on rumen microbes, calcium salts of FA were developed as a protection mechanism making the FA more rumen inert (Jenkins and Palmquist, 1982) with little effect on rumen fermentation or FA

digestibility (Jenkins and Palmquist, 1984). In calcium salt products, calcium is bound to the carboxyl end of the free FA. However, high concentrations of linoleic acid in a calcium salt product led to reduced milk fat concentration at low rumen pH (Palmquist, 1984), indicating that calcium salts do not offer complete protection for rumen microbes because of dissociation of the calcium salt at low rumen pH.

Saturated FA prills, usually containing high concentrations of palmitic, stearic acid, or a combination of the two, are considered relatively rumen inert because they contain only small concentrations of unsaturated FA. Digestibility of saturated FA prills may be impacted by prill size with increased surface area corresponding to higher digestibility (Elliott et al., 1994; Eastridge and Firkins, 2000) with saturated free FA historically having higher digestibility than saturated triglycerides (Elliott et al., 1994; Pantoja et al., 1995). Early research comparing the effects of feeding saturated FA indicated differences in production responses dependent on chain length of fat supplement (Steele and Moore, 1968), which will be discussed in more detail in the milk fat synthesis section.

Tallow, contains approximately 25% palmitic acid, 20% stearic acid, and greater than 50% unsaturated FA, mostly as oleic acid (NRC, 2001). The FA profile of tallow is somewhat dependent on the dietary FA profile of the ingredients fed to the animals from which tallow is rendered. Historically tallow has been considered moderately unsaturated; however, due to an increase in the use of distillers grains and other feed ingredients with high concentrations of linoleic acid, the current composition of animal fat, from which tallow is derived, contains more linoleic acid (Duckett et al., 2008). Changes in FA profile of a fat supplement may impact rumen biohydrogenation, dry matter intake, and/or milk fat synthesis.

Rumen

Hydrolysis of esterified FA to non-esterified FA occurs rapidly in the rumen by bacterial enzymes (Palmquist and Jenkins, 1980). Glycerol and any sugars released from glycolipids are fermented to volatile FA (VFA) whereas FA are not extensively metabolized in the rumen. Through biohydrogenation, rumen bacteria convert unsaturated to saturated FA as a protection mechanism (Jenkins, 1993). Rumen biohydrogenation requires a free carboxyl end; therefore hydrolysis of esterified FA is required prior to biohydrogenation (Palmquist and Jenkins, 1980). Due to an excess of hydrogen present in the rumen from anaerobic fermentation, biohydrogenation occurs relatively quickly. Recent work done with in vitro cultures indicates that free PUFA are more disruptive to fiber digesting bacteria (ex. *B. fibrosolvens*) than either monounsaturated FA or FA methyl esters (Maia et al., 2010). Potential mechanisms for the disruption of cell integrity associated with free FA include uncoupling of proton-motive force, increasing ion leakage, or decoupling of intra-membrane pathways (Maia et al., 2010).

Although the end product of rumen biohydrogenation is a completely saturated FA (stearic acid), incomplete biohydrogenation leads to the production and accumulation of biohydrogenation intermediates. Biohydrogenation of oleic acid produces *cis* and *trans* isomers of C18:1 as well as stearic acid directly (Mosley et al., 2002; Jenkins et al., 2006). PUFA are biohydrogenated to saturated FA through a series of steps involving isomerization and hydrogenation with different types of bacteria responsible for different steps in the biohydrogenation pathway (Harfoot and Hazelwood, 1997).

Currently, seven different CLA isomers are identified from the initial steps of biohydrogenation of linoleic acid (Lee and Jenkins, 2011). Several of these CLA isomers: *trans*-10, *cis*-12 C18:2 (Baumguard et al. 2000); *cis*-10, *trans*-12 C18:2 (Sæbø et al., 2005), and *trans*-

9, *cis*-11 C18:2 (Perfield et al., 2005), have been linked to changes in FA metabolism in the mammary gland and adipose tissue. Reductions in rumen pH are specifically linked to a *trans*-10 shift in rumen biohydrogenation producing *trans*-10, *cis*-12 CLA instead of the normal isomer produced, *cis*-9, *trans*-11 CLA (Kim et al., 2002; Jenkins et al., 2006). Incomplete rumen biohydrogenation and accumulation of intermediates may be related to lactic acid, causing reduced rumen pH, creating a lag in biohydrogenation and slowing down the rate of stearic acid formation (Maia et al., 2010).

The potential reduction in fiber digestibility when oil is supplemented is thought to be from one or a combination of four proposed mechanisms: 1.) coating of the fiber with fat preventing microbial interaction; 2.) the toxic effect of unsaturated FA on certain microbial populations; 3.) inhibition of microbial activity on cell membranes by FA; 4.) reduced cation availability due to the formation of insoluble complexes with FA (Palmquist and Jenkins, 1980). In vitro analysis of cellulolytic degradation indicated that both a reduction in rumen pH and the addition of tallow to cotton fiber depressed the cellulolytic capability of microorganisms (Stewart, 1977). Unsaturated FA supplemented at 10% of diet DM reduced the ratio of acetate to propionate by > 50% however, using calcium salts as a protection mechanism mediated the effects of unsaturated FA on rumen fermentation (Chalupa et al., 1986). Additionally, saturated FA fed at 10% of diet DM had little effect on rumen fermentation characteristics (Chalupa et al., 1986). When calcium salts of palm oil and prilled fat supplements included up to 3.5% of diet DM in dairy cow diets, no effects were observed for DM or NDF digestibility (Grummer, 1988). Although unsaturated FA appear to have a more detrimental effect on fiber digestibility than saturated FA, the effects may be minimized by using calcium salts of unsaturated FA. In several studies analyzing the effects of unsaturated FA on rumen fermentation and fiber digestibility the

concentration of FA far exceeds the level commonly fed on commercial dairy farms, therefore results need to be considered in context.

Small Intestine

As digesta exits the rumen it is at a near neutral rumen pH and most of the free FA are found as salts of FA, as potassium, sodium, or calcium. Low abomasal pH causes the dissociation of a majority of the free FA from the mineral salts. Due to the extensive hydrolysis and changes in FA structure due to biohydrogenation, FA reaching the small intestine are mostly saturated free FA (Moore and Christie, 1984). The high proportion of 18-carbon unsaturated dietary FA compared to other FA found in most feedstuffs leads to a majority of the FA reaching duodenum to be stearic acid with a small proportion of FA present as phospholipids from bacterial membranes. Digesta reaching the duodenum has a pH of less than 3 (Moore and Christie, 1984) and esterified FA reaching the duodenum are rapidly hydrolyzed (Doreau and Ferlay, 1994). Prior to absorption, FA must be released from association with particulate matter of the digesta. In the duodenum, bile and pancreatic secretions are added to digesta at a nearly continuous rate independent of flow rate of digesta (Noble, 1981). Bile provides bile salts and lecithin while pancreatic secretions provide phospholipase to convert lecithin to lysolecithin; both bile salts and pancreatic secretions are required for micelle formation. In ruminants, lysolecithin acts as an amphiphile, having both hydrophilic and lipophilic properties, to help in the formation of micelles for absorption of FA. Freeman (1969 and 1984) reported that lysolecithin is the most effective emulsifier for stearic acid. Other emulsifiers include oleic acid, however the concentrations normally found in the duodenum may not be sufficient and the amphiphilic properties are not as effective for stearic acid absorption. Once micelles are formed,

a majority of FA absorption occurs in the jejunum of the small intestine. Micelles facilitate the transfer of water-insoluble FA across the unstirred water layer of intestinal epithelial cells.

Any free PUFA in intestinal epithelial cells are preferentially incorporated into phospholipids and cholesterol esters as a way to prevent PUFA from being oxidized as fuel (Moore and Christie, 1984). Both linoleic and linolenic acids cannot be produced by mammalian tissue and therefore they are conserved for required membrane structures and cholesterol esters. In intestinal epithelial cells, free FA are combined with glycerol to form triglycerides and packaged with cholesterol, phospholipids, and apoproteins into lipoproteins as chylomicrons or VLDL. The size of these lipoproteins precludes their direct transfer into the venous bloodstream and therefore they are first secreted into the lymph to be delivered into the bloodstream close to the heart for transport to other organs (Moore and Christie, 1984). Unlike other nutrients that are absorbed from the intestinal epithelial cells, long chain (>14 carbon) FA do not proceed to the liver directly for first-pass metabolism. Therefore, dietary FA do not significantly contribute to fat accumulation in the liver seen around parturition (Drackley 1999). Previous research indicates the digestibility of saturated FA decreases as chain length increases while level of unsaturation increases digestibility of FA (Steele and Moore, 1968; Andrews and Lewis, 1970). Increasing fat inclusion in the diet may result in reduced FA digestibility (Palmquist, 1991; Khorasani et al., 1992). Specifically, increasing fat supplementation reduced stearic acid digestibility compared to other FA (Weisbjerg et al., 1992). Due to the potential negative effects of fat supplements on DMI, fiber digestibility, and reduced FA digestibility inclusion of fat supplements do not always supply more digestible energy to dairy cattle. In order to accurately determine if FA increase energy available to the cow and increase the FA available for incorporation into milk fat, individual FA digestibility needs to be considered. To measure FA

digestibility, duodenally cannulated cows must be used to measure the amount of individual FA available for absorption. As a result of the requirement for duodenally cannulated cows, a limited number of studies have measured individual FA digestibility. In chapter 3, results from published studies that measured individual FA digestibility are summarized.

Liver

A majority of research on the liver's role in FA metabolism has been during the periparturient period of dairy cattle. As intake decreases with a subsequent dramatic increase in energy demands for milk synthesis, mobilized body fat will enter the blood stream as NEFA. Circulating NEFA are readily available for complete oxidation by a variety of tissues (Contreras et al. 2010). Approximately 25% of total plasma NEFA is extracted from blood by the liver regardless of NEFA supply (Emery et al., 1992) and used as a fuel source, stored as TAG, or repackaged into VLDL. Both hepatic blood flow and plasma NEFA concentrations increase dramatically during early lactation compared to the dry period (Reynolds et al. 2003). Rates of triglyceride formation are similar however; rates of secretion of triglycerides are much slower in ruminants compared to non-ruminants (Graulet et al. 1998). High plasma NEFA concentration and limited ability of the ruminant liver to export triglycerides can result in an accumulation of fat in the liver of dairy cattle.

Work done in cows in early lactation indicates that the liver may preferentially take up different FA with increased concentrations of palmitic and oleic acid and reduced concentrations of stearic acid in liver TAG compared to pre calving values (Douglas et al., 2007). Loften et al. (2014) hypothesized that during negative energy balance stearic acid is metabolized for energy or utilized for milk FA synthesis and is not accumulated in the liver or adipose tissue. Research done in established lactation trials indicate that increasing palmitic through the diet or abomasal

infusions increased both milk fat yield and concentration, compared to stearic acid supplementation (Steele and Moore, 1968; Enjalbert et al., 2000). These differences between supplements in transfer to milk FA may be due to differences in digestibility, preferential use of stearic acid as an energy source, or storage of stearic acid in adipose tissue.

Adipose

Rumen biohydrogenation of unsaturated FA results in the majority of FA available for uptake by adipose tissue by lipoprotein lipase being saturated FA. Stearoyl-CoA desaturase (SCD) is the enzyme responsible for converting stearic to oleic acid in adipose tissue with the activity of this enzyme partially dependent on dietary FA composition (Dryden et al., 1973; Chang et al., 1992). As more unsaturated FA are fed to ruminants, less conversion of stearic to oleic acid is required as comparatively more unsaturated FA are available in circulation. An additional enzyme active in adipose tissue is FA elongase, capable of converting palmitic to stearic acid (St. John et al., 1991) further altering the composition of FA absorbed compared to FA stored in adipose tissue. During periods of negative energy balance there is a coordinated regulation in adipose tissue reducing rates of lipogenesis and increasing lipolysis (McNamara and Hillers, 1989). Lipolysis of FA is achieved through hormone sensitive lipase which is considered the rate-limiting enzyme (Yeaman, 1994). Thus, following calving the expression of hormone sensitive lipase increases in order to meet energy demands (Sumner and McNamara, 2007). Due to the lack of ATP citrate-lyase and NADP-malate dehydrogenase ruminant adipose tissue is unable to use substrates that generate mitochondrial acetyl-CoA (Bauman, 1976). Therefore, substrates such as glucose that enter the TCA cycle as pyruvate are unable to be used for FA synthesis. Acetate and β-hydroxybuterate are used as precursors for FA synthesis in adipose tissue.

Dietary Factors that Impact Milk Fat

Milk fat is reduced when two factors occur in the rumen: 1.) an altered rumen environment that changes fermentation generally caused by reduced rumen pH and 2.) the inclusion of unsaturated FA leading to the production of specific biohydrogenation intermediates. Factors that can contribute to reduced rumen pH include increasing the fermentability of carbohydrates thus increasing the acid load in the rumen (Oba and Allen, 2003) or reducing particle size (Grant et al., 1990). Additionally monensin, an ionophore approved for use in lactating dairy cattle, has been shown to inhibit complete biohydrogentation and may contribute to reduced milk fat yield. In conjunction with diets low in physically effective fiber, monensin increased the severity of MFD (Dubuc et al., 2009). Some research indicates that the amount of fat in the diet that is non-esterified (free FA), compared to esterified (TAG) may negatively impact milk fat synthesis. Cooke et. al (2007) fed cottonseed with varying amounts of free FA and observed a reduction in milk fat concentration when diets higher in free FA were fed, potentially indicating that free FA have a more negative effect on rumen biohydrogenation rates or pathways. The type of forage present in the diet has also been shown to impact milk fat yield when oilseeds or unsaturated fat supplements were fed. Corn silage based diets compared to a higher alfalfa based diets have been shown to have more negative effects on rumen fermentation and therefore reduced milk fat yield and/or concentration (Smith and Harris, 1993; Onetti et al., 2002).

Synthesis of Milk Fatty Acids

The FA incorporated into milk fat are produced by two mechanisms; either the incorporation of preformed FA > 16 carbons in length or the production of short and medium chain FA < 16 carbons (*de novo* synthesis) in the mammary gland (Lock and Bauman, 2004).

Preformed FA are derived from dietary FA absorbed from the small intestine or mobilized FA from adipose tissue and enter the mammary gland from circulating triglycerides in plasma. FA synthesized *de novo* in the mammary gland are produced from the precursors acetate and β-hydroxybuterate that are produced in the rumen from the fermentation of feed. FA of 16 carbons in length can come from either source (Lock and Bauman, 2004).

While adipose tissue is able to convert palmitic acid to stearic acid using elongase, the mammary gland is incapable of producing stearic acid from palmitic acid (Palmquist, 2006). The mammary gland does however add a double bond using SCD that is capable of converting myrisitc (C14:0), palmitic (C16:0), and stearic acid (C18:0) to myristoleic (*cis*-9 C14:1), palmitoleic (*cis*-9 C16:1) and oleic acid (*cis*-9 C18:1), respectively (Dils, 1983). Increasing the level of unsaturation decreases the melting point. For example stearic acid, with a melting point of 157°F can be converted to oleic acid with a melting point of 55°F in the mammary gland. Enjalbert et al. (2000) determined that palmitic acid has greater transfer efficiency to the mammary gland than stearic acid using duodenal infusions of both FA. Recent studies feeding palmitic acid-enriched fat supplements have observed increases in milk fat yield when comparing a palmitic acid-supplemented diet to a control (Lock et al., 2013; Piantoni et al., 2013) and when comparing palmitic to stearic acid (Rico et al., 2013).

Synthesis of de novo milk fatty acids

To produce milk FA from 4 to 16 carbons in length in the mammary gland, acetate is converted to acetayl CoA by acetyl CoA synthetase. Next, acetyl-CoA carboxylase (ACC) converts acetyl-CoA to malonyl-CoA in an irreversible reaction (Bauman and Davis, 1974). The production of malonyl-CoA is considered the rate-limiting step for de novo synthesis of milk FA and the activity of ACC is considerably lower than the activity of other FA synthesis enzymes.

Malonyl-CoA is the starting point for de novo FA synthesis and can be elongated, in two carbon increments, by the enzyme FA synthase (FAS). Although the mechanism responsible for regulating termination of FA synthesis, resulting in FA of differing chain length, are not completely known, changing the concentrations of acetyl CoA and malonyl CoA results in different FA profiles. For example, increasing the ratio of acetyl CoA to malonyl CoA produces greater concentrations of short- and medium-chain FA *in vitro* (Bauman and Davis, 1974).

Preformed milk fatty acids

The triglycerides contained within chylomicrons and VLDL in plasma are the primary source of milk FA >16 carbons in length taken up by the mammary gland (Palmquist, 2006) with NEFA only contributing FA to milk fat when concentrations of plasma NEFA are high, usually only occurring during periods of negative energy balance in early lactation (Miller et al., 1991). Mammary lipoprotein lipase (LPL) is the enzyme responsible for hydrolyzing triglycerides and enabling uptake of FA by the mammary gland. Mammary LPL hydrolyzes the FA in the *sn*-1 position and will not hydrolyze a 2-monoglyceride (Mendelson et al., 1977). Free FA and diacylglycerol are taken up by mammary epithelial cells and used for triglyceride synthesis in the mammary gland. Although the exact mechanism is unknown, uptake of FA across the plasma membrane is thought to be regulated by the gradient of FA concentration across the basolateral membrane and facilitated by membrane associated FA binding proteins (Glatz et al., 1997). Mammary LPL activity increases post calving while adipose LPL activity is reduced (Shirley et al., 1973) with this coordinated change controlled by the release of prolactin (Liesman et al., 1988).

Triglyceride synthesis

The endoplasmic reticulum of mammary epithelial cell is the site of triglyceride synthesis of milk fat. The primary pathway used for synthesis of triglycerides in the mammary gland is the *sn*-glycerol 3 phosphate pathway where both *de novo* and preformed FA are incorporated onto the glycerol-3 phosphate backbone (Dils, 1983). Glycerol phosphate acyl transferase (GPAT) is responsible for adding a fatty acyl-CoA to the *sn*-1 position of glycerol-3 phosphate and acyl glycerol phosphate acyl transferase (AGPAT) adds the second fatty acyl-CoA to the *sn*-2 position. The final fatty acyl-CoA is added to the *sn*-3 position forming the triglyceride. Milk fat is composed of 95% triglycerides, 2% diacylglycerol and small concentrations of phospholipids, cholesterol esters, and free FA (Jensen and Newberg, 1995). Due to the complexity of ruminant FA metabolism, over 400 different FA have been identified in milk fat (Jensen, 2002).

Although substantial variation does exist in the profile of FA in milk fat, the triglycerides in the mammary gland need to be able to be secreted into droplets that can be incorporated into milk and be fluid at body temperature (Dils, 1986). Therefore, the melting point of milk fat is relatively constant even with the variation in FA that differ in melting point. The melting point of triglycerides can be reduced three ways: 1) increasing unsaturated FA; 2) increasing short-chain FA; and 3) preferentially positioning short-chain FA at the *sn-3* position of the glycerol backbone (Dils, 1986). The location of FA on the glycerol backbone is not random with individual FA being preferentially located at different sn-positions. Short- and medium-chain FA are preferentially esterified to the *sn*-3 position. Over 98% of C4:0 and 93% of C6:0 are esterified on the *sn*-3 position. The *sn*-2 position contains greater than 50% of all C10:0 to C14:0 milk FA. Distribution of palmitic acid is fairly uniform between the *sn*-1 and *sn*-2 position while

stearic acid is primarily esterified to *sn*-1 at 56% with a smaller proportion esterified to *sn*-3. Oleic acid is esterified to either the *sn*-1 or *sn*-3 position of triglycerides (Jensen, 2002).

Milk Fat Depression Theories

Alterations in rumen biohydrogenation produce FA that are potent regulators of mammary synthesis of milk fat; increases in these biohydrogenation intermediates explain most cases of diet-induced MFD (Bauman et al., 2011). The identification that C18:1 *trans* FA were increased in cows with low milk fat lead Davis and Brown (1970) to consider that *trans* FA originated from incomplete biohydrogenation of unsaturated FA and may be partially responsible for milk fat depression. This biohydrogenation theory was further developed by Bauman and Griinari, who discovered that milk fat depression was caused by the inhibition of lipid synthesis in the mammary gland by specific FA intermediates that are produced by the incomplete biohydrogentation of unsaturated FA (Bauman et al., 2011). To date, three conjugated linoleic acid isomers; *trans*-10, *cis*-12 C18:2 (Baumgard et al., 2000), *cis*-10, *trans*-12 C18:2 (Sæbø et al., 2005) and *trans*-9, *cis*-11C18:2 (Perfield et al., 2005) have been identified that directly inhibit fat synthesis in the mammary gland.

Trans-10 C18:1 was identified as a potential contributor to diet-induced MFD through abomasal infusion of a mixture C18:1 isomers (Shingfield et al., 2009). Using principal component analysis, *trans*-7 C18:1 has been connected to lowered MFD at a greater potency than *trans*-10 C18:1 (Kadegowda et al., 2008). There is not consensus in the field of which FA directly cause MFD and which are elevated in milk, but are not directly linked to MFD. He and Armantano (2011) indicated that a high oleic safflower treatment resulted in a greater than three fold increase in *trans*-10 C18:1 from control without a significant decrease in milk fat yield. Abomasal infusions of *trans*-10 C18:1 resulted in no reduction in milk fat compared to a control

(Lock et al., 2007). This suggests that although a positive correlation exists between the concentration of *trans*-10 C18:1 and reduced milk fat yield, it is not a causal relationship.

An alternative theory is that increases in insulin caused by increased propionate produced in the rumen causes uptake of acetate and β-hydroxybutyrate into adipose tissue. While adipose tissue is responsive to changes in insulin levels, the mammary gland is not, the result would be that milk FA precursors would be preferentially partitioned to adipose tissue and away from the mammary gland, thus reducing milk fat synthesis. Early work done using hyperinsulinemic-euglycymic clamps while abomasally infusing glucose found no effect of insulin on milk fat yield (Griinari et al., 1997). Whereas, Corl (2006) using hyperinsulinemic-euglycemic clamps reported reduced milk fat yield through a reduction in preformed milk FA while *de novo* synthesized milk FA yield remained the same. Typically, diet-induced MFD causes a reduction in *de novo* synthesized milk FA to a greater extent then preformed milk FA, indicating that tissue competition for lipogenic intermediates may not be the cause for diet-induced MFD. We are uncertain if dietary factors that increase insulin would produce the same results on milk fat synthesis as hyperinsulinemic-euglycemic clamps. In several studies where high concentrate diets were fed, plasma insulin concentrations were elevated and milk fat synthesis was reduced (Grum et al., 1996; van Knegsel et al., 2007). However, we are uncertain if the effect of insulin on milk fat yield was causative or merely correlated.

Regulation of Milk Fatty Acids

Sutton (1932) first observed changes in milk fat as a result of supplementing corn oil, with added corn oil increasing the iodine value of milk fat, indicating a greater proportion of unsaturated FA in milk fat. Feeding unsaturated FA has an inhibitory effect on *de novo* synthesis in the mammary gland reducing the yields of short and medium FA in milk fat (Grummer, 1991).

He and Armentano (2011) summarized changes in milk FA yield from nine oil supplementation studies and reported that when unprotected vegetable oil was supplemented in low fat diets it resulted in a reduction in de novo synthesized milk FA yield and increased or maintained preformed milk FA yield. This change in FA profile indicates that *de novo* synthesis may be inhibited to a greater extent than the incorporation of preformed FA into milk fat by increasing unsaturated fat. However, a relationship exists between *de novo* synthesized and 18-carbon FA, as a drastic reduction in *de novo* synthesized milk FA can limit the incorporation of 18-carbon FA into triglycerides (Glasser et al., 2008). This relationship between *de novo* and preformed FA can be observed in short term infusions of *trans*-10, *cis*-12 C18:2 resulting in a decrease in milk fat yield by reducing both *de novo* and preformed milk FA (Baumgard et al. 2002; Harvatine and Bauman, 2006).

Feeding diets designed to cause MFD causes a decrease in mRNA abundance and enzymatic activity of several key mammary lipogenic enzymes (Piperova et al., 2000; Peterson et al., 2003). During diet-induced MFD the enzymes down regulated included ACC, FAS, GPAT, AGPAT and mammary LPL (Peterson et al., 2003). The reduction in abundance of mRNA and activity of these enzymes would reduce the inclusion of both *de novo* and preformed milk FA. During diet induced MFD the gene expression of sterol response element binding protein-1 (SREBP1) decreases with decreasing milk fat yield (Harvatine and Bauman 2006). SREBP1 is a global transcription factor that regulates both *de novo* synthesis and incorporation of preformed FA, highlighting the regulation in the mammary gland for milk fat synthesis. Bionaz and Loor (2008) recently suggested that the increase in peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear receptor protein that regulates the expression of genes, at parturition corresponds to increases in gene expression for FA uptake, transport, and synthesis,

potentially indicating a role for PPARγ in the regulation of milk fat synthesis. However, the role of PPARγ throughout lactation on milk fat synthesis and specifically diet-induced MFD is currently unknown.

Energy Partitioning

Abomasal infusion of *trans*-10, *cis*-12 C18:2 resulted in increased expression of genes related to lipid synthesis in adipose tissue (Harvatine et al., 2009) consistent with observations that cows producing reduced milk fat concentrations have increased body condition score (Van Soest, 1963). In mid-lactation, when cows are in a positive energy balance, an increase in the production of *trans*-10, *cis*-12 C18:2 in the rumen, causing a reduction in milk fat yield, would lead to a reduction in milk energy output. If increasing the production of *trans*-10, *cis*-12 C18:2 had no substantial effect on intake or digestibility of other nutrients, the increased energy would be retained as body tissue. The changes in energy output as milk fat and increase in energy as body tissue potentially indicates the role of *trans*-10, *cis*-12 C18:2 as a mediator of energy partitioning. Furthermore, it is well established that insulin inhibits lipolysis while stimulating lipogenesis in adipose tissue (Bauman, 2000). Infusions of excess glucose, increasing plasma insulin, resulted in no effect on milk energy output; however, the glucose infusions increased BW and back fat thickness in mid lactation dairy cows (Al-Trad et al., 2009). It is unknown if changes in energy partitioning due to changes in milk energy output are a result of the specific action of *trans*-10, *cis*-12 C18:2 on adipose tissue or whether the reduction in gene expression of lipogenic genes in the mammary gland during MFD increases the availability of FA for uptake by adipose tissue. The changes in gene expression in adipose tissue may be a result of increased availability of FA because less FA are taken up by the mammary gland. Furthermore, it is

unknown what role, if any, insulin plays on partitioning energy during diet-induced MFD or reduced milk energy output situations in mid-lactation dairy cattle.

Using equations from the NRC for NE requirement of BW gain and changes in milk energy output, we produced theoretical changes in both BW and BCS as a result of reduced milk fat concentration (Figure 2.1). The model used cows at 180 DMI producing 45 kg/d of milk and weighing 680 kg with a BCS of 3.0. Milk yield decreased by 2% per week up to 300 DIM to mimic a normal lactation curve while change in BW and BCS were adjusted per NRC equations. With a modest reduction in milk fat concentration of 0.2% units (i.e. 3.7% to 3.5% reduction in milk fat concentration) the increase in BW from the change in milk fat output over 120 d is 12 kg with an increase in BCS of approximately 0.20 pts. Whereas, with a more severe change in milk fat concentration of 0.6% units (i.e. 3.7% to 3.1% reduction in milk fat concentration) the increase in BW due to the change in milk fat output over 120 d is 40 kg with an increase in BCS of approximately 0.75 pts. Currently, the exact mechanisms that cause the repartitioning of energy during MFD are unknown. However, the reduction in milk fat yield with no change in dietary energy intake results in excess energy available for body tissue, potentially deposition of adipose tissue.

Conclusions

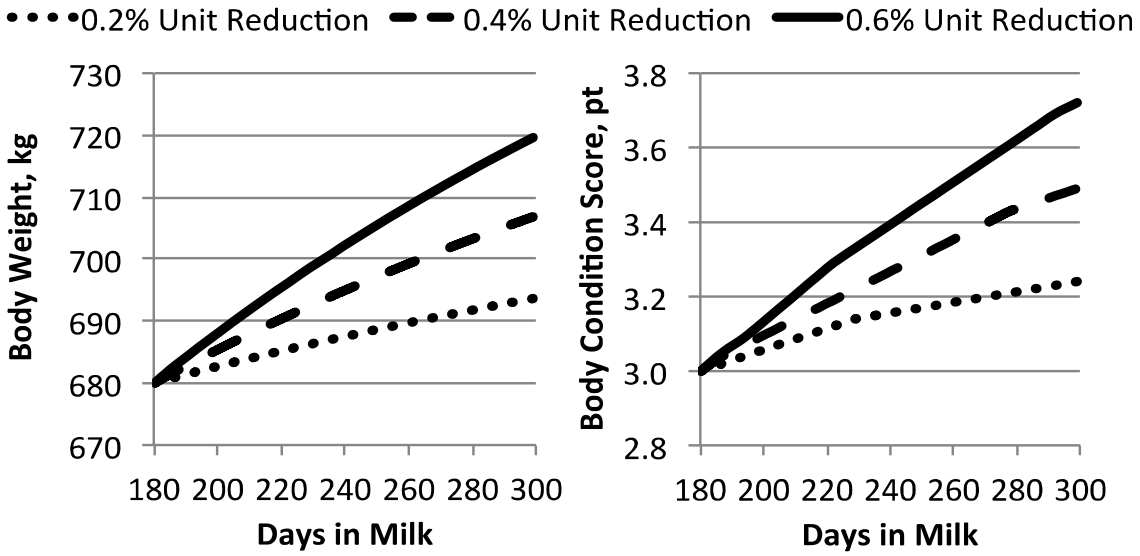
Although considerable research has been done on FA metabolism in dairy cows there are still substantial gaps in our understanding. The following research chapters try to explore these areas and contribute to our current understanding of FA metabolism in lactating dairy cows. We used meta-analysis to determine individual FA digestibility estimates compiling all available published papers. Additionally we were able to derive prediction equations to determine which dietary factors impact individual FA digestibility to better understand the biology of FA

digestion. Two research trials evaluated the effects of diets on energy partitioning to determine if we can feed diets that will increase the yield of milk fat and reduce the deposition of body fat. We compared a non-esterified to an esterified FA to determine if differences exist in biohydrogenation intermediates and milk fat synthesis. Finally, we fed increasing amounts of a stearic acid-enriched supplement to determine if there was an optimal level of stearic acid for FA digestibility and incorporation into milk FA. These research chapters although broad in objectives all deal with increasing our understanding of metabolism and digestion of FA in lactating dairy cattle.

APPENDIX

APPENDIX

Figure 2.1. Theoretical impact of reduced milk fat on BW and BCS gain using NRC equations.



REFERENCES

REFERENCES

Al-Trad, B., K. Reisberg, T. Wittek, G. B. Penner, A. Alkaassem, G. Gabel, M. Furil, and J. R. Aschenbach. 2009. Increasing intravenous infusions of glucose improve body condition but not lactation performance in midlactation dairy cows. J. Dairy Sci. 92:5645-5658.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598-1624.

Allen, M.S., B.J. Bradford, and K.J. Harvatine. 2005. The cow as a model to study food intake regulation. Annu. Rev. Nutr. 25:523–547.

Andrews, R. J. and D. Lewis. 1970. The utilization of dietary fats by ruminants. II. The effect of fatty acid chain length and unsaturation on digestibility. J. Agri. Sci. 75: 55-60.

Bauchart, D., Verite, R., Remond, B. 1984. Long-chain fatty acid digestion in lactating cows fed fresh grass from spring to autumn. Can. J. Anim. Sci. 64:330–331.

Bauman, D. E. 1976. Intermediary metabolism of adipose tissue. Fed. Proc. 35:2308-2313.

Bauman, D. E. 2000. Regulation of nutrient partitioning during lactation: Homeostasis and homeorhesis revisited. In: P.B. Cronje (Ed.) Ruminant Physiology, Digestion, Metabolism, Growth and Reproduction. Proceedings 9th International Symposium on Ruminant Physiology. Commonwealth Agricultural Bureau International, New York, NY; 311–318.

Bauman, D. E., and C. L. Davis. 1974. Biosynthesis of milk fat. In: B. L. Larson, and V. R. Smith (Eds.) Lactation: A Comprehensive Treatise. Vol. 2. Academic Press, New York, pp. 31-75.

Bauman, D. E., K. J. Harvatine, and A. L. Lock. 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Ann. Rev. Nutr. 31: 299-319.

Baumgard, L. H., B. A. Corl, D. A. Dwyer, A. Sæbø, and D. E. Bauman. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Physiol. Regul. Integr. Comp. Physiol. 278:R179-R184.

Baumgard, L. H., E. Matitashvili, B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002. Trans-10, cis-12 conjugated linoleic acid decreases lipogenic rates and genes involved in milk lipid synthesis in dairy cows. J. Dairy Sci. 85:2155-2163.

Bell, A.W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J Anim Sci 73:2804-2819.

Bionaz, M. and J. J. Loor. 2008. Gene networks driving bovine milk fat synthesis during the lactation cycle. BMC Genomics 9:366.

Chalupa, W., B. Vecchiarelli, A. E. Elser, D. S. Kronfeld, D. Sklan, and D. L. Palmquist. 1986. Ruminal fermentation in vivo as influenced by long-chain fatty acids. J. Dairy Sci. 69:1293-1301.

Chang, J. H. P., D. K. Lunt, and S. B. Smith. 1992. Fatty acid composition and fatty acid elongase and stearoyl-CoA desaturase activities in tissues of steers fed high oleate sunflower seed. J. Nutr. 122: 2074-2080.

Contreras, G. A., N. J. O'Boyle, T. H. Herdt, and L. M. Sordillo. 2010. Lipomobilization in periparturient dairy cows influences the composition of plasma nonesterified fatty acids and leukocyte phospholipid fatty acids. J. Dairy Sci. 93:2508-2516.

Cooke, K. M., J. K. Bernard, C. D. Wildman, J. W. West and A. H. Parks. 2007. Performance and ruminal fermentation of dairy cows fed whole cottonseed with elevated concentrations of free fatty acids in the oil. J. Dairy Sci. 90:2329-2334.

Corl, B. A., S. T. Butler, W. R. Butler, and D. E. Bauman. 2006. Short communication: regulation of milk fat yield and fatty acid composition by insulin. J. Dairy Sci. 89:4172-4175.

Davis, C. L., and R. E. Brown. 1970. Low-fat milk syndrome. In A. T. Phillipson (Ed.), Physiology of Digestion and Metabolism in the Ruminant. Oriel Press, Newcastle upon Tyne, UK. Pp. 545-565.

Dils, R. R. 1983. Milk fat synthesis. In: T. B. Memphan (Ed.), Biochemistry of lactation. Elsevier, Amsterdam. 1983: 141.

Dils, R. R. 1986. Comparative aspects of milk fat synthesis. J. Dairy Sci. 69:904-910.

Douglas, G. N., J. Rehage, A. D. Beaulieu, A. O Bahaa, and J. K. Drackley. 2007. Prepartum nutrition alters fatty acid composition in plasma, adipose tissue, and liver lipids of periparturient dairy cows. J. Dairy Sci. 90:2941-2959.

Doreau, M. and A. Ferlay. 1994. Digestion and utilization of fatty-acids by ruminants. Anim. Feed Sci. Technol. 45:379-396.

Drackley, J. K. 1999. Biology of dairy cows during the transition period: the final frontier? J. Dairy Sci. 82:2259-2273.

Drackley, J. K., T. H. Klusmeyer, A. M. Trusk, and J. H. Clark. 1992. Infusion of long-chain fatty acids varying in saturation and chain length into the abomasum of lactating dairy cows. J. Dairy Sci. 75:1517-1526.

Dryden, F. D. and J. A. Marchello. 1973. Influence of dietary fats upon carcass lipid composition in the bovine. J. Anim. Sci. 37: 33-39.

Dubuc, J., D. DuTremblay, M. Brodeur, T. Duffield, R. Bagg, J. Baril, and L. DesCôteaux. 2009. A randomized herd-level field study of dietary interactions with monensin on milk fat percentages in dairy cows. J. Dairy Sci. 92:777-781.

Duckett, S. K., S. L. Pratt, and E. Pavon. 2008. Corn oil or corn grain supplementation to steers grazing endophyte-free tall fescue. II. Effects on subcutaneous fatty acid content and lipogenic gene expression. J. Anim. Sci. 87:1120-1128.

Eastridge, M. L., and J. L. Firkins. 2000. Feeding tallow triglycerides of different saturation and particle size to lactating dairy cows. Anim. Feed Sci. Tech. 83:249-259.

Elliott, J. P., T. R. Overton, and J. K. Drackley. 1994. Digestibility and effects of three forms of mostly saturated fatty acids. J. Dairy Sci. 77:789-798.

Emery, R.S., J.S. Liesman, and T.H. Herdt. 1992. Metabolism of long-chain fatty acids by ruminant liver. J. Nutr. 122:832-837.

Enjalbert, F., M. C. Nicot, C. Bayourthe, and R. Moncoulon. 2000. Effects of duodenal infusions of palmitic, stearic, or oleic acids on milk composition and physical properties of butter. J. Dairy Sci. 83:1428–1433.

Freeman, C. P. 1969. Properties of fatty acids in dispersions of emulsified lipid and bile salt and the significance of these properties in fat absorption in the pig and the sheep. British J. Nutr. 23:249-263.

Freeman, C. P. 1984. Digestion, absorption and transport of fats - non-ruminant animals. In: Fats in Animal Nutrition. pp. 105-122. Butterworths, London, UK.

Glasser, F., A. Ferlay, M. Doreau, P. Schmidely, D. Sauvant, and Y. Chilliard. 2008. Long-chain fatty acid yield in relation to duodenal flows and de novo synthesis. J. Dairy Sci. 91: 2771-2785.

Glatz, J. F., J. J. Luiken, F. A. van Nieuwenhoven, and G. J. Van der Vusse. 1997. Molecular mechanism of cellular uptake and intracellular translocation of fatty acids. Prostaglandins Leukot. Essent. Fatty Acids. 57:3-9.

Grant, R.J., V. F Colenbrander, and D. R. Mertens. 1990. Milk fat depression in dairy cows: role of particle size of alfalfa hay. J. Dairy Scci. 73:1823-1833.

Graulet, B., D. Gruffat, D. Durand, and D. Bauchart. 1998. Fatty acid metabolism and very low density lipoprotein secretion in liver slices from rats and preruminant calves. J. Biochem. 124:1212-1219.

Griinari, J. M., M. A. McGuire, D. A. Dwyer, D. E. Bauman, D. M. Barbano, and W. A. House. 1997. The role of insulin in the regulation of milk protein synthesis in dairy cows. J. Dairy Sci. 80:2361-2371.

Grum, D. E., J. K. Drackley, L. R. Hansen, and J. D. Cremin, Jr. 1996. Production, digestion, and hepatic lipid metabolism of dairy cows fed increased energy from fat or concentrate. J. Dairy Sci. 79:1836-1849.

Grummer, R. R. 1988. Influcence of prilled fat and calcium salt of palm oil fatty acids on ruminal fermentation and nutrient digestibility. J. Dairy Sci. 71:117-123.

Grummer, R.R. 1991. Effect of feed on the composition of milk fat. J. Dairy Sci. 74: 3244-3257.

Harfoot, C. G., and G. P. Hazlewood. 1997. Lipid metabolism in the rumen. Page 382 in the Rumen Microbial Ecosystem. 2nd ed. P. N. Hobson and C. S. Stewart, ed. Blackie Academic & Professional, New York.

Harvatine, K. J., and D. E. Bauman. 2006. Update on milk fat: cellular mechanism for inhibition of milk fat synthesis by rumen biohydrogenation intermediates. Proc. Cornell Nutr. Conf. pp. 67-73.

Harvatine, K. J., J. W. Perfield II, and D. E. Bauman. 2009. Expression of enzymes and key regulators of lipid synthesis is upregulated in adipose tissue during CLA-induced milk fat depression in dairy cows. J. Nutr. 139:821-825.

He, M., and L. E. Armentano. 2011. Effect of fatty acid profile in vegetable oils and antioxidant supplementation on dairy cattle performance and milk fat depression. J. Dairy Sci. 94: 2481-2491.

Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76: 3851-3863.

Jenkins, T. C. 2000. Feeding oleamide to lactating jersey cows effects on lactation performance and milk fatty acid composition. J. Dairy Sci. 83:332-337.

Jenkins, T. C. and D. L. Palmquist. 1982. Effect of added fat and calcium on in vitro formation of insoluble fatty acid soaps and cell wall digestibility. J. Anim. Sci. 55: 957-963.

Jenkins, T. C. and D. L. Palmquist. 1984. Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations. J. Dairy Sci. 67: 978-986.

Jenkins, T. C., A. A. AbuGhazaleh, S. Freeman, and E. J. Thies. 2006. The production of 10-hydroxystearic and 10-ketostearic acids is an alternative route of oleic acid transformation by the ruminal microbiota in cattle. J. Nutr. 136:926-931.

Jensen, R. G. 2002. Invited review: the composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 85:295-350.

Jensen, R. G., and D. S. Newberg. 1995. Bovine milk lipids. In R. G. Jensen (Ed.) Handbook of Milk Composition. Academic Press, San Diego, CA. Pp. 543-575.

Kadegowda, A. K. G., L. S. Piperova, and R. A. Erdman. 2008. Principal component and multivariate analysis of milk long-chain fatty acid composition during diet-induced milk fat depression. J. Dairy Sci. 91:749-759.

Khan, N. A., Cone, J. W., Pellikaan, W. F., Khan, M. A., Struik, P. C., Hendriks, W. H. 2011. Changes in fatty acid content and composition in silage maize during grain filling. J. Sci. Food Agric. 91, 1041–1049.

Khan, N. A., J. W. Cone, V. Fievez, W. H. Hendriks. 2012. Causes of variation in fatty acid content and composition in grass and maize silages. Anim. Feed Sci. Technol. 174:36-45.

Khorasani, G. R., G. de Boer, P. H. Robinson, and J. J. Kennelly. 1992. Effect of canola fat on ruminal and total tract digestion, plasma hormones, and metabolites in lactating dairy cows. J. Dairy Sci. 75:492-501.

Kim, Y. J., R. H. Liu, J. L. Rychlik, and J. B. Russell. 2002. The enrichment of a ruminal bacterium (Megasphaera elsdenii YJ-4) that produces the trans-10, cis-12 isomer of conjugated linoleic acid. J. Appl. Microbiol. 92:976-982.

Klein, C. M., J. C. Ploetz, T. C. Jenkins, and A. L. Lock. 2013. Variability in the concentrations of free and esterified fatty acids in corn silage and byproduct feeds. J. Dairy Sci. 96 (Suppl.1):150. *Abstract*.

Lee, Y. J., and T. C. Jenkins. 2011. Biohydrogenation of linoleic acid to stearic acid by the rumen microbial population yields multiple intermediate conjugated diene isomers. J. Nutr. 141:1145-1450.

Lock, A. L., and D. E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids 39:1197–1206.

Lock, A. L., C. Tyburczy, D. A. Dwyer, K. J. Harvatine, F. Destaillats, Z. Mouloungui, L. Candy, and D. E. Bauman. 2007. *Trans*-10 octadecenoic acid does not reduce milk fat synthesis in dairy cows. J. Nutr. 137:71-76.

Lock, A.L., C.L. Preseault, J.E. Rico, K.E. DeLand, and M.S. Allen. 2013. Feeding a C16:0-enriched fat supplement increased the yield of milk fat and improved conversion of feed to milk. J. Dairy Sci. 96:6650-6659.

Loften, J. R., J. G. Linn, J. K. Drackley, T. C. Jenkins, C. G. Soderholm, and A. F. Kertz. 2014. Invited review: palmitic and stearic acid metabolism in lactating dairy cows. J. Dairy Sci. 97:1-14.

Maia, M. R. G., L. C. Chaudhary, C. S. Bestwick, A. J. Richardson, N. McKain. T. R. Larson. I. A. Graham, and R. J. Wallace. 2010. Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, *Butyrivibrio fibrosolvens*. BMC Microbiology. 10:52-61.

McNamara, J. P., and J. K. Hillers. 1989. Regulation of bovine adipose tissue metabolism during lactation. Relationships of lipid synthesis and lipolysis with energy intake and utilization. J. Dairy Sci. 72:407-418.

Mendelson, C. R., O. Zinder, J. E. Blanchette-Mackie, S. S. Chernick, and R. O. Scow. 1977. Lipoprotein lipase and lipid metabolism in mammary gland. J. Dairy Sci. 60:666-676.

Miller, P. S., B. L. Reis, C. C. Calvert, E. J. DePeters, and R. L. Baldwin. 1991. Relationship of early lactation and bovine somatotropin on nutrient uptake by cow mammary gland. J. Dairy Sci. 74:3800-3806.

Moore, J. H., R. C. Noble, W. Steele, and J. W. Czerkawski. 1969. Differences in the metabolism of esterified and unesterified linoleic acid by rumen micro-organisms. Br. J. Nurt. 23: 869-878.

Moore, J. H. and W. W. Christie. 1984. Digestion, absorption and transport of fats in ruminant animals. In: J. Wiseman (Ed.) Fats in Animal Nutrition. pp. 123-149. Butterworths, London, UK.

Mosley, E. E., G. L. Powell, M. B. Riley, and T. C. Jenkins. 2002. Microbial biohydrogenation of oleic acid to *trans* isomers *in vitro*. J. Lipid Res. 43:290-296.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Acad. Sci., Washington D.C.

Noble, R. C. 1981. Digestion, transport and absorption of lipids. In W. W. Christie (Ed.) Lipid Metabolism in Ruminant Animals. pp. 57-93. Pergamon Press Ltd. Oxford, UK.

Noble, R.C., J. H. Moore, and C. G. Harfoot. 1974. Observations on the pattern of biohydrogenation of esterified and unesterified linoleic acid in the rumen. Br. J. Nutr. 31: 99-108.

Oba, M. and M. S. Allen. 2003. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. J. Dairy Sci. 86:174-183.

Onetti, S. G., R. D. Shaver, M. A. McGuire, D. L. Palmquist, and R. R. Grummer. 2002. Effect of supplemental tallow on performance of dairy cows fed diets with different corn silage:alfalfa silage ratios. J. Dairy Sci. 85:632-641.

Palmquist, D. L. 1984. Calcium soaps of fatty acids with varying unsaturation as fat supplements for lactating cows. Can. J. Anim. Sci. 64:240-241.

Palmquist, D. L. 1991. Influence of source and amount of dietary fat on digestibility in lactating cows. J. Dairy Sci. 74:1354-1360.

Palmquist, D. L., and T. C. Jenkins. 1980. Fat in lactation rations: review. J. Dairy Sci. 63:1-14.

Pantoja, J., J. L. Firkins, M. L. Eastridge. 1995. Site of digestion and milk production by cows fed fats differing in saturation, esterification, and chain length. J. Dairy Sci. 78:2247-2258.

Perfield II, J., A. Lock, A. Sæbø, J. M. Griinari, and D. E. Bauman. 2005. *Trans*-9, *cis*-11 conjugated linoleic acid (CLA) reduces milk fat synthesis in lactating dairy cows. J. Dairy Sci. 88:211.

Peterson, D. G., E. A. Matitashvili, and D. E. Bauman. 2003. Diet-induced milk fat depression in dairy cows results in increased *trans*-10, *cis*-12 CLA in milk fat and coordinate suppression of mRNA abundance for mammary enzymes involved in milk fat synthesis. J. Nutr. 133:3098-3102.

Piantoni, P., A. L. Lock, and M. S. Allen. 2013. Palmitic acid increased yields of milk and milk fat and nutrient digestibility across production level of lactating cows. J. Dairy Sci. 96:7143-7154.

Piperova, L. S., B. B. Teter, I Bruckental, J. Sampugna, S. E Mills, M. P. Yarawecz, J. Fritsche, K. Ku. and R. A. Erdman. 2000. Mammary lipogenic enzyme activity, trans fatty acids and conjugated linoleic acids are altered in lactating dairy cows fed a milk fat-depressing diet. J. Nutr. 130:2568-2574.

Relling, A. E., and C. K. Reynolds. 2007. Feeding rumen-inert fats differing in their degree of saturation decreases intake and increases plasma concentrations of gut peptides in lactating dairy cows. J. Dairy Sci. 90:1506-1515.

Reynolds, C. K., P. C. Aikman, B. Lupoli, D. J. Humphries, and D. E. Beever. 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. J. Dairy Sci. 86:1201-1217.

Rico, J. E., M. S. Allen, and A. L. Lock. 2013. Compared with stearic acid, palmitic acid increased the yield of milk fat and improved feed efficiency across production level of cows. J. Dairy Sci. 97:1057-1066.

Rouse, R. H., 2003. Feeding fats quality and handling characteristics. Multi-state Poultry Meeting, May 20-22, 2003.

Sæbø, A., P. C. Sæbø, J. M. Griinari, and K. J. Shingfield. 2005. Effect of abomasal infusions of geometric isomers of 10, 12 conjugated linoleic acid on milk fat synthesis in dairy cows. Lipids. 40: 823-832.

Shingfield K. J., A. Saebø, P. C. Saebø, V. Toivonen, and J. M. Griinari. 2009. Effect of abomasal infusions of a mixture of octadecenoic acids on milk fat synthesis in lactating cows. J. Dairy Sci. 92:4317-4329.

Shirley, J. E., R. S. Emery, E M. Convey, and W. D. Oxender. 1973. Enzymatic changes in bovine adipose and mammary tissue, serume and mammary tissue hormonal changes with initiation of lactation. J. Dairy Sci. 56: 569-574.

Smith, W. A., and B. Harris, Jr. 1993. The influence of type of forage on the production response of lactation dairy cows supplemented with different types of fat. Prof. Anim. Sci. 8:7-21.
Spiehs, M. J., M. H. Whitney, and G. C. Shurson. 2002. Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. J. Anim. Sci. 80:2639-2645.

St. John, L. C., D. K. Lunt, and S. B. Smith. 1991. Fatty acid elongation and desaturation enzyme activities of bovine liver and subcutaneous adipose tissue microsomes. J. Anim. Sci. 69:1064-1073.

St. Pierre, N. Assessing the value of components on the farm level. Proceedings from 22nd Discover Conference: Milk Components: Opportunities for Maximizing Farm Gate Returns. October 10-13, 2011. Itasca, IL.

Steele, W., and J. H. Moore. 1968. The effects of a series of saturated fatty acids in the diet on milk fat secretion in the cow. J. Dairy Res. 35:361-370.

Stein, H. H., and G. C. Shurson. 2009. Board-invited review: The use and application of distillers grains with solubles (DDGS) in swine diets. J. Anim. Sci. 87:1292-1303.

Stewart, C. S. 1977. Factors affecting the cellulolytic activity of rumen contents. Appl. Environ. Microb. 33:497-502.

Sumner, J. M., and J. P. McNamara. 2007. Expression of lipolytic genes in the adipose tissue of pregnant and lactating Holstein dairy cattle. J. Dairy Sci. 90:5237-5246.

Sutton, T. S., J. B. Brown, E. W. Johnston. 1932. The effect of corn oil on milk and butter fat production and on the composition of butter fat in the dairy cow. J. Dairy Sci. 15:209-211.

van Knegsel, A. T. M., H. van den Brand, E. A. M. Graat, J. Dijkstra, R. Jorritsma, E. Decuypere, S. Tamminga and B. Kemp. 2007b. Dietary energy source in dairy cows in early lactation: metabolites and metabolic hormones. J. Dairy Sci. 90:1477-1485.

Van Soest, P. J. 1963. Ruminant fat metabolism with particular reference to factors affection low milk fat and feed efficiency. A review. J. Dairy Sci. 46:204-216.

Weisbjerg, M. R., C. F. Borsting, and T. Hvelplund. 1992. Fatty acid metabolism in the digestive tract of lactating cows fed tallow in increasing amounts at two feed levels. Acta. Agric. Scand., Sect. A, Animal Sci. 42:106-114.

Weiss, W. P., and D. J. Wyatt. 2004. Digestible energy values of diets with different fat supplements when fed to lactating dairy cows. J. Dairy Sci. 87:1446-1454.

Yeaman, S. J. 1994. The multifunctional role of hormone-sensitive lipase in lipid metabolism. Adv. Enzyme Regul. 34:355-370.

CHAPTER 3

INTESTINAL DIGESTIBILITY OF LONG CHAIN FATTY ACIDS IN LACTATING DAIRY COWS: A META ANALYSIS

INTRODUCTION

Digestion and metabolism of FA in ruminants has gained interest from both a research and industry perspective due to several factors. First, dietary FA supplements have been used to increase the metabolizable energy density of rations in lactating dairy cattle (Jenkins and Jenny, 1989; Chilliard, 1993). Depending on stage of lactation and energy balance, an increase in metabolizable energy could increase milk production, increase milk fat yield, and/or increase body reserves as long as dietary FA supplements do not reduce dry matter intake or negatively impact fiber digestibility. Second, increased recognition of the bioactive properties of specific FA has led to increased interest in fat supplementation in ruminant diets. For example, supplemented omega-3 FA improved reproductive performance in early lactation animals (Abayasekara and Wathes, 1999; Ambrose et al., 2006) and C16:0 supplementation improved milk fat yield compared to C18:0 supplementation (Rico et al., 2013). Third, basal diets can vary dramatically in FA profile and content; therefore, research on FA digestion and metabolism is important even when no supplemental fat is included in diets. The ability to understand and model FA digestibility will be useful for diet formulation strategies and provide information for optimal FA supplementation.

Metabolism of FA begins in the rumen through the processes of hydrolysis and biohydrogenation. Hydrolysis of fat occurs when triglycerides and glycolipids are converted into glycerides and FA by microbial lipases (Jenkins, 1993). Unsaturated FA, which are toxic to certain rumen microbes, undergo biohydrogenation to form saturated FA (Maia et al., 2010).

Because of these changes in the rumen, feed-to-feces digestibility estimates for individual FA, appropriate for nonruminants, are not applicable for ruminants. When they leave the rumen, the majority of FA are saturated, with no significant modification or absorption in the omasum or abomasum (Moore and Christie, 1984). A small amount of triglycerides and phospholipids exit the rumen and are hydrolyzed in the small intestine (Doreau and Ferlay, 1994). The low pH of the digesta entering the duodenum and the high concentration of taurocholic acid in the bile promote the solubility of FA (Harrrison and Leat, 1975). Pancreatic and bile secretions are added to the digesta in the duodenum, and are responsible for solubilizing FA (Bauchart, 1993). Pancreatic and bile secretions are at an apparent steady state in ruminants and not subject to large fluctuations dependent on meal composition (Noble, 1981). A majority of absorption of FA occurs in the jejunum; therefore, in order to accurately measure the digestibility of individual FA, duodenal flows of FA are needed to determine the total amount of each FA available to the ruminant. FA may also become biohydrogenated in the large intestine (Pantoja et al., 1996), which may result in an over prediction of unsaturated FA digestibility and an under prediction of saturated FA digestibility. Therefore, there may be differences in digestibility estimates when digesta samples are collected from the ileum compared to feces.

Previous reports have indicated that the digestibility of saturated FA decreases with increasing chain length and unsaturation increases digestibility of FA (Steele and Moore, 1968; Andrews and Lewis, 1970). Digestibility of FA might decrease as more fat is included in the diet (Palmquist, 1991; Weisbjerg et al., 1992). Specifically, the reduction in digestibility as fat is included in diets has been linked to the lower digestibility of C18:0 (Weisbjerg et al., 1992). Based on previous reports of differences in long chain FA digestibility and the importance of correctly modeling FA to understand the metabolism of FA in dairy cows, our objective was to

perform a meta-analysis to determine intestinal digestibility of individual long chain FA in lactating dairy cattle. Secondarily, our objective was to use meta-regression to evaluate whether digestibility estimates of individual FA differed dependent on FA type, concentration, and other potential biological drivers.

MATERIALS AND METHODS

Our initial selection criteria for inclusion into the dataset were studies that reported individual FA digestibility measurements in lactating dairy cows using duodenally cannulated cows. We collected data from twenty studies representing 80 treatment means that were published in peer-reviewed journals. Five studies were removed because no measurement of variation was reported; a measurement of variation is required in order to properly weight the studies (Borenstein et al., 2009). Of the remaining 15 studies with 62 treatments, one treatment was removed because it contained a saturated triglycerides as partially hydrogenated tallow (Pantoja et al., 1996), which has been previously reported as poorly digested in ruminants (Elliott et al., 1996; Weiss and Wyatt, 2004). The 61 remaining treatment means included 46 containing supplemental fat and 16 containing no supplemental fat source. A list of studies and treatments reported is in Table 3.1. Treatments were separated into categories for supplemental fat type including control diets (no supplemental fat added) and diets supplemented with animal-vegetable fat, calcium-salts of FA, tallow, vegetable oil, seed meal, whole seeds, and other.

Comprehensive Meta-Analysis v 2.0 software was used to analyze the data (Biostat, Englewood, NJ). Digestibility estimates were obtained using a random effects model, which accounted for variation both within and among studies. Studies were weighted based on the inverse of the sum of both the within and among study variance. Digestibility of C18:0 was used as the comparison to all other FA because of equal saturation compared to C16:0 and equal chain

length compared to C18:1, C18:2, and C18:3. If digestibility estimates were reported for individual isomers of unsaturated FA, a weighted average of digestibility based on flow through the duodenum was used. For example, if both *cis* and *trans* isomers of C18:1 were reported, we combined the data to represent a single value for C18:1 digestibility.

Our data set included digestibility estimates from both ileal and fecal collections. Using fecal digestion does not account for biohydrogenation that could occur in the large intestine. Only 4 studies with 18 treatment comparisons used ileally cannulated cows to measure FA digestibility in the small intestine. Therefore, we further examined if site of collection impacted digestibility estimates for individual FA by evaluating studies that used similar methods of collection together.

Figures of trial-adjusted values for digestibility of FA were developed based on SAS code described by St-Pierre (2001). Including the random effect of study in the model accounts for variation among trials and improves the accuracy of equations produced.

Meta-regression was used to determine dietary variables or measurements taken from the duodenum that influence digestibility estimates using JMP version 10.0.2 (SAS Institute, Cary, NC). Studies were weighted by the inverse of the standard error squared (St.-Pierre, 2001; Borenstein et al., 2009). In studies from which the standard error was less than half of the mean standard error, the standard error was set to half of the mean standard error across all studies to prevent over weighting (Firkins et al., 2001). The original model including all variables for duodenal flow was:

$Y = \beta_0 + \text{Trial} + \beta_1\text{Total Flow (g/d)} + \beta_2\text{C16:0 (\% of FA)} + \beta_3\text{C16:0}^2\text{ (\% of FA)} + \beta_4\text{C18:0 (\% of FA)} + \beta_5\text{C18:0}^2\text{ (\% of FA)} + \beta_6\text{C18:1 (\% of FA)} + \beta_7\text{C18:1}^2\text{ (\% of FA)} + \beta_8\text{C18:2 (\% of FA)} + \beta_9\text{C18:2}^2\text{ (\% of FA)} + \beta_{10}\text{C18:3 (\% of FA)} + \beta_{11}\text{C18:3}^2\text{ (\% of FA)} + \text{error}$

Where the individual FA are % of total FA flow through the duodenum.

The original model including all dietary variables was:

$Y = \beta_0 + Trial + \beta_1 DMI\ (kg/d) + \beta_2 DMI^2\ (kg/d) + \beta_3 Forage\ (\%\ of\ Diet) + \beta_4 Forage^2\ (\%\ of\ Diet) + \beta_5 DMI\ (kg/d) \times Forage\ (\%\ of\ Diet) + \beta_6 C16{:}0\ (\%\ of\ diet\ DM) + \beta_7 C18{:}0\ (\%\ of\ diet\ DM) + \beta_8 C18{:}1\ (\%\ of\ diet\ DM) + \beta_9 C18{:}2\ (\%\ of\ diet\ DM) + \beta_{10} C18{:}3\ (\%\ of\ diet\ DM) + \beta_{11} FA\ in\ Y^2\ (\%\ of\ diet\ DM) + \beta_{12} DMI\ (kg/d) \times FA\ in\ Y\ (\%\ of\ diet\ DM) + \beta_{13} Forage\ (\%\ of\ Diet) \times FA\ in\ Y\ (\%\ of\ diet\ DM) + Fat\ type + error.$

Where FA in Y would correspond to the FA digestibility that is being estimated. For example if Y = C16:0 digestibility then FA in Y = C16:0 as % of diet DM.

Variables were removed from the model using backwards elimination with significance criteria of $P < 0.05$. For variables that included interactions or quadratic terms, the linear terms remained in the model regardless of significance (St. Pierre, 2001). The random effect of trial was included in the model and digestibility estimates were weighted by the inverse of the standard error term squared to improve the accuracy of the model. Including trial in the model reduced the RMSE and CV compared to the without-trial RMSE and CV. However, we recognize the variance of a study is unknown until the completion of a study and therefore we also report the RMSE and CV when study was not included in the model. The without trial RMSE and CV would be used as an indication of variation around the digestibility estimates of FA when the variance of the study is unknown or the study has not been completed. The lower RMSE and CV for each FA with trial included emphasizes the importance of having trial as a random effect in the model and weighting by a measurement of variation to improve the confidence of estimates (Oldick et al., 1999). Collinearity of significant variables was measured

by the variance inflation factor (VIF). Variables were considered highly correlated when VIF was > 10 and removed from the model as explained by St. Pierre and Glamocic (2000).

RESULTS

Dietary variables, FA intakes, and duodenal flows of individual FA used in our analysis are reported in Table 3.2. Total FA flow through the duodenum was positively linearly related to total intake of FA ($P < 0.0001$; Figure 3.1).

We initially evaluated all 61 treatments for apparent FA digestibility estimates. We chose to compare all other FA to C18:0 as we considered C18:0 to be the most appropriate FA to compare to both C16:0 and 18-carbon unsaturated FA. Digestibility was not different for C16:0 compared with C18:0 ($P = 0.34$, Table 3.3). Additionally, C18:2 was not different than C18:0 ($P = 0.18$). However, both C18:1 and C18:3 had increased digestibility compared with C18:0 ($P = 0.002$ and $P = 0.03$, respectively).

Using apparent digestibilities of individual FA evaluated from studies that measured digestibility from the duodenum to the ileum we observed no differences between C16:0 and C18:0 ($P = 0.29$, Table 3.4). Unsaturated FA when compared with C18:0 had numerically higher digestibility; with C18:1 and C18:3 digestibility estimates tended to be greater than C18:0 (both $P = 0.06$) and no difference between C18:2 and C18:0 ($P = 0.87$). From studies that measured digestibility from the duodenum to feces, there were no differences between C16:0 and C18:0 ($P = 0.10$, Table 3.5). However, all unsaturated FA were higher than C18:0 ($P = 0.0001$, $P = 0.003$, and $P = 0.003$ for C18:1, C18:2 and C18:3, respectively).

Lastly, individual FA digestibilities were evaluated for treatments that contained no supplemental fat (i.e. control treatments) to determine if digestibility differed as a result of fat

supplementation. Digestibility of C16:0 was similar to C18:0 ($P = 0.24$; Table 3.6). All unsaturated FA were also similar to C18:0 digestibility ($P > 0.50$ for C18:1, C18:2 and C18:3).

Total FA duodenal flow was negatively associated with total FA digestibility estimates ($P = 0.01$; Figure 3.2). To determine which variables impact individual FA digestibility, we developed best-fit equations from either duodenal (Table 3.7) or dietary variables (Table 3.8). Duodenal measurements were evaluated to determine post-rumen factors that impact apparent FA digestibility (Table 3.7). For C16:0 digestibility, the linear and quadratic terms for flows of C16:0, C18:1, C18:2 and C18:3 were all included in the initial model. Adjusting C16:0 digestibility by trial to account for the variation that exists between studies showed that C16:0 digestibility is not significantly impacted by flow of C16:0 through the duodenum ($P = 0.74$; Figure 3.3).

Remaining variables that explained variation in apparent C18:0 digestibility included total flow of FA, the concentration of C18:0, and C18:1 flow to the duodenum. Figure 3.4 shows the impact of C18:0 flow through the duodenum on C18:0 digestibility. Because flow of C18:0 through the duodenum increased, digestibility of C18:0 linearly decreased ($P < 0.001$; Figure 3.4). The reduction in digestibility appears to occur regardless of type of fat supplemented. This relationship between C18:0 duodenal flow and C18:0 digestibility also remained when considering only the control diets. With flow of C18:0 reaching the duodenum ranging from approximately 50 to 450 g/d, the digestibility of C18:0 was linearly reduced ($P = 0.05$; Figure 3.5). The digestibility of C18:0 was the only FA digestibility estimate in which total flow of FA was significant and included in the best-fit equation (Table 3.7).

The digestibility of all unsaturated FA were negatively related to the concentration of C18:0 flow through the duodenum (Table 3.7). The digestibility of C18:1 (both *cis* and *trans*

isomers included) was also explained by the quadratic coefficient for flow of C18:0, the linear and quadratic coefficients for flow of C18:2 through the duodenum and the linear coefficient for flow of C18:3. Digestibility of C18:2 was explained by the quadratic flow of C18:0, and the flow of C18:1 and C18:2 through the duodenum. The variation in digestibility of C18:3 was explained by the linear and quadratic flow of C16:0 in addition to the flow of C18:0.

Prediction equations based on dietary variables are reported in Table 3.8. Digestibility of C16:0 was dependent on the concentration of C16:0 and C18:1 as % of diet DM. DMI and forage concentration also significantly explained the variation in C16:0 digestibility ($P < 0.05$). Total C16:0 intake (DMI × Dietary C16:0 % DM), the interaction between DMI and forage concentration, and the interaction between dietary C16:0 % DM by forage concentration were also significant. The fat type supplemented altered digestibility of C16:0, with calcium-salts of FA-supplemented diets having higher digestibility and the control and whole seeds-supplemented diets having the lowest digestibility for total C16:0 reaching the duodenum.

Apparent digestibility of C18:0 was dependent on the concentration of C18:0, C18:1, and C18:2 as % of DM. Total C18:0 intake (DMI × Dietary C18:0 %) also explained variation observed in C18:0 digestibility. Although DMI was not significant, the term remained in the model because of the significant interactions that contained DMI.

Apparent digestibility of C18:1 was impacted by dietary concentration of C18:1 as % DM and by the type of fat supplemented. Calcium salts of FA and vegetable oil-supplemented diets had higher digestibility estimates, whereas whole seed-supplemented diets had the lowest digestibility for total C18:1 reaching the duodenum. Digestibility of C18:2 was only significantly impacted by the type of fat supplemented. Similarly to C18:1, calcium-salts of FA and vegetable oil-supplemented diets had the highest estimates while whole seed-supplemented diets had the

lowest digestibility of the fat types analyzed. Digestibility estimates of C18:3 were impacted by the dietary concentrations of C16:0 and the forage concentration of the diets.

DISCUSSION

Our data set contained studies with a wide range of dietary conditions and intakes of individual FA. We compared the digestibility of every FA to the digestibility of C18:0. The concentration of C18:0, regardless of degree of saturation of dietary fat type, represents the FA at largest concentrations leaving the rumen and available for absorption. Additionally, C18:0 is a logical comparison for both C16:0, both being saturated, and for C18:1, C18:2, and C18:3 because of equal chain length. Although we observed significant differences between C18:0 and unsaturated FA using meta-analysis, the differences in digestibility estimates were modest. Comparison of digestibility estimates across different sites of collection (estimates from Table 3.4 and Table 3.5) should only be made with caution because no study measured digestibility using both ileal and fecal collection; thus, trial-adjusted digestibility estimates might not be independent from random effects among trials. The most valid way to evaluate differences is by comparing individual FA against C18:0 within site of collection. However, generation of best-fit equations showed that only C18:0 digestibility was significantly reduced as flow of total FA increased. Additionally, C18:0 concentration significantly negatively impacted the apparent digestibility of all 18-carbon FA.

The positive linear relationship between intake of FA and duodenal flow of FA is similar to what has previously been reported in a review by Doreau et al. (1997). Similar to our meta-analysis results, they reported no difference between C16:0 and C18:0 digestibility. However, contrary to our results for C18:0 digestibility being impacted by duodenal flow, which is related

to intake of 18-carbon FA, Doreau et al. (1997) reported that intestinal digestibility of C18:0 was not associated with FA intake. Glasser et al. (2008) generated equations to predict duodenal flow of 18-carbon FA using dietary variables. They reported that for all unsaturated 18-carbon FA, absorption was a linear function of duodenal flow of FA. However, absorption of C18:0 plateaued at high duodenal flows of C18:0, indicating reduced digestibility at higher duodenal flow. Apparent 18-carbon FA digestibilites reported by Glasser et al. (2008) were in the same range (~70-82%) as what we observed; however, they reported individual estimates for specific isomers of unsaturated FA, whereas we combined all isomers into one estimate due to a limited number of observations accounting for individual 18-carbon isomers in lactating dairy cow studies.

The reduction in C18:0 digestibility with increasing flow through the duodenum is in agreement with results from individual studies that reported reductions in C18:0 digestibility in fat supplemented diets compared to control diets (e.g., Tice et al., 1994; Pantoja et al., 1996; Loor et al., 2002). In our data set, control diets with no added fat had C18:0 digestibility estimates that were equivalent to all other FA digestibility estimates (Table 3.6). Most likely, this is due to the reduced flow of C18:0 to the duodenum and unlimited physiological capacity to emulsify and absorb 18:0. However, we still observed a reduction in C18:0 digestibility in the control diets as duodenal flow of C18:0 increased (Figure 3.5). A potential reason for the reduction in C18:0 digestibility as flow increases is if lysolecithin were limiting. Lysolecithin is an effective amphiphile for C18:0, increasing the flux of lipids into micelles and therefore increasing absorption (Freeman, 1969). If C18:0 flow exceeded the capacity of either the lecithin in bile or the phospholipase excreted from the pancreas, digestibility of C18:0 may be reduced. Heath and Morris (1963) reported that FA absorption was almost nonexistent when bile

secretions were blocked in ruminants. However, supplementing additional lysolecithin to diet containing supplemental soybeans and soybean oil reduced milk fat yield compared to a diet containing no additional lysolecithin (Rico et al., 2014). If additional lysolecithin increased uptake of C18:0 we would expect to see an increase in milk fat yield, specifically in preformed milk FA. Freeman (1969) observed a reduction in stearic acid incorporation into the micelle when pH > 6.4 in the presence of 1.0 mEq/L of calcium ions. There may be other mechanisms responsible for the reduction of digestibility of C18:0 as flow increased: physical structure of the FA, availability of absorption sites, or availability of FA within a fat supplement.

Harrison and Leat (1972) used radiolabeled FA in sheep to determine the percent absorption and absorption rates between the small intestine and lymph fluid for individual FA. Compared to C18:0, C16:0 increased the rate of absorption and therefore the total percent of FA absorbed. Potentially, these differences in absorption between FA that only differ in chain-length are due to the increased ability of C16:0 to form micelles. Across our data set, we observed no effect of C16:0 flow on C16:0 digestibility; however, Piantoni et al (2013) fed a 99% pure C16:0 supplement and reported a reduction in 16-carbon total tract digestibility, potentially due to the physical size of the fat supplement reducing solubilization in the small intestine. Further research is required to determine if C16:0 digestibility is impacted with increased duodenal flow, especially given the increased use of C16:0-eneriched fat supplements in dairy cattle diets.

The type of fat included in the diet impacted digestibility for three out of the five (C16:0, C18:1 and C18:2) FA reported. When whole seeds were fed we observed reduced individual FA digestibility, whereas when calcium salts of FA and vegetable oils and were fed, we observed increased individual FA digestibility. A possible reason for these discrepancies in digestibility might be availability of the fat when reaching the small intestine. Calcium salts of FA and

vegetable oils are readily available for emulsification and absorption in the small intestine while the FA in whole seeds is contained within the hard seed coat that is resistant to degradation in the rumen (Mohamed et al., 1988; Reddy et al., 1994). While whole seeds may limit the negative effects of unsaturated FA in the rumen, their reduced digestibility in the small intestine might limit their effectiveness as a fat source to include in lactating dairy cattle diets.

Using meta-analysis limits the scope of interpretation of results because not all of the studies were performed the same way with cows of the same genetic potential, diets, fat types, stage of lactation etc. The method of collection and analysis were different so the results have to be considered in context. However, by including studies across a wide range of feeding strategies then we can be more confident that the mean response is representative of what would actually occur in a future study so long as the dataset is reasonably balanced and the random effect of study averages such sources of variation to decrease their potential bias (St. Pierre, 2001). A limited number of studies have reported individual FA digestibilities in lactating dairy cattle. Only one of these studies reported both duodenum to ileum and duodenum to fecal digestibility to compare the two methods for determining FA digestibility (Pantoja et al., 1996); however, this study did not report the standard error for both methods of digestibility estimates. Therefore no inferences can be made comparing method of calculating digestibility between fecal and ileal collection.

Our meta-analysis results indicate there are minimal differences in the digestibility of individual FA. However, these represent the weighted mean value and masked important differences in apparent digestibility associated with limiting absorption capacity associated with increased supply to the small intestine. Further insight on both dietary and duodenal factors that impact individual FA digestibility was gained using meta-regression and plotting out digestibility

by duodenal flows of individual FA. Characteristics of the diet, type of fat supplemented, and amount of individual FA reaching the duodenum may all impact individual FA digestibility estimates.

Although C18:0 digestibility was reduced as flow increased when analyzing all of the treatments and just the control treatments, our analysis is based on associations and should be followed up by direct hypothesis testing. For example, studies providing additional lysolecithin to C18:0-enriched fat supplemented diets would provide further insight. Additionally, protection methods of FA in the rumen to increase the flow of unsaturated FA may have benefits to the cow in terms of providing essential FA as well as increasing the efficiency of digestion from feeding a fat supplement. That C16:0 did not have a reduction in digestibility over a wide range of duodenal flows probably indicates unique properties and potential uses for increasing C16:0 inclusion in lactating cattle diets, provided the characteristics of the fat supplement allow for adequate intestinal absorption.

Although we demonstrated that individual FA digestibility is dependent on several dietary and duodenal variables, the differences in FA digestibility should be considered in proper context. The amount of FA that are included in the diet is relatively small for lactating dairy cattle, and changes in FA digestibility therefore have minimal effects on overall DM digestibility and digestible energy intake. Even significant reductions in individual FA digestibility estimates may have little impact on reducing total DM digestibility compared to reductions in digestibility of more abundant feed ingredients. Potentially of greater importance, fat supplements may impact on DMI (Allen, 2001) and digestibility of other nutrients (Palmquist, 1991). Changes in intake and digestibility of other nutrients from fat supplements would impact digestible energy available for milk production and/or body reserves.

CONCLUSIONS

Digestion and metabolism of FA in ruminants is complex and dependent on coordination of several processes. We observed minimal differences in FA digestibility using meta-analysis techniques to compile results from 15 studies feeding a variety of fat supplements. In control diets that contained no additional fat, there were no differences in the digestibility of individual FA. However, generation of best-fit equations and trial adjusted plots indicate that C18:0 flow through the duodenum negatively impacts digestibility of several FA. The most severe reduction in digestibility for C18:0 acid is observed at high concentrations of C18:0 flow through the duodenum. The mechanisms that inhibit C18:0 absorption when at high concentrations are unknown and warrant further investigation.

APPENDIX

APPENDIX

Table 3.1. Description of studies included in the analysis.

Study	n cows/ treatment	Description of treatment; treatment category (FA %)
Christensen et al., 1998	4	1. Low fat no nicotinic acid; Control (2.77) 2. Low fat + nicotinic acid; Control (2.77) 3. High fat no nicotinic acid; Tallow (5.86) 4. High fat + nicotinic acid; Tallow (5.86)
Weisbjerg et al., 1992	4	1. Low feed level no added tallow; Control (2.6) 2. Low feed level 4% tallow; Tallow (6.6) 3. Low feed level 6% tallow; Tallow (8.4) 4. High feed level no added tallow; Control (2.6) 5. High feed level 2% tallow; Tallow (4.7) 6. High feed level 4% tallow; Tallow (6.6) 7. High feed level 6% tallow; Tallow (8.5)
Wu et al., 1991 and Ohajuruka et al., 1991	5	1. Control; Control (2.53) 2. Calcium salt low (Megalac); Ca Salt (5.25) 3. Calcium salt high (Megalac); Ca Salt (7.29) 4. Animal vegetable low; AVF (4.54) 5. Animal vegetable high; AVF (6.46)
Ferlay et al., 1993	3	1. Control; Control (1.9) 2. Calcium salts of rapeseed; Ca Salt (8.5) 3. Oil of rapeseed; Vegetable oil (7.7)
Klusmeyer et al., 1991	4	1. Soybean meal no fat; Control (2.57) 2. Fish meal no fat; Control (2.76) 3. Soybean meal + calcium salts (Megalac); Ca Salt (5.73) 4. Fish meal + calcium salts (Megalac); Ca Salt (5.95)
Murphy et al., 1987	3	1. Control; Control (3.58) 2. Rapeseed meal; Seed meal (7.04) 3. Rapeseed meal; Seed meal (10.37)
Pantoja et al., 1996	6	1. Control; Control (2.1) 2. Tallow and soyhulls; Tallow (6.4) 3. Animal vegetable fat low forage; AVF (7.1) 4. Animal vegetable fat low forage + soyhulls; AVF (6.1) 5. Animal vegetable fat high forage:; AVF (6.9)
Pires et al., 1997	4	1. Tallow; Tallow (5.12) 2. Whole raw cottonseed; Whole seed (5.24) 3. Ground raw cottonseed; Seed meal (5.09) 4. Whole roasted cottonseed; Whole seed (5.31) 5. Ground roasted cottonseed; Seed meal (5.40)

Table 3.1. (cont'd)

Study	n cows/ treatment	Description of treatment; treatment category (FA %)
Tice et al., 1994	5	1. Calcium salt; Calcium salt (4.91) 2. Whole raw soybeans; Whole seed (5.43) 3. Whole roasted soybeans; Whole seed (5.73) 4. Cracked roasted soybeans; Seed meal (5.67) 5. Ground roasted soybeans; Seed meal (5.47)
Enjalbert et al., 1997	3	1. Control; Control (1.9) 2. Calcium salts of palm fatty acids; Ca Salt (4.8) 3. Calcium salts of rapeseed fatty acids; Ca Salt (4.8)
Bauchart et al., 1987	5	1. Control; Control (1.91) 2. Milk; Other (9.05)
Avila et al., 2000	4	1. Control; Control (3.2) 2. Tallow; Tallow (4.8) 3. Blend; AVF (5.1) 4. Yellow grease; AVF (5.0)
Loor et al., 2002	4	1. Control; Control (2.7) 2. Canola oil; Vegetable oil (4.8) 3. Canola oil and canolamide; Other (4.8) 4. Canolamide; Other (4.9)
Loor et al., 2004	4	1. Low concentrate; Control (1.6) 2. Low concentrate with linseed oil; Vegetable oil (4.7) 3. High concentrate; Control (1.9) 4. High concentrate with linseed oil; Vegetable oil (5.1)
Loor et al., 2005	3	1. Fish oil; Other (4.1) 2. Linseed oil; Vegetable oil (6.6) 3. Sunflower oil; Vegetable oil (6.6)

Table 3.2. **Data set minimum, maximum, mean, and standard deviation values.**

Variable	N	Mean	Min	Max	SD
Dietary Fat, % DM	61	5.06	1.6	10.4	1.98
DMI, kg/d	61	17.9	8.44	26.9	4.56
Forage, % DM	61	52.4	35	65	8.21
FA Intake, g/d					
C16:0	61	176	34.7	528	114
C18:0	61	47.0	4.2	147	37.0
C18:1	61	236	21.5	621	153
C18:2	61	243	34.4	676	137
C18:3	56	77.6	8.9	480	95.3
FA Intake, g/100 g of FA					
C16:0	61	20.5	7.07	42.1	8.11
C18:0	61	5.24	2.03	12.9	3.33
C18:1	61	26.1	13.3	50.4	10.2
C18:2	61	30.0	4.5	61.0	12.8
C18:3	56	9.9	2.0	49.6	9.89
FA Duodenal Flow, g/d					
C16:0	61	161	23.4	526	97.5
C18:0	56	380	56.5	740	171
C18:1	56	149	17.5	485	102
C18:2	61	55.6	6	212	45.2
C18:3	56	8.77	0.3	31.7	7.57
FA Duodenal Flow, g/100 g of FA					
C16:0	59	19.5	8.88	39.7	7.08
C18:0	54	46.3	14.4	71.4	10.6
C18:1	54	16.4	6.43	38.7	7.50
C18:2	59	6.66	1.27	18.8	3.67
C18:3	54	1.10	0.07	3.01	0.75

Table 3.3. Apparent digestibility estimates of individual FA from 15 studies including both methods of collection (ileal and fecal) in lactating dairy cattle.

FA	n	Estimate	SE	Lower Limit	Upper Limit	*P*-value[1]
16:0	61	76.0	1.04	74.0	78.1	0.34
18:0	61	73.3	2.68	68.0	78.5	---
18:1	56	81.6	0.57	80.5	82.7	0.002
18:2	61	77.7	1.92	73.9	81.5	0.18
18:3	48	79.2	0.59	78.0	81.3	0.03
Total	61	74.7	1.68	71.5	78.0	0.64

[1] *P*-value associated with comparing individual FA digestibility against C18:0.

Table 3.4. Apparent digestibility estimates of individual FA for studies using ileal collection to determine FA digestibility in lactating dairy cattle.

FA	n	Estimate	SE	Lower Limit	Upper Limit	*P*-value[1]
16:0	18	77.1	1.46	74.2	79.9	0.29
18:0	18	72.8	3.82	65.3	80.3	---
18:1	13	80.2	0.85	78.5	81.8	0.06
18:2	18	73.5	2.16	69.3	77.8	0.87
18:3	10	80.5	1.68	77.2	83.8	0.06
Total	18	74.5	1.44	71.7	77.3	0.68

[1] *P*-value associated with comparing individual FA digestibility against C18:0.

Table 3.5. Apparent digestibility estimates of individual FA for studies using fecal collection to determine FA digestibility in lactating dairy cattle.

FA	n	Estimate	SE	Lower Limit	Upper Limit	*P*-value[1]
16:0	43	76.5	0.64	75.3	77.8	0.10
18:0	43	73.7	1.56	70.7	76.8	---
18:1	43	80.8	0.93	79.0	82.6	0.001
18:2	43	79.9	1.34	77.3	82.6	0.003
18:3	38	78.8	0.65	77.5	80.0	0.003
Total	43	75.0	1.24	72.6	77.4	0.52

[1] *P*-value associated with comparing individual FA digestibility against C18:0.

Table 3.6. Apparent digestibility estimates of individual FA for comparisons containing no supplemental fat, control diets in lactating dairy cattle.

FA	n	Estimate	SE	Lower Limit	Upper Limit	*P*-value[1]
16:0	16	76.7	1.88	73.0	80.4	0.24
18:0	16	81.9	4.02	74.0	89.8	---
18:1	15	82.3	1.31	79.7	84.9	0.93
18:2	16	77.3	5.90	65.7	88.8	0.52
18:3	13	79.5	1.24	77.1	81.9	0.57
Total	16	77.8	3.95	70.1	85.6	0.53

[1] *P*-value associated with comparing individual FA digestibility against C18:0.

Table 3.7. Meta-regression for duodenal measurements to determine best-fit equations for individual FA digestibility coefficients (%) in lactating dairy cattle.

	Dependent variable		Independent variables			+ Trial[1]		- Trial[2]	
FA	Intercept	SEM	Variable, g/d	Coefficient	SEM	RMSE[3]	CV, %	RMSE	CV, %
C16:0	78.83	4.06	% C16:0 Flow	0.249	0.122	1.32	1.71	4.34	5.74
			(% C16:0 Flow)^2	-0.025	0.009				
			% C18:1 Flow	-0.241	0.152				
			(% C18:1 Flow)^2	0.032	0.009				
			% C18:2	-1.268	0.284				
			% C18:3	4.315	1.393				
C18:0	122.46	10.04	Total Flow	-0.02	0.004	1.90	2.56	11.21	15.26
			% C18:0 Flow	-0.44	0.155				
			% C18:1 Flow	-0.67	0.279				
C18:1	103.97	5.21	% C18:0 Flow	-0.376	0.080	1.21	1.45	4.91	6.10
			(% C18:0 Flow)^2	-0.006	0.003				
			% C18:2 Flow	-1.304	0.415				
			(% C18:2 Flow)^2	-0.102	0.047				
			% C18:3 Flow	4.832	1.576				
C18:2	89.318	9.95	% C18:0 Flow	-0.324	0.142	1.27	1.60	9.47	12.26
			(% C18:0 Flow)^2	-0.013	0.005				
			% C18:1 Flow	0.812	0.191				
			% C18:2 Flow	-1.381	0.389				
C18:3	93.614	7.19	% C16:0 Flow	-0.102	0.180	1.19	1.50	8.30	10.94
			(% C16:0 Flow)^2	0.028	0.012				
			% C18:0 Flow	-0.350	0.100				

[1] Variance from trial was known.
[2] Variance from trial was unknown and the random effect of study was not included in the model.
[3] Root mean square error.

Table 3.8. Meta-regression for dietary measurements to determine a best-fit equation for apparent digestibility of individual FA (%) in lactating dairy cattle.

	Dependent variable		Independent variables			+ Trial[1]		- Trial[2]	
FA	Intercept	SEM	Variable	Coefficient	SEM	RMSE[3]	CV, %	RMSE	CV, %
C16:0	105.98	8.69	Dietary C16:0 (% DM)	-4.197	1.799	0.96	1.24	5.63	7.44
			Dietary C18:1 (% DM)	-3.794	0.726				
			DMI (kg/d)	-0.419	0.274				
			DMI × Dietary C16:0 (% DM)	-1.208	0.283				
			Forage %	-0.271	0.101				
			DMI × Forage %	-0.102	0.043				
			Dietary C16:0 (% DM) × Forage %	-0.692	0.200				
			Fat Type[4]						
			AVF	76.60	2.09				
			Ca Salts	82.94	3.12				
			Control	68.22	2.07				
			Other	77.24	2.65				
			Seed meal	72.99	2.45				
			Tallow	73.01	2.18				
			Vegetable oil	75.84	1.97				
			Whole seeds	68.42	3.29				
C18:0	100.29	7.34	Dietary C18:0 (% DM)	-15.055	3.817	1.33	1.80	8.35	11.40
			Dietary C18:1 (% DM)	-5.005	0.829				
			Dietary C18:2 (%DM)	-5.443	1.320				
			DMI	-0.486	0.388				
			Dietary C18:0 (% DM) × DMI	-1.670	0.569				
C18:1	83.08	2.06	Dietary C18:1 (% DM)	-2.544	0.768	1.02	1.23	6.56	8.14
			Fat Type[4]						
			AVF	82.67	2.33				
			Ca Salts	86.85	1.98				

Table 3.8. (cont'd)

	Dependent variable		Independent variables			+ Trial[1]		- Trial[2]	
FA	Intercept	SEM	Variable	Coefficient	SEM	RMSE[3]	CV, %	RMSE	CV, %
C18:2 (con't)			Control	78.67	1.90				
			Other	83.69	2.36				
			Seed meal	75.52	2.54				
			Tallow	77.75	2.56				
			Vegetable oil	85.42	1.96				
			Whole seed	64.18	4.48				
C18:2	77.45	2.45	Fat Type[4]			1.45	1.83	9.33	12.04
			AVF	74.76	4.25				
			Ca Salts	82.91	3.23				
			Control	75.87	2.70				
			Other	81.93	4.94				
			Seed meal	80.19	3.74				
			Tallow	74.30	3.58				
			Vegetable oil	84.53	3.31				
			Whole seeds	65.12	5.36				
C18:3	85.97	5.40	Dietary C16:0 % DM	3.021	1.491	1.35	1.70	8.76	11.55
			Forage %	- 0.238	0.095				

[1] Variance from trial was known.
[2] Variance from trial was unknown and the effect of study was not included in the model.
[3] Root mean square error.
[4] Coefficients and standard errors for the class variable (Fat Type) represent the least square means for apparent digestibility of individual FA when specific fat types were supplemented.

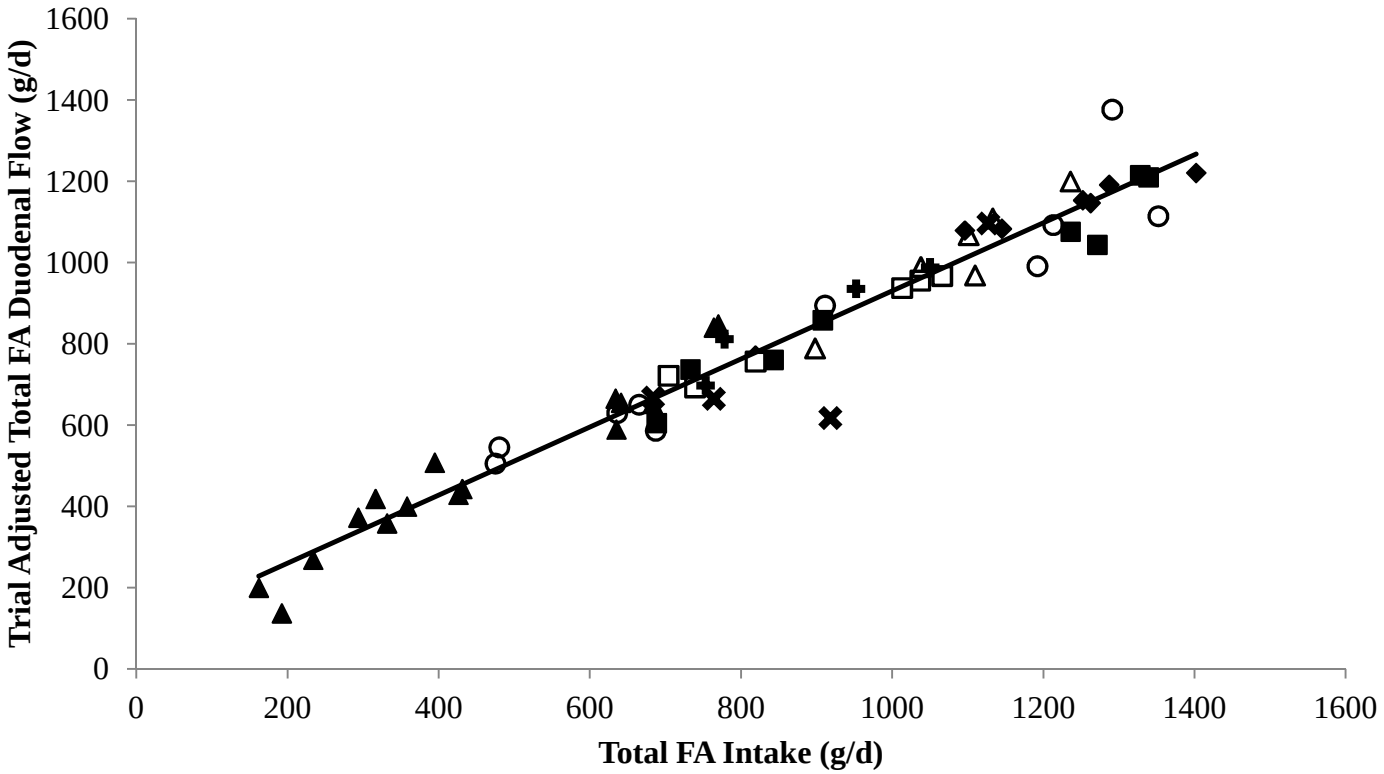


Figure 3.1. Relationship between trial-adjusted total fatty acid duodenal flow (g/d) and total fatty acid intake (g/d) for 61 observations from 15 studies. Total FA duodenal flow (g/d) = 92.89 (SE = 29.01) + 0.838 (SE = 0.054) × total FA intake (g/d); (P < 0.001; intercept P = 0.006). Control treatments represented by black triangles; animal-vegetable fat treatments represented by black diamonds; calcium salt treatments represented by black squares; tallow treatments represented by open circles; vegetable oil treatments represented by open triangles; seed meal treatments represented by open squares; whole seed treatments represented by black addition sign; and other treatments represented by black multiplication sign.

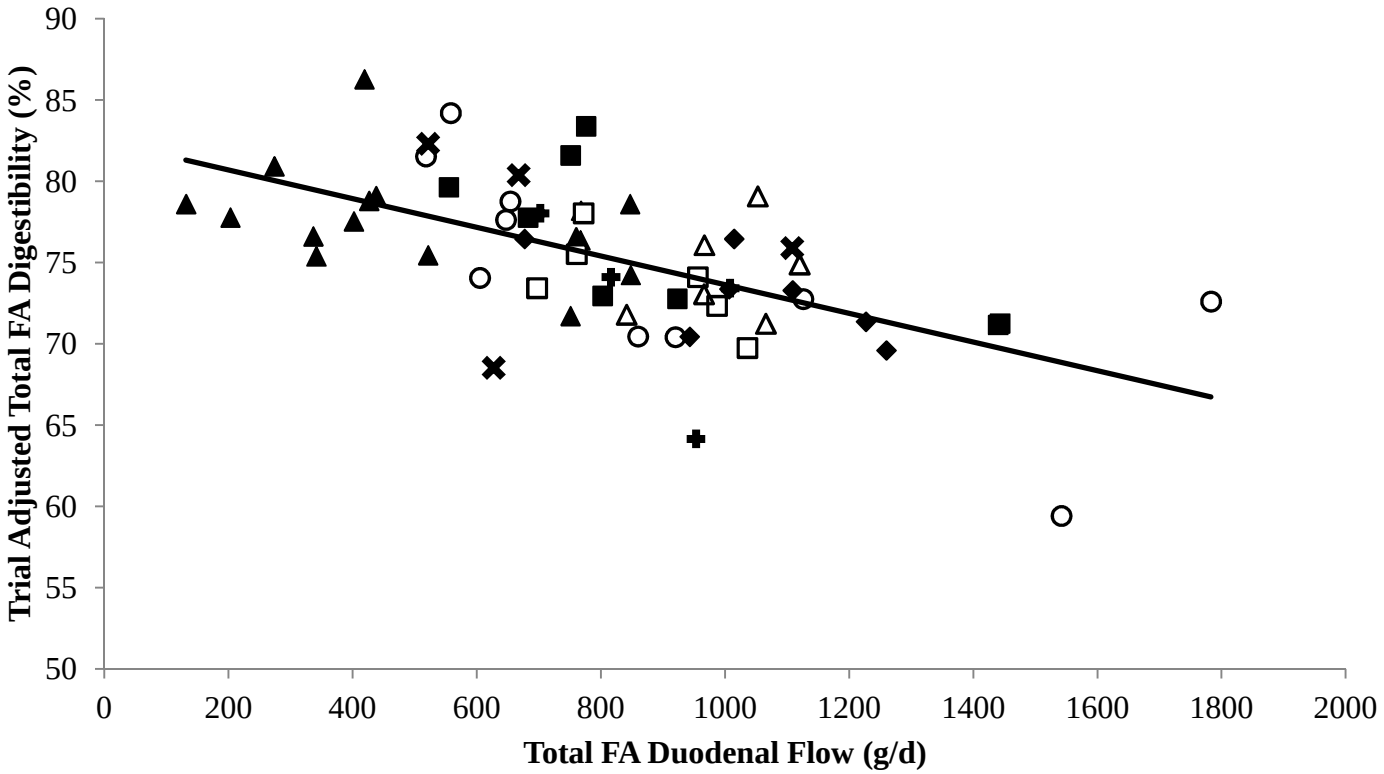


Figure 3.2. Relationship between trial adjusted total FA digestibility (%) and total fatty acid duodenal flow (g/d) for 61 observations from 15 studies. Total FA digestibility (%) = 82.46 (SE = 2.15) - 0.0088 (SE = 0.0031) × total FA duodenal flow (g/d) (P = 0.01). Control treatments represented by black triangles; animal-vegetable fat treatments represented by black diamonds; calcium salt treatments represented by black squares; tallow treatments represented by open circles; vegetable oil treatments represented by open triangles; seed meal treatments represented by open squares; whole seed treatments represented by black addition sign; and other treatments represented by black multiplication sign.

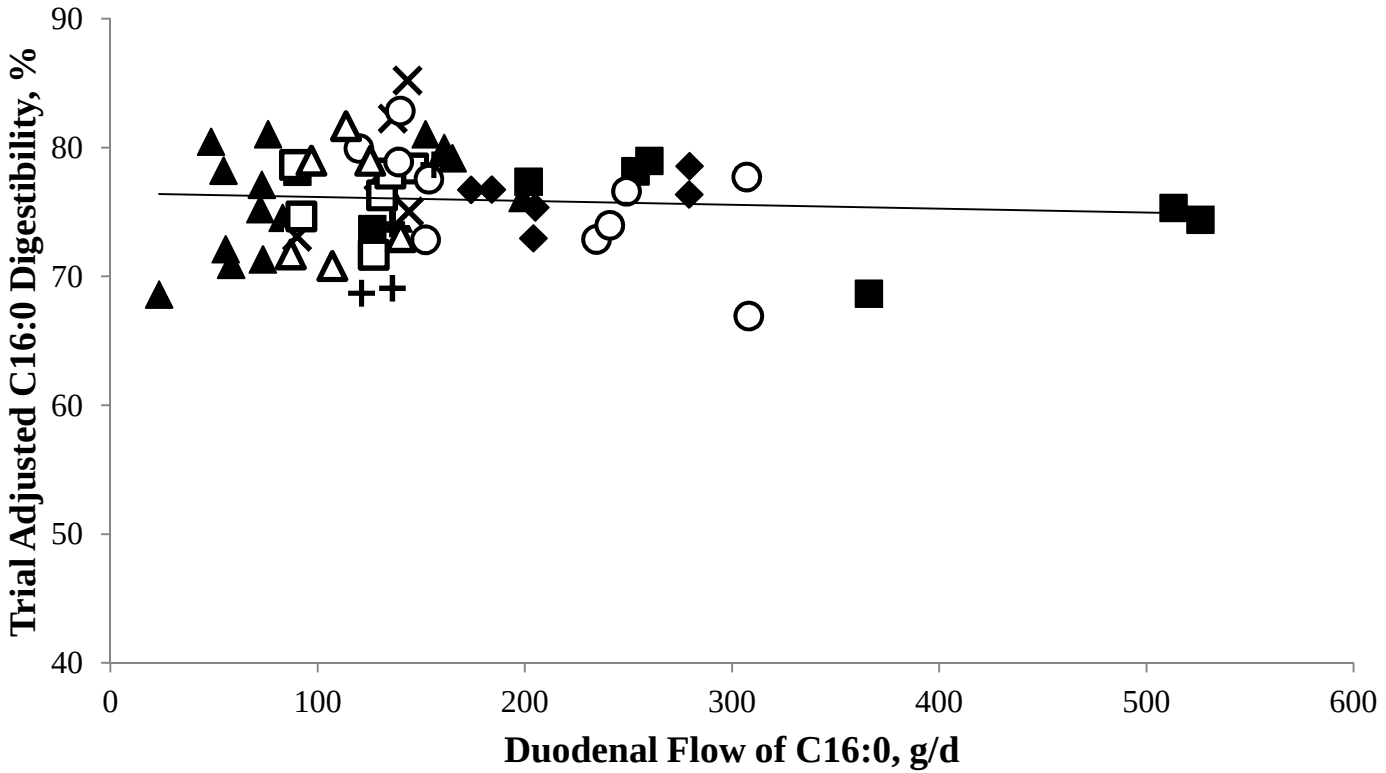


Figure 3.3. Relationship between trial adjusted C16:0 digestibility and duodenal flow of C16:0. Trial adjusted C16:0 digestibility (%) = 76.46 (SE = 1.83) – 0.0030 (SE = 0.0090) × duodenal flow of C16:0 (g/d) (P = 0.74). Control treatments represented by black triangles; animal-vegetable fat treatments represented by black diamonds; calcium salt treatments represented by black squares; tallow treatments represented by open circles; vegetable oil treatments represented by open triangles; seed meal treatments represented by open squares; whole seed treatments represented by black addition sign; and other treatments represented by black multiplication sign.
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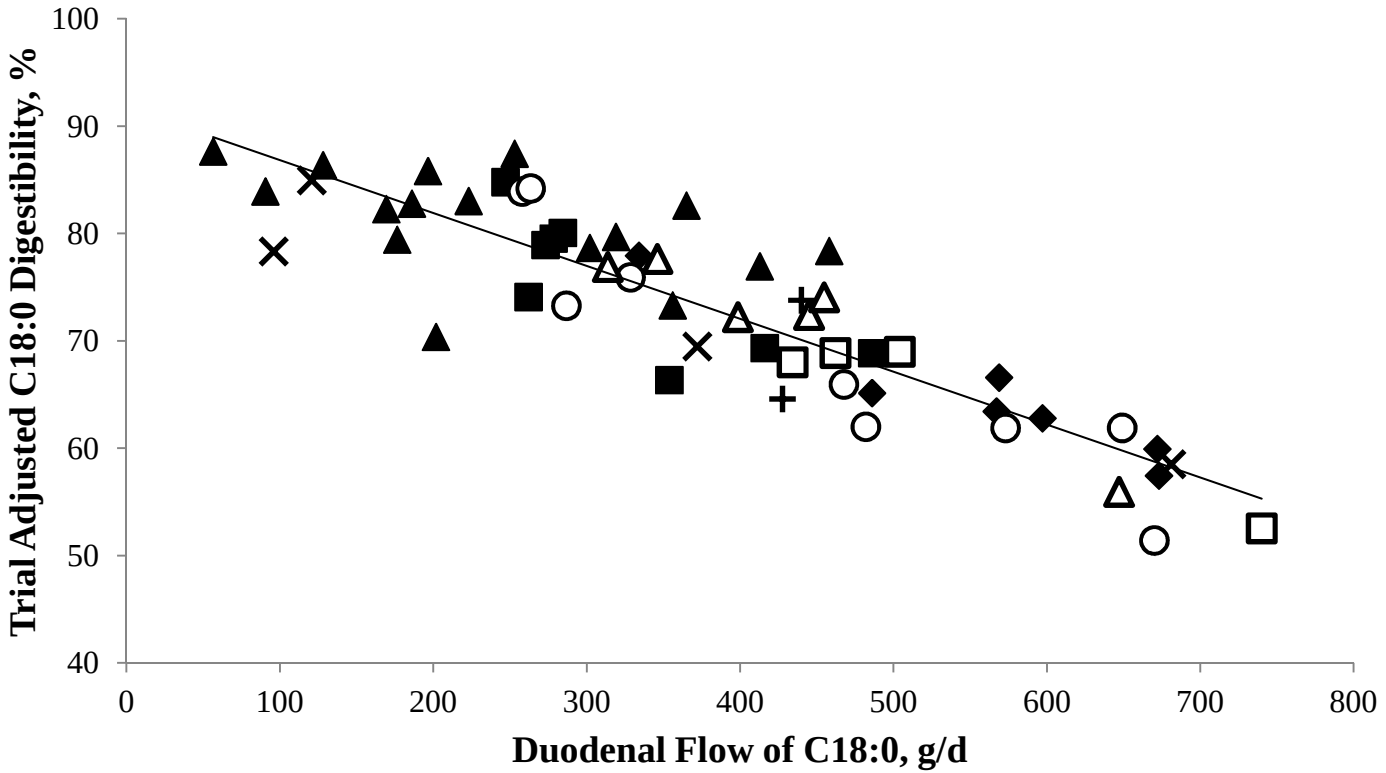

Figure 3.4. Relationship between trial adjusted C18:0 digestibility and duodenal flow of C18:0. Trial adjusted C18:0 digestibility (%) = 91.76 (SE = 1.98) – 0.0493 (SE = 0.0098) × duodenal flow of total C18:0 (g/d); $P < 0.001$). Control treatments represented by black triangles; animal-vegetable fat treatments represented by black diamonds; calcium salt treatments represented by black squares; tallow treatments represented by open circles; vegetable oil treatments represented by open triangles; seed meal treatments represented by open squares; whole seed treatments represented by black addition sign; and other treatments represented by black multiplication sign.

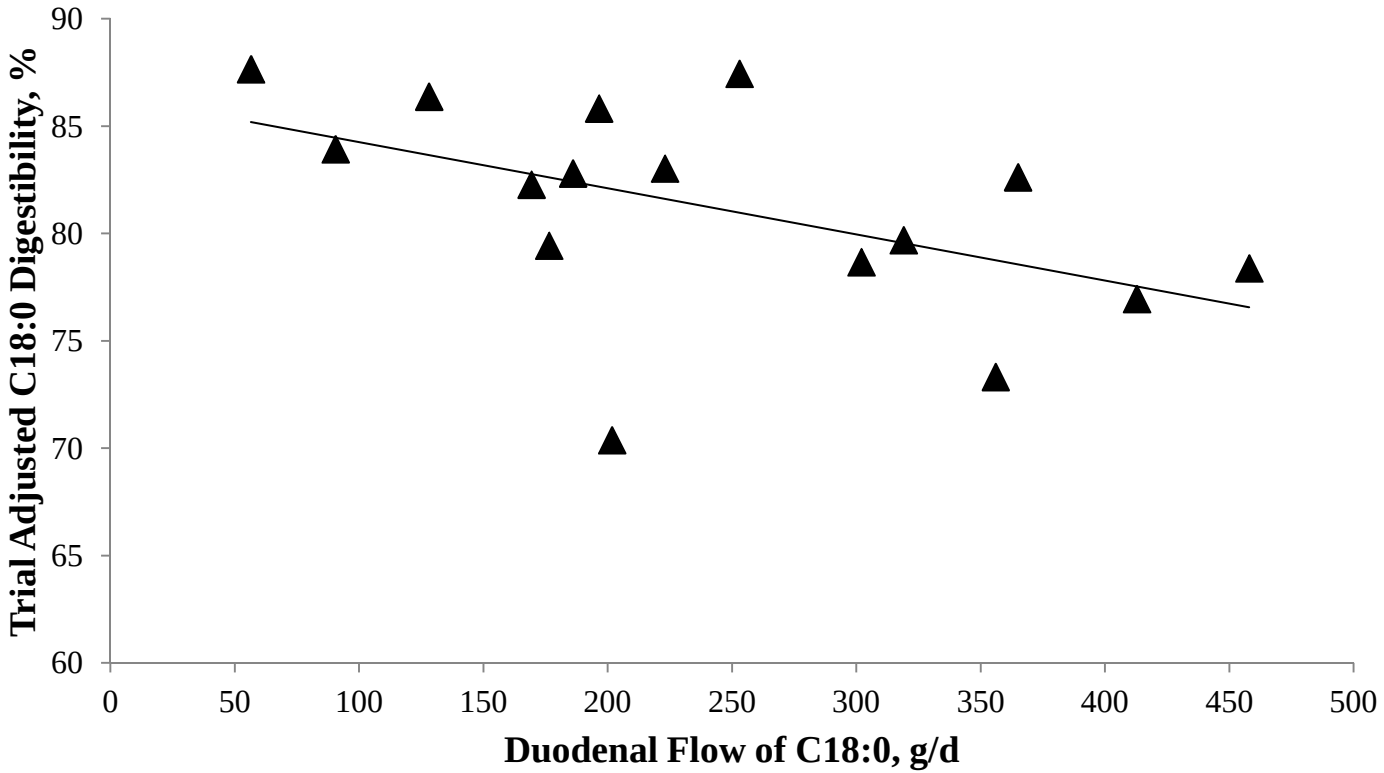

Figure 3.5. Relationship between trial adjusted C18:0 digestibility and duodenal flow of C18:0 for control diets. Trial adjusted C18:0 digestibility (%) = 86.41 (SE = 2.62) – 0.0215 (SE = 0.0098) × duodenal flow of total C18:0 (g/d); (*P* = 0.05).

REFERENCES

REFERENCES

Abayasekara, D. R., and D. C. Wathes. 1999. Effects of altering dietary fatty acid composition on prostaglandin synthesis and fertility. Prostaglandins Leukot. Essent. Fatty Acids. 61:275–287

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598-1624.

Ambrose, D. J., J. P. Kastelic, R. Corbett, P. A. Pitney, H. V. Petit, J. A. Small, and P. Zalkovic. 2006. Lower pregnancy losses in lactating dairy cows fed a diet enriched in alpha-linolenic acid. J. Dairy Sci. 89:3066-3074.

Andrews, R. J. and D. Lewis. 1970. The utilization of dietary fats by ruminants. II. The effect of fatty acid chain length and unsaturation on digestibility. J. Agri. Sci. 75: 55-60.

Avila, C.D., E.J. DePeters, H. Perez-Monti, S.J. Taylor, and R.A. Zinn. 2000. Influences of saturation ratio of supplemental dietary fat on digestion and milk yield in dairy cows. J. Dairy Sci. 83:1505–1519.

Bauchart, D. 1993. Lipid absorption and transport in ruminants. J. Dairy Sci. 76:3864-3881.

Bauchart, D., M. Doreau, and A. Kindler. 1987. Effect of fat and lactose supplementation on digestion in dairy cows. 2. Long-chain fatty acids. J. Dairy Sci. 70:71–80.

Borenstein, M., L. V. Hedges, J. P. T. Higgins, and H. R. Rothstein. 2009. Introduction to Meta-Analysis. John Wiley & Sons Ltd. West Sussex, UK.

Chilliard, Y. 1993. Dietary fat and adipose tissue metabolism in ruminants, pigs, and rodents: a review. J. Dairy Sci. 76, 3897-3931.

Christensen, R.A., J.H. Clark, J.K. Drackley, and S.A. Blum. 1998. Fatty acid flow to the duodenum and in milk from cows fed diets that contained fat and nicotinic acid. J. Dairy Sci. 81:1078–1088.

Doreau, M. and A. Ferlay. 1994. Digestion and utilization of fatty-acids by ruminants. Anim. Feed Sci. Technol. 45:379-396.

Elliott, J. P., J. K. Drackley, and D. J. Weigel. 1996. Digestibility and effects of hydrogenated palm fatty acid distillate in lactating dairy cows. J. Dairy Sci. 79: 1031-1039.

Freeman, C. P. 1969. Properties of fatty acids in dispersions of emulsified lipid and bile salt and the significance of these properties in fat absorption in the pig and the sheep. British J. Nutr. 23:249-263.

Freeman, C. P. 1984. Digestion, absorption and transport of fats - non-ruminant animals. In: Fats in Animal Nutrition. pp. 105-122. Butterworths, London, UK.

Harrison, F. A, and W. M. F. Leat. 1972. Absorption of palmitic, stearic, and oleic acids in the sheep in the presence or absence of bile and/or pancreatic juice. J. Physiol. 225:565-576.

Harrison, F. A., and W. M. F. Leat. 1975. Digestion and absorption of lipids in non-ruminant and ruminant animals: A comparison. Proc. Nutr. Soc. 34: 203-210.

Heath, T. J., and B. Morris. 1963. The role of bile and pancreatic juice in the absorption of fat in ewes and lambs. British J. Nutr. 17: 465-474.

Jenkins, T. C. and B. F. Jenny. 1989. Effect of hydrogenated fat on feed intake, nutrient digestion, and lactation performance of diary cows. J. Dairy Sci. 72: 2316.

Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76: 3851-3863.

Jenkins, T. C. 1994. Feeding fat to dairy cattle. Pp. 100-109 in Proc. Dairy Heard Management Conf. University of Georgia, Athens, GA.

Klusmeyer, T.H., G.L. Lynch, J.H. Clark, and D.R. Nelson. 1991. Effects of calcium salts of fatty acids and proportion of forage in diet on ruminal fermentation and nutrient flow to duodenum of cows. J. Dairy Sci. 74:2220–2232.

Loor, J. J., J. H. Herbein, and T. C. Jenkins . 2002. Nutrient digestion, biohydrogenation, and fatty acid profiles in blood plasma and milk fat from lactating Holstein cows fed canola oil or canolamide. Anim. Feed Sci. Techno. 97:65-82.

Loor, J.J., K. Ueda, A. Ferlay, Y. Chilliard, and M. Doreau. 2004. Biohydrogenation, duodenal flow, and intestinal digestibility of trans fatty acids and conjugated linoleic acids in response to dietary forage:concentrate ratio and linseed oil in dairy cows. J. Dairy Sci. 87:2472–2485.

Loor, J.J., M. Doreau, J.M. Chardigny, A. Ollier, J.L. Sebedio, and Y. Chilliard. 2005. Effects of ruminal or duodenal supply of fish oil on milk fat secretion and profiles of trans-fatty acids and conjugated linoleic acid isomers in dairy cows fed maize silage. Anim. Feed Sci. and Techn. 119:227–246.

Maia, M.R., L.C. Chaudhary, C.S. Bestwick, A.J. Richardson, N. McKain, T.R. Larson, I.A. Graham, and R.J. Wallace. 2010. Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, Butyrivibrio fibrisolvens. BMC Microbiology. 10:52.

Mohamed, O. E., L. D. Satter, R. R. Grummer, and F. R. Ehle. 1988. Influence of dietary cottonseed and soybean on milk production and composition. J. Dairy Sci. 71:2677-2688.

Moore, J. H. and W. W. Christie. 1984. Digestion, absorption and transport of fats in ruminant animals. In: Fats in Animal Nutrition. pp. 123-149. Butterworths, London, UK.

Noble, R. C. 1981. Digestion, transport and absorption of lipids. In W. W. Christie (Ed.) Lipid Metabolism in Ruminant Animals. pp. 57-93. Pergamon Press Ltd. Oxford, UK.

Ohajuruka, O.A., Z. Wu, and D.L. Palmquist. 1991. Ruminal Metabolism, Fiber, and Protein Digestion by Lactating Cows Fed Calcium Soap or Animal-Vegetable Fat. J. Dairy Sci. 74:2601–2609.

Oldick, B. S., J. L. Firkins, and N. R. St-Pierre. 1999. Estimation of microbial nitrogen flow to the duodenum of cattle based on dry matter intake and diet composition. J. Dairy Sci. 82:1497-1511.

Palmquist, D. L. 1991. Influence of source and amount of dietary fat on digestibility in lactating cows. J. Dairy Sci. 74:1354-1360.

Pantoja, J., J.L. Firkins, and M.L. Eastridge. 1996. Fatty acid digestibility and lactation performance by dairy cows fed fats varying in degree. J. Dairy Sci. 79:429–437.

Pires, A.V., M.L. Eastridge, J.L. Firkins, and Y.C. Lin. 1997. Effects of heat treatment and physical processing of cottonseed on nutrient digestibility and production performance by lactating cows. J. Dairy Sci. 80:1685–1694.

Reddy, P. V., J. L., Morrill, and T. G. Nagaraja. 1994. Release of free fatty acids from raw or processed soybeans and subsequent effects on fiber digestibilities. J. Dairy Sci. 77:3410-3416.

Rico, D. E., J. Y. Ying, and K. J. Harvatine. 2014. Effects of lysolecithin on milk fat synthsisis and milk fatty acid profile of cow fed diets differing in fiber and unsaturated fatty acid concentration. J. Anim. Sci. 92:E-Suppl. 1843. *Abstract*.

Steele W. and J. H. Moore. 1968. The digestibility coefficients of myristic, palmitic and stearic acids in the diet of sheep. J. Dairy Res. 35: 371-376.

St-Pierre, N. R. 2001. Invited Review: Integrating Quantitative Findings from Multiple Studies Using Mixed Model Methodology. J. Dairy Sci. 84:741-755.

St-Pierre, N. R., and D. Glamocic. 2000. Estimating unit costs of nutrients from market prices of feedstuffs. J. Dairy Sci. 83:1402-1411.

Tice, E.M., M.L. Eastridge, and J.L. Firkins. 1994. Raw soybeans and roasted soybeans of different particle sizes. 2. Fatty acid utilization by lactating cows. J. Dairy Sci. 77:166–180.

Weisbjerg, M. R., C. F. Borsting, and T. Hvelplund. 1992. Fatty acid metabolism in the digestive tract of lactating cows fed tallow in increasing amounts at two feed levels. Acta. Agric. Scand., Sect. A, Animal Sci. 42:106-114.

Weiss, W. P., and D. J. Wyatt. 2004. Digestible energy values of diets with different fat supplements when fed to lactating dairy cows. J. Dairy Sci. 87: 1446-1454.

Wu, Z., O.A. Ohajuruka, and D.L. Palmquist. 1991. Ruminal Synthesis, Biohydrogenation, and Digestibility of Fatty Acids by Dairy Cows1. J. Dairy Sci.74:3025–3034.

CHAPTER 4

MILK PRODUCTION RESPONSES TO A CHANGE IN DIETARY STARCH CONCENTRATION VARY BY PRODUCTION LEVEL IN DAIRY CATTLE

INTRODUCTION

Corn grain is typically included in dairy cattle diets to increase the energy density of the ration providing glucose precursors for milk production and substrates for microbial protein production. There is increasing competition for corn because of increasing utilization for human food and biofuels. Identifying alternative feedstuffs that provide energy and maintain milk component yields will decrease dependence on high starch ingredients such as corn. Non-forage fiber sources (NFFS) have been researched as alternatives to starch for lactating dairy cattle (e.g. Firkins and Eastridge, 1992; Ipharraguerre et al., 2002; Voelker and Allen, 2003; Mahjoubi et al., 2009). For example, soyhulls have been effectively utilized in diets due to their high energy density, low filling effect, and highly digestible NDF. Soyhulls, however, are not as rapidly digestible in the rumen as starch sources, so they could improve rumen pH (Firkins, 1997), but they yield fewer glucose precursors compared with corn.

Previous studies have compared the effects of NFFS and starch on milk production. Ipharraguerre et al. (2002) determined the effect of replacing dry ground corn with soyhulls at concentrations from 0 to 40% diet DM in low producing dairy cattle (30 kg/d), and Voelker and Allen (2003) determined the effect of replacing high-moisture corn with beet pulp at concentrations up to 24% diet DM. In both experiments, substitution of NFFS for corn did not affect milk yield, and in one case increased milk fat yield (Ipharraguerre et al., 2002). Both studies, however, observed a reduction in DMI at the highest levels of NFSS inclusion, likely because physical fill was beginning to limit feed intake.

Cows varying in milk production have previously been reported to respond differently to diets differing in the ratio of forage to concentrate (Voelker at al., 2002) and the ruminal fermentability of starch (Bradford and Allen, 2004). A low-forage diet increased DMI linearly as preliminary milk yield increased compared to a high-forage diet. However, 3.5% FCM increased quadratically as preliminary milk yield increased but the response was positive only for cows over ~ 45 kg/d of milk (Voelker at al., 2002). Compared with dry corn, high-moisture corn decreased 3.5% FCM and fat yields for low producing cows but increased 3.5% FCM and fat yields for high producing cows (Bradford and Allen, 2004). Although treatment responses to changes in ratio of forage to concentrate and ruminal starch fermentability differed with level of milk production, we are uncertain if the same is true for changes in the concentration of starch in the diet with the same concentration of forage NDF across levels of milk production.

Based on these previous findings, our objectives were twofold; first, to determine the effects of starch concentration in diets with the same concentration of forage NDF on DMI, nutrient digestibility, yields of milk and milk components, and feed efficiency, and second to evaluate if responses to dietary treatments differ for cows varying in milk yield. We hypothesized that high-producing cows respond more favorably to a high starch diet compared with low-producing cows because the requirement for glucose precursors increases with milk yield.

MATERIALS AND METHODS

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University. Thirty-two mid-lactation (115 ± 22 DIM) Holstein cows (7 primiparous, 25 multiparous) from the Michigan State University Dairy Field

Laboratory were randomly assigned to treatment sequence in a crossover design experiment with 28-d periods preceded by a 14-d preliminary period.

Treatments consisted of diets containing either ground corn grain (CG) or soyhulls (SH) at 30% diet DM. The diet fed during the preliminary period was intermediate between the two diets. Diets were based on corn silage and alfalfa silage as the major forage components and formulated to contain 24% forage NDF and 16% CP (Table 4.1). Minerals and vitamins were formulated according to NRC (2001) recommendations. The DM concentration of forages was determined twice weekly and diets were adjusted when necessary. All cows were housed in the same tie-stall throughout the entire experiment and milked twice daily (0430 and 1530 h). Access to feed was blocked from 1000 to 1200 h to allow for collecting orts and offering feed. Cows were fed 115% of expected intake at 1200 h daily. Water was available ad libitum in each stall, and stalls were bedded with sawdust and cleaned twice daily.

Milk yield was recorded for each milking during the last 3 d of the preliminary period when cows were offered the common diet. For production and digestibility responses, samples and data were collected during the last 5 d of each treatment period (d 24 to 28). Samples of all diet ingredients (0.5 kg) and orts from each cow (12.5%) were collected daily and composited by period for analysis. Milk yield was recorded and two milk samples were collected at each milking. One aliquot was collected in a sealed tube with preservative (bronopol tablet; D&F Control Systems, San Ramon, CA) and stored at 4^{o}C for milk component analysis. The second aliquot was stored without preservative at -20^{o}C until analyzed for FA composition. Fecal (~ 400 g) samples were collected every 15 h for the last 5 d of each period resulting in 8 samples per cow per period, representing every 3 h of a 24 h period to account for diurnal variation. Fecal samples were stored at -20^{o}C until dried, ground, and composited on an equal DM basis.

Diet ingredients, orts, and fecal samples were dried at 55°C in a forced-air oven for 72 h for DM determination. Dried samples were ground with a Wiley mill (1 mm screen; Arthur H. Thomas, Philadelphia, PA). Diet ingredients were analyzed for NDF, indigestible NDF, CP, and starch as described by Kammes and Allen (2012). Indigestible NDF was measured as NDF after 240 h in vitro fermentation (Goering and Van Soest, 1970) using rumen fluid from a non-lactating mature cow fed dry hay. The concentration of FA in feed ingredients was determined as described by Lock et al. (2013).

Individual milk samples were analyzed for fat, true protein, and lactose concentration by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan Herd Improvement Association (Universal Lab Services, Lansing MI). Yields of 3.5% FCM, ECM (NRC, 2001), and milk components were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each collection period.

Milk samples used for analysis of FA composition were composited based on milk fat yield (d 24-28 of each treatment period). Milk lipids were extracted and FA methyl esters (FAME) prepared according to our methods described previously (Lock et al., 2013). Quantification of FA composition covering ~70 FA in the range C4:0 to C24:0 was determined using GLC with a CP-Sil 88 WCOT fused silica column (100 m × 0.25 mm i.d. × 0.2 μm film thickness; Varian Inc., Lake Forest, CA) as described by Lock et al. (2013). Yield of individual FA (g/d) in milk fat were calculated by using milk fat yield and FA concentration to determine yield on a mass basis using the molecular weight of each FA while correcting for glycerol content and other milk lipid classes (Piantoni et al., 2013).

We determined energy partitioning during treatment periods from weekly milk samples taken from four consecutive milkings and analyzed for fat, protein, and lactose concentrations

(Universal Lab Services, Lansing MI), BW measurements five times per wk following the morning milking, and BCS determined by three trained investigators on a 5-point scale (in 0.25 point increments; Wildman et al., 1982) on the last day of each period. These values were used to calculate milk energy output, metabolic BW, and body tissue gain throughout treatment periods.

Milk energy output (MCal/d) for a cow was estimated according to NRC (2001) with an adjustment for the coefficient from crude protein to true protein: Milk energy output (Mcal/d) = [9.29 × fat (kg) + 5.63 × true protein (kg) + 3.95 × lactose (kg)],

where each component was based on the average output of a cow during the 28-d period. Metabolic BW (MBW) was $BW^{0.75}$, where BW was the mean BW for the cow during the 28-d period. Mean daily BW change (kg) was calculated for each cow within period by linear regression after two iterations of removing outliers. Energy expended for body energy gain (Mcal/d) was estimated according to NRC (2001):

Body energy gain (Mcal/d) = [(2.88+1.036 × BCS) × ΔBW], where BCS was the average BCS for a cow during the 28-d period.

Energy concentration of the diet was calculated as the sum of milk energy output, MBW, and body energy gain divided by DMI for each cow on each diet. Energy partitioning was predicted based on observed performance: % to milk, maintenance, or body tissue = [milk energy output, 0.08 × MBW, or body energy gain /(milk energy output + 0.08 × MBW + body energy gain) × 100], where % to milk, maintenance, or body tissue is the percent of energy partitioned to milk production, maintenance requirement, or body energy gain, respectively.

All data were analyzed using the fit model procedure of JMP (version 10; SAS Institute, Cary, NC). Preliminary milk was used as a covariate and was calculated as the mean daily milk

production for the last 3 days of the preliminary period. Data were analyzed using the following model:

$Y_{ijk} = \mu + C_i + P_j + T_k + pMilk + T_k \times pMilk + pMilk^2 + T_k \times pMilk^2 + e_{ijk}$

where Y_{ijk} = the dependent variable, μ = the overall mean, C_i = random effect of cow (i = 1 to 32), P_j = fixed effect of period (j = 1 or 2), T_k is the fixed effect of treatment (k = 1 or 2), pMilk is the linear effect of preliminary milk yield, $T_k \times pMilk$ is the interaction between treatment and the linear effect of preliminary milk yield, $pMilk^2$ is the quadratic effect of preliminary milk yield, $T_k \times pMilk^2$ is the interaction between treatment and the quadratic effect of preliminary milk yield, and e_{ijk} = the residual error.

The interaction between period and treatment was initially included in the model and removed because $P > 0.20$ for all variables of interest. Interactions between treatment and preliminary milk yield were removed from the model using the same criteria of $P > 0.20$. Treatment terms were declared significant at $P \leq 0.05$ and trends were declared at $P \leq 0.10$. All data are expressed as least square means and standard error of the means, unless otherwise specified.

Three cows were excluded from our results, one cow had mastitis during the preliminary period causing a severe reduction in milk yield and her data was not used because of our interest in preliminary milk by treatment interactions. Two cows were excluded because they were highly influential outliers using Cook's D test (Cook and Weisberg, 1982) for three or more production variables > 0.20.

RESULTS

Diets contained 50% forage on a DM basis, with a 1:1 ratio of corn silage to alfalfa silage and 5% wheat straw (Table 4.1). The SH diet contained 30% soyhulls, by reducing ground corn and a small reduction in soybean meal to maintain similar protein concentration in both diets. Dietary NDF was 27% and 44% and starch was 30% and 12% DM for CG and SH, respectively. Total dietary FA were relatively low for both diets at 2.3% for CG and 1.7% for SH. At the end of the covariate period, BW of cows was 679 ± 77 kg, milk yield was 41.0 ± 9.7 kg/d, milk fat concentration was 3.78% ± 0.56%, and milk protein concentration was 3.08% ± 0.28% (mean ± SD).

The SH treatment reduced DMI and milk yield by 6% and 10%, respectively (both $P < 0.001$; Table 4.2). We detected a treatment by preliminary milk yield interaction for milk yield, with the difference in milk yield for SH compared with CG increasingly greater for cows with higher milk yield (linear interaction $P < 0.001$; Figure 4.1). We observed similar treatment by preliminary milk yield interactions for yields of fat, protein, lactose, ECM, and 3.5% FCM (all linear interactions $P < 0.05$). There was no treatment by preliminary milk yield interaction for DMI.

The SH treatment decreased the yields of milk fat and protein by 11% and 14% (both $P < 0.001$) compared with CG. Similarly, SH decreased the concentration of milk protein by 4% ($P < 0.001$) but did not affect the concentration of milk fat ($P = 0.17$) compared with CG. The SH treatment reduced milk:feed (ECM/DMI) 4% ($P = 0.01$). Treatment did not affect BW ($P = 0.11$) or BCS ($P = 0.62$; Table 4.3).

Dietary energy values based on animal performance indicated that the CG treatment tended to contain ~ 5% more Mcal NE_L/kg than the SH treatment ($P = 0.07$, Table 4.3).

Calculated milk energy output was greater for CG than SH ($P < 0.001$) and was increasingly greater for cows with greater preliminary milk yield ($P = 0.04$ for pMilk x treatment interaction). Daily change in BW (kg/d) and body energy gain (Mcal/d) were similar for treatments (both $P > 0.10$). The percentages of energy partitioned to milk ($P = 0.99$) and used for body energy gain ($P = 0.30$) were similar for both treatments, while the percentage of energy used for maintenance was higher for SH ($P = 0.002$).

Treatment altered the concentration and yield of FA in milk fat according to source (<16 carbon FA from de novo synthesis in the mammary gland, >16 carbon FA originating from extraction from plasma, and 16-carbon FA originating from mixed sources; Table 4.4). On a concentration basis, SH decreased de novo synthesized FA ($P < 0.001$) and preformed milk FA ($P = 0.04$), but increased 16-carbon FA (< 0.001). On a yield basis, SH decreased both de novo and preformed milk FA (both, $P < 0.001$) with no difference in 16-carbon milk FA (Table 4.4). A significant treatment by preliminary milk interaction was detected for preformed milk FA yield (P=0.02), which parallels the interaction observed for milk yield. The SH treatment decreased the concentration and yield of *trans*-10 C18:1 in milk (data not shown) but the concentration and yield of *trans*-10, *cis*-12 C18:2 was below detectable limits for all samples.

Apparent total tract digestibility of DM and NDF was affected by treatment (Table 4.5). Digestibility of NDF increased for SH compared with CG (53 vs. 40%; $P < 0.001$) for all cows with a greater difference in NDF digestibility between treatments for higher producing cows (interaction $P < 0.01$; Figure 4.2). The interaction was because NDF digestibility decreased as preliminary milk yield increased for CG ($P < 0.001$), but there was no relationship between NDF digestibility and preliminary milk yield for SH ($P = 0.61$, Figure 4.2). Digestibilities of starch

and CP were unaffected by treatment ($P = 0.81$ and $P = 0.29$, respectively; Table 4.5) but SH decreased DM digestibility compared with CG (66% vs. 68%, $P < 0.001$).

DISCUSSION

Previous research has evaluated the effects of diets high in NFFS (relatively low in starch) compared with higher starch diets containing corn grain (e.g. Ipharraguerre et al., 2002; Voelker and Allen, 2003). These studies reported that NFFS could be included in the diet at high levels (30 and 24% DM, respectively) with no detrimental effects on production parameters. However, the cows in these studies had only moderate milk yield (30 and 36 kg/d, respectively). We postulated that results might differ for higher producing cows with greater glucose requirements. Variation in production response, dependent on level of milk production, has been previously reported in diets differing in ratio of forage to concentrate (Voelker et al., 2001) and starch source (Bradford and Allen, 2004). Therefore, in our current study, cows with a wide range of milk yield (28 to 62 kg/d) during the preliminary period were used to evaluate effects of diets differing in starch concentrations.

The forage composition of both diets was the same so forage NDF remained constant. The SH treatment reduced DMI compared with CG, consistent with previous results when soyhulls were substituted for corn grain at 30% of diet DM (Ipharaguerre et al., 2002). This is likely because some cows were limited by physical fill. Although NFFS generally have little effect on DMI when substituted in the diet for grains (Allen, 2000), soyhulls likely digest and pass from the rumen more slowly than corn grain and physical fill might already have been limiting DMI for some cows. In our current experiment, forage NDF concentration (~ 24%) was relatively high given the mean milk yield of these cows (40 kg/d ECM for the CG treatment).

Forage NDF concentration is filling relative to other diet fractions and ruminal fill is a greater limitation to DMI as milk yield increases (Allen, 2000).

Failure to detect an interaction for DMI between preliminary milk yield and treatment was not expected. When fed a low-forage diet, compared to a high-forage diet, DMI was greater for higher producing cows than lower producing cows (Voelker et al. 2002). Higher producing cows were able to consume more and meet their energy demands on the low forage diet because they were not limited by physical fill. We expected that for our current study, if SH reduced DMI, the reduction would be greater as preliminary milk yield of cows increased because rumen fill likely limits feed intake to a greater extent for these cows (Allen, 2000). Additionally, we thought that increased propionate production from CG compared with the SH treatment might decrease DMI more for lower-producing cows by stimulating hepatic oxidation (Allen et al., 2009). However, rumen distention might have dominated control of feed intake from the high forage NDF concentration of the treatment diets even for the lower producing cows and propionate supply might not have been excessive because dry corn is not highly fermentable in the rumen (Allen, 2000). Even though we had a wide range in milk production, the cows were relatively close in DIM. Whether using cows with greater variation in stage of lactation may have resulted in a treatment by preliminary milk interactions for DMI is not known.

Our two treatments contained extreme concentrations of starch relative to diets typically fed to lactating cows. The SH treatment with 12% starch decreased milk production relative to the CG diet with 30% starch. However, the cows in this experiment had a wide range of milk yield and the reduction in milk yield was much less for lower producing cows with a lower requirement for glucose precursors. At the low end of the range in milk yield (< 30 kg/d), cows were able to maintain milk yield on the 12% compared with the 30% starch diet. The preliminary

milk by treatment interactions for yields of fat, lactose, ECM and 3.5% FCM were all driven by milk yield, with a greater response to the CG treatment for higher producing cows and the lower producing cows able to maintain production on the SH treatment.

High producing cows generally have lower plasma insulin concentration and are less insulin sensitive than lower-producing cows which favors energy partitioning to milk compared to adipose tissue (De Koster and Opsomer, 2013). The CG treatment likely increased the supply of glucose precursors and glucose, allowing greater lactose production and milk yield but also greater insulin secretion. Greater plasma insulin concentration is more likely to partition more energy to adipose tissue, particularly for lower producing cows that are more insulin sensitive than high-producing cows.

The reduction in milk protein concentration from the SH diet might have been the result of decreased dietary starch concentration. Less starch likely reduced microbial protein, and in turn, the amount of protein available for milk protein synthesis (Weiss et al., 2012). Additionally, the SH diet had 4% less CP than the CG diet and resulted in 6% less DMI, both contributing to less protein available for milk protein synthesis. Furthermore, higher starch diets would likely cause an increase in insulin concentrations, and insulin seems to play a role in milk protein synthesis (Griinari et al., 1997; Mackle et al., 1999).

We expected to see a reduction in milk fat concentration when cows were fed the CG diet. The high starch and relatively low fiber content of the CG diet are risk factors for milk fat depression (Bauman et al., 2011). However, we did not observe a reduction in milk fat with CG. This may have been due to: 1) the very low concentration of FA in CG (2.26% DM) limiting the supply of PUFA to significantly alter rumen fermentation and produce biohydrogenation intermediates associated with milk fat depression; 2) moderate ruminal fermentability of starch

in the CG treatment limiting fluctuations in rumen pH; and/or 3) high rumen buffering capacity from the high concentration of forage NDF maintaining ruminal pH. Notably, we did not detect *trans*-10, *cis*-12 C18:2 in any of our milk samples although we observed increases in *trans*-10 C18:1 for CG, suggesting that the CG diet did alter biohydrogenation pathways to a limited extent.

The increase in NDF digestibility with the SH treatment in our study is similar to results reported by Voelker and Allen (2003) with 24% beet pulp compared with high moisture corn increasing NDF digestibility. Although DM digestibility was increased with added beet pulp diets (Voelker and Allen, 2003), we observed a reduction in DM digestibility with added NFFS (soyhulls) despite higher NDF digestibility, indicating that the NDF from soyhulls was less digestible than starch from corn grain. The difference in the intercept in Figure 4.2 (50 vs. 55% for CG and SH, respectively) of NDF digestibility is because the NDF from the soyhulls in SH was more digestible than the NDF in the forages, which supplied the majority of the NDF in CG. However, the change in slope for the treatments could be due to a number of factors. Cows on CG had higher intake and this change could relate to higher passage rates, which could have reduced the amount of time the NDF was available in the rumen for digestion. Additionally, high starch diets might have reduced ruminal pH or altered the microbial population enough to depress fiber digestion.

CONCLUSIONS

While we observed distinct differences in treatment means for many production and digestibility parameters, preliminary milk by treatment interactions gave further insight on responses to diets varying in dietary starch concentration. Higher producing cows benefited

more from the high starch diet while lower producing cows were able to maintain production with a low starch diet. The finding that cows at different levels of milk production responded differently to dietary starch content indicates that grouping cows by production level would enable optimum allocation of feeds.

APPENDIX

APPENDIX

Table 4.1. Ingredients and nutrient composition of experimental diets[1].

	Treatments	
	CG	SH
Ingredients, % of DM		
Corn silage	22.5	22.5
Alfalfa silage	22.5	22.5
Wheat straw	5.0	5.0
Ground corn	30.0	3.6
Soyhulls	---	30.0
Soybean meal	17.0	13.7
Vitamin & mineral mix[2]	2.0	2.0
Limestone	0.50	---
Sodium bicarbonate	0.50	0.50
Dicalcium phosphate	---	0.25
Nutrient Composition, % of DM		
DM[3]	53.9	53.6
NDF	27.2	43.9
Forage NDF	23.6	23.9
Starch	30.1	12.2
CP	16.5	15.9
FA	2.26	1.69

[1] Experimental diets fed to 32 cows in a crossover design with 28-d periods.
[2] Vitamin and mineral mix contained 34.1% dry ground shell corn, 25.6% white salt, 21.8% calcium carbonate, 9.1% Biofos, 3.9% magnesium oxide, 2% soybean oil, and < 1% of each of the following: manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, iodine, cobalt carbonate, vitamin E, vitamin A, vitamin D, and selenium.
[3]Expressed as percent of as fed.

Table 4.2. Dry matter intake, milk production, milk components, feed efficiency, body weight, and body condition score for cows fed treatment diets (n=29; data collected d 24 to 28 of each treatment period).

	Treatments[1]			*P*-values[2]	
Variable	CG	SH	SEM	Trt	Trt × pMilk
DMI, kg/d	28.0	26.4	0.57	<0.001	NS
Milk Yield, kg/d					
Milk	38.7	34.8	0.83	< 0.001	< 0.001
3.5% FCM[3]	40.1	36.5	0.93	< 0.001	0.003
ECM[4]	40.3	36.3	0.89	< 0.001	< 0.001
Milk Components					
Fat, kg/d[5]	1.45	1.29	0.05	< 0.001	0.02
Fat, %	3.77	3.85	0.10	0.17	NS
Protein, kg/d	1.27	1.09	0.03	< 0.001	< 0.001
Protein, %	3.32	3.18	0.05	< 0.001	NS
Lactose, kg/d	1.88	1.68	0.05	< 0.001	< 0.001
Lactose, %	4.85	4.83	0.03	0.42	NS
ECM/DMI	1.45	1.39	0.04	0.01	NS

[1] Treatments contained 30% dried ground corn (CG) or 30% soyhulls (SH) on a DM basis.
[2] *P*-value associated with treatment differences (CG vs. SH; Trt) and the linear interaction between treatment and preliminary milk yield (Trt × pMilk).
[3] Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].
[4] Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)].
[5] Significant treatment by quadratic preliminary milk yield interaction (*P* = 0.05).

Table 4.3. Energy partitioning[1] for cows fed treatment diets (n=29).

	Treatments[2]			*P*-value[3]	
Nutrients	CG	SH	SEM	Trt	Trt × pMilk
BW[4]	691	689	13.9	0.11	NS
BCS[4]	2.76	2.75	0.08	0.62	NS
Change in BW, kg/d	0.54	0.34	0.10	0.19	NS
Change in BCS, pt/28 d	0.10	0.06	0.04	0.45	NS
Calculated energy values					
Dietary Energy, Mcal/kg	1.63	1.55	0.04	0.07	NS
Milk, Mcal/d	28.5	26.0	0.60	< 0.001	0.04
BW Change, kg/d	0.54	0.34	0.10	0.19	NS
Body Energy Gain, Mcal/d	3.13	1.93	0.57	0.16	NS
Maintenance, Mcal/d	10.8	10.7	0.16	0.13	NS
Partitioning Milk, % energy intake	67.1	67.1	1.34	0.99	NS
BW Gain, % energy intake	6.93	4.67	1.46	0.30	NS
Maintenance, % energy intake	25.9	28.2	0.77	0.002	NS

[1] Based on animal performance throughout the 28 d periods.
[2] Treatments contained 30% dried ground corn (CG) or 30% soyhulls (SH) on a DM basis.
[3] *P*-value associated with treatment differences (CG vs. SH; Trt) and the linear interaction between treatment and preliminary milk yield (Trt × pMilk).
[4] Mean throughout the 28 d period.

Table 4.4. Milk fatty acid concentration and yield by sources of fatty acids of cows fed treatment diets (n=29).

	Treatments[1]			*P*-values[2]	
Sources of milk fatty acids[3]	CG	SH	SEM	Trt	Trt × pMilk
FA concentration, (g/100 g)					
De novo	29.1	28.8	0.31	< 0.001	NS
Mixed	35.4	37.9	0.41	< 0.001	NS
Preformed	34.7	34.0	0.44	0.04	NS
FA yield, (g/d)					
De novo	403	351	14.6	< 0.001	NS
Mixed	480	476	19.8	0.69	NS
Preformed	465	421	11.7	< 0.001	0.02

[1] Treatments contained 30% dried ground corn (CG) or 30% soyhulls (SH) on a DM basis.
[2] *P*-value associated with treatment differences (CG vs. SH; Trt) and the linear interaction between treatment and preliminary milk yield (Trt × pMilk).
[3] De novo = milk FA < 16 carbons in length; mixed = milk FA 16-carbons in length; preformed = milk FA > 16 carbons in length.

Table 4.5. Nutrient digestibility of cows fed treatment diets (n=29).

	Treatments[1]			*P*-value[2]	
Nutrients	CG	SH	SEM	Trt	Trt × pMilk
DM	68.3	65.5	0.37	< 0.001	NS
NDF	39.6	53.4	0.65	< 0.001	0.01
Starch	94.2	94.1	0.36	0.81	NS
CP	68.9	68.3	0.44	0.29	NS

[1] Treatments contained 30% dried ground corn (CG) or 30% soyhulls (SH) on a DM basis.
[2] *P*-value associated with treatment differences (CG vs. SH; Trt) and the linear interaction between treatment and preliminary milk yield (Trt × pMilk).

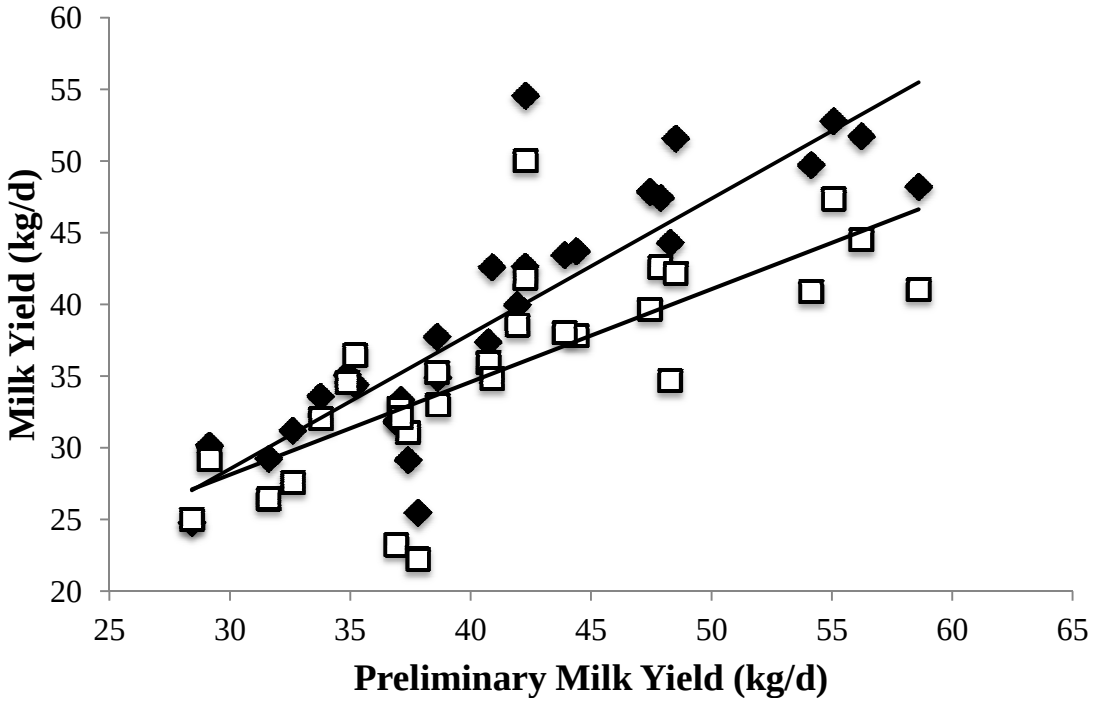

Figure 4.1. **Relationship between milk yield (kg/d) and preliminary milk yield (kg/d) for cows fed 30% dry ground corn (CG) or 30% soyhulls (SH; n=29).** CG represented by black diamonds, Milk yield (kg/d) = 0.19 + 0.94 × preliminary milk yield (kg) ($R^2 = 0.75$; $P < 0.001$). SH represented by open squares, Milk yield (kg/d) = 8.72 + 0.65 × preliminary milk yield (kg) ($R^2 = 0.56$; $P < 0.001$).

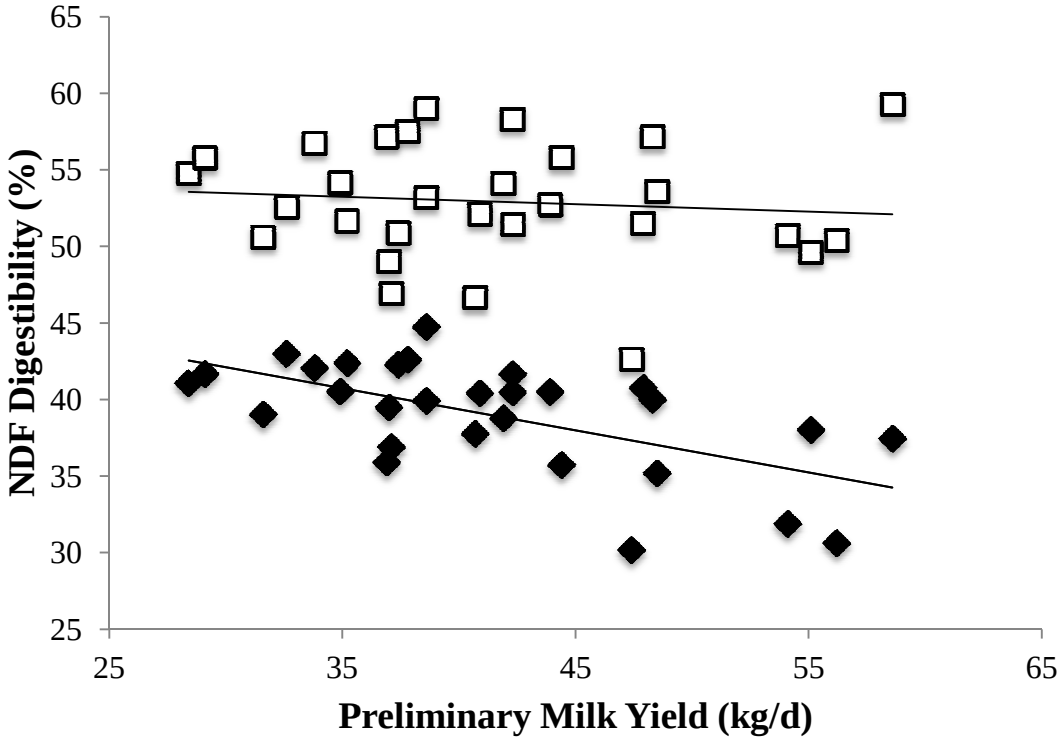

Figure 4.2. **Relationship between NDF digestibility (%) and preliminary milk yield (kg/d) for cows fed 30% dry ground corn (CG) or 30% soyhulls (SH; n=29).** CG represented by black diamonds, NDF digestibility % = 50.33 – 0.27 × preliminary milk yield (kg) (R^2 = 0.37; *P* < 0.001). SH represented by open squares, NDF digestibility % = 54.94 – 0.05 × preliminary milk yield (kg) (R^2 = 0.01; *P* = 0.61).

REFERENCES

REFERENCES

Allen, M.S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624.

Allen, M. S., B. J. Bradford, and M. Oba. 2009. Board Invited Review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim. Sci. 87:3317-3334.

AOAC. 1990. Official Methods of Analysis. 15th ed. AOAC International, Arlington, VA.

Bradford, B. J., and M. S. Allen. 2004. Milk fat responses to a change in diet fermentability vary by production level in dairy cattle. J. Dairy Sci. 87:3800-3807.

Cook, R. D. and S. Weisberg. 1982. Monographs on Statistics and Applied Probability: Residuals and Influence in Regression. Chapman and Hall, New York, NY.

De Koster, J. D., and G. Opsomer. 2013. Insulin resistance in dairy cows. Vet. Clin. North Am. Food Anim. Pract. 29: 299-322.

Firkins, J. L. 1997. Effects of feeding nonforage fiber sources on site of fiber digestion. J. Dairy Sci. 80: 1426-1437.

Firkins, J. K. and M. L. Eastridge. 1992. Replacement of forage or concentrate with combinations of soyhulls, sodium bicarbonate, or fat for lactating diary cows. J. Dairy Sci. 75: 2752-2761.

Glasser, F., M. Doreau, A. Ferlay, Y. Chilliard. 2007. Technical Note: Estimation of milk fatty acid yield from milk fat data. J. Dairy Sci. 90: 2302-2304.

Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. USDA-ARS, Washington, DC.

Griinari, J. M., M. A. McGuire, D. A. Dwyer, D. E. Bauman, D. M. Barbano, and W. A. House. 1997. The role of insulin in the regulation of milk protein synthesis in dairy cows. J. Dairy Sci. 80:2361-2371.

Harvatine, K.J., J.W. Perfield, and D.E. Bauman. 2009. Expression of enzymes and key regulators of lipid synthesis is upregulated in adipose tissue during CLA-induced milk fat depression in dairy cows. J. Nutr. 139:849–854.

Ipharraguerre, I. R., R. R. Ipharraguerre, and J. H. Clark. 2002. Performance of lactating dairy cows fed varying amounts of soyhulls as a replacement for corn grain. J. Dairy Sci. 85:2905-2912.

Ipharraguerre, I. R., and J. H. Clark. 2003. Soyhulls as an alternative feed for lactating dairy cows: a review. J. Dairy Sci. 86: 1052-1073.
Kammes, K. L. and M. S. Allen. 2012. Nutrient demand interacts with grass particle length to affect digestion responses and chewing activity in dairy cows. J. Dairy Sci. 95: 807-823.

Kronfeld, D. S., Major metabolic determinants of milk volume, mammary efficiency, and spontaneous ketosis in dairy-cows. J. Dairy Sci. 65:2204-2212.

Lock, A. L., T. R. Overton, K. J. Harvatine, J. Giesy, and D. E. Bauman. 2006. Milk fat depression: Impact of dietary components and their interaction during rumen fermentation. Proc. Cornell Nutr. Conf. p. 75-85.

Lock, A.L., C.L. Preseault, J.E. Rico, K.E. DeLand, and M.S. Allen. 2013. Feeding a C16:0-enriched fat supplement increased the yield of milk fat and improved conversion of feed to milk. J. Dairy Sci. 96:6650-6659.

Mackle, T. R., D. A. Dwyer, K. L. Ingvartsen, P. Y. Chouinard, J. M. Lynch, D. M. Barbano, and D. E. Bauman. 1999. Effects of insulin and amino acids on milk protein concentration and yield from dairy cows. J. Dairy Sci. 82:1512-1524.

Mahjoubi, E., H. Amanlou, D. Zahmatkesh, M. Ghelich Khan, N. Aghaziarati. 2009. Use of beet pulp as a replacement for barley grain to manage body condition score in over-conditioned late lactation cows. Anim. Feed Sci. Tech. 153:60-67.

Mertens, D. R. 1994. Regulation of forage intake. Forage quality, evaluation, and utilization. Am. Soc. Agron., Madison, WI. p. 450-493.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Acad. Sci., Washington D.C.

Pantoja, J., J. L. Firkins, M. L. Eastridge, and B. L. Hull. 1994. Effects of fat saturation and source of fiber on site of nutrient digestion and milk production by lactating dairy cows. J. Dairy Sci. 77: 2341-2356.

Schauff, D. J., J. H. Clark, and J. K. Drackley. 1992. Effects of feeding lactating dairy cows diets containing extruded soybeans and calcium salts of long-chain fatty acids. J. Dairy Sci. 75: 3003-3019.

Sukhija, P. S., and D. L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36: 1202-1206.

Ulberth, F. and F. Schrammel. 1995. Accurate quantitation of short-, medium-, and long-chain fatty acid methyl esters by split-injection capillary gas-liquid chromatography. J. Chromatography. 704: 455-463.

Voelker, J. A. and M. S. Allen. 2003. Pelleted beet pulp substituted for high-moisture corn: 1. Effects on feed intake, chewing behavior, and mil production of lactating dairy cows. J. Dairy Sci. 86: 3542-3552.
Weiss, W.P., D.E. Shoemaker, L.R. McBeth, and N.R. St-Pierre. 2012. Effects on lactating dairy cows of oscillating dietary concentrations of unsaturated and total long-chain fatty acids. J. Dairy Sci. 96:506–514. doi:10.3168/jds.2012-5835.

Wildman, E. E., G. M. Jones, P. E. Wagner, and R. L. Bowman. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65: 495-501.

CHAPTER 5

EFFECTS OF PARTLY REPLACING DIETARY STARCH WITH FIBER AND FAT ON MILK PRODUCTION AND ENERGY PARTITIONING

INTRODUCTION

In established lactation, cows are usually in positive energy balance and the goals are to maximize milk and component yields and reduce excessive conditioning. During milk fat depression (MFD), there is a decreased priority for milk production and an increased priority for storage of energy as adipose (Van Soest, 1963; Griinari and Bauman, 2006). Therefore diets that cause MFD result in decreased fat-corrected milk yield per unit of feed. As a result, diet-induced MFD is detrimental in the short term because of decreased milk income over feed costs and may have negative effects in the long term because of condition gain and health problems associated with excessively conditioned cows. Cows that gain excessive body condition in mid and late lactation are at high risk for culling in the subsequent lactation due to increased incidence of metabolic diseases and reproductive failure (NRC, 2001; Roche et al., 2009).

In studies comparing high starch to high fat diets, the higher fat diets partitioned more energy towards milk, whereas the higher starch diets had reduced milk fat output and increased BW (Grum et al., 1996; van Knegsel et al., 2007a). In both studies, increased dietary starch resulted in higher plasma insulin concentrations, and it is well established that insulin inhibits lipid mobilization and stimulates lipogenesis in adipose tissue (Bauman, 2000). Additionally, milk fat synthesis is reduced by specific FA intermediates produced in the rumen by altered rumen biohydrogenation (Bauman et al., 2011). Increasing dietary starch, due to or independent of reduced rumen pH, can increase the formation of biohydrogenation intermediates associated with MFD (Bauman et al., 2011). Specifically, *trans*-10, *cis*-12 C18:2 has been shown to reduce

gene expression of lipogenic enzymes in the mammary gland while up-regulating lipogenic gene expression in adipose tissue (Harvatine et al. 2009). Therefore, increased rumen synthesis and outflow of *trans*-10, *cis*-12 C18:2 can alter the partitioning of energy from the mammary gland to adipose tissue.

Previous studies comparing high starch to high fat diets were in lower producing (< 30 kg/d of milk production; Grum et al., 1996) or early lactation (van Knegsel et al., 2007a) cows, while our interest is in production responses to diets differing in starch, NDF, and fat in post-peak higher producing cows. Our objectives, therefore, were to quantify the effect of diets designed to alter milk fat yield on production, BW gain, body condition, and feed efficiency. Our hypothesis was that post-peak cows fed a high starch vs. a high fiber and fat diet would allocate more energy towards body gain due to reductions in milk fat yield through the production of biohydrogenation intermediates and/or increased plasma insulin concentration that reduce milk fat synthesis and increase adipose tissue gain.

MATERIALS & METHODS

The Institutional Animal Care and Use Committee at Michigan State University approved experimental procedures. Thirty-two mid-lactation (102 ± 22 DIM; mean ± SD) Holstein cows (16 primiparous, 16 multiparous) fed a common diet for a minimum of 14 d were selected from the Michigan State University Dairy Field Laboratory. Production data was collected the last 3 d of the preliminary period for covariate values. Cows were blocked by milk yield and parity and then randomly assigned to treatment sequence in a crossover design experiment with 28-d periods. Samples were taken throughout the period for calculations of energy gain and

partitioning, but only samples collected during the final 5 d of each experimental period were used for production measurements, digestibility, and plasma metabolites and hormones.

Treatments consisted of a high fiber and fat diet (HFF) containing a 50:50 ratio of forage to concentrate containing a C16:0-enriched fat supplement at 2.5% of diet DM or a high starch diet (HS) containing a 40:60 ratio of forage to concentrate containing a mixture of dry ground and high moisture corn (Table 5.1). Although the diets differed in nutrient composition, diets were formulated to contain a similar predicted energy density according to NRC (2001). Mineral and vitamins were formulated according to NRC (2001) recommendations. DM concentration was determined twice weekly for forages, and diets were adjusted when necessary. All cows were housed in the same tie-stall (assigned randomly) throughout the entire experiment and milked twice daily (0500 and 1600 h). Access to feed was blocked from 1000 to 1200 h to allow for collection of orts and offering of new feed. Cows were fed 115% of expected intake at 1200 h daily. Water was available ad libitum in each stall, and stalls were bedded with sawdust and cleaned twice daily.

Samples and data for production variables, digestibility estimates, and plasma measurements were collected during the last 5 d of each treatment period (d 24 to 28). Samples of all diet ingredients (0.5 kg) and orts from each cow (12.5%) were collected daily and composited by period for analysis. Milk yield was recorded and two milk samples were collected at each milking. One aliquot was collected in a sealed tube with preservative (bronopol tablet; D&F Control Systems, San Ramon, CA) and stored at 4^{o}C for milk component analysis. The second aliquot was stored without preservative at -20^{o}C until analyzed for FA composition.

Fecal (~ 400 g) and blood (~ 15 ml) samples were collected every 15 h for the last 5 d of each period resulting in 8 samples per cow per period, representing every 3 h of a 24-h period to

account for diurnal variation. Feces were stored in a sealed plastic cup at −20°C until dried and composited on equal DM basis for each cow period. Blood was collected by coccygeal venipuncture into three evacuated tubes; two contained potassium EDTA as an anticoagulant and the other contained potassium oxalate as an anticoagulant and sodium fluoride as a glycolytic inhibitor. Blood was stored on ice until centrifugation at 2,000 × g for 15 min at 4°C (within 30 min of sample collection). Plasma was transferred into microcentrifuge tubes and stored at -20^{o}C until composited by cow period.

On the last d of the preliminary period and last d of each treatment period, subcutaneous fat thickness was determined at two locations, the 12th intercostal space and the sacral region between the tuber coxae (hooks) and tuber ischia (pins) via ultrasound (Aloka SSD-500V Ultrasound equipped with a 172-mm Linear Body Composition Transducer). Ultrasound images were analyzed by the National Centralized Ultrasound Processing Lab (Ames, IA), and change in subcutaneous fat thickness was calculated as the difference between a measurement and the previous measurement. Three trained investigators determined BCS on a 5-point scale (in 0.25-point increments; Wildman et al., 1982) on the last day of the preliminary period and each treatment period.

Diet ingredients, orts, and fecal samples were dried at 55°C in a forced-air oven for 72 h for DM determination. Dried samples were ground with a Wiley mill (1 mm screen; Arthur H. Thomas, Philadelphia, PA). Feed ingredients, orts, and feces were analyzed for NDF, CP, and starch concentrations as described by Kammes and Allen (2012). The concentration of FA in feed ingredients, orts, and feces were determined as described by Lock et al. (2013). Indigestible NDF, used for estimating fecal output and thus digestibility of nutrients (Cochran et al., 1986), was estimated as NDF after 240-h in vitro fermentation (Goering and Van Soest, 1970) using

rumen fluid from a non-lactating mature cow fed dry hay; flasks were reinoculated at 120 h to ensure a viable microbial population.

Individual milk samples were analyzed for fat, true protein, and lactose concentration by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan Herd Improvement Association (Universal Lab Services, Lansing MI). Yields of 3.5% FCM, milk energy, and milk components were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each collection period.

Milk samples used for analysis of FA composition were composited based on milk fat yield (d 25-28 of each period). Milk lipids were extracted, and FA-methyl esters were prepared and quantified using GLC according to our methods described previously (Lock et al., 2013). Yield of individual FA (g/d) in milk fat were calculated by using milk fat yield and FA concentration to determine yield on a mass basis using the molecular weight of each FA while correcting for glycerol content and other milk lipid classes (Piantoni et al., 2013).

All plasma samples were analyzed in duplicate with a CV of < 5% between duplicates. Commercial kits were used to determine plasma concentrations of NEFA (NEFA-HR (2) kit; Wako Chemicals, Richmond, VA) and triglycerides (L-Type Triglyceride M kit; Wako Chemicals, Richmond, VA). Insulin was measured with a Bovine Insulin ELISA using a solid phase two-site enzyme immunoassay (Mercodia, Uppsala, Sweden). Glucose was quantified using a glucose oxidase method (PGO Enzyme Product No. P7119; Sigma Chemical Co.).

We determined energy partitioning throughout treatment periods using weekly milk samples taken from four consecutive milkings and analyzed for fat, protein, and lactose concentrations and BW measurements taken three times per wk following the morning milking.

Data were used to calculate milk energy output, metabolic BW, and body tissue gain through treatment periods.

Milk energy output (Mcal/d) was calculated according to NRC (2001) with an adjustment in the coefficient to account for the difference between crude protein and true protein:

Milk energy output (Mcal/d) = [9.29 × fat (kg) + 5.63 × true protein (kg) + 3.95 × lactose (kg)], where each component was based on the average output of a cow during the 28-d period.

Metabolic BW (MBW) was estimated as $BW^{0.75}$, where BW was the mean BW for a cow during the 28-d period. Mean daily BW change (kg/d) was calculated for each cow within period by linear regression after two iterations of removing outliers. Energy expended for body tissue gain (Mcal/d) was estimated according to NRC (2001):

Body tissue gain (Mcal/d) = [(2.88+1.036 × BCS) × ΔBW],

where BCS was the average BCS for a cow during a 28-d period.

Apparent NE_L concentration of the diet was calculated for individual cows for each treatment from performance data:

Apparent NE_L of diet = [Milk energy output + (0.08 × MBW) + body tissue gain] / DMI

Energy partitioning was estimated based on observed performance:

% to milk, maintenance, or body tissue =

[milk energy output, 0.08 × MBW, or body tissue gain /(milk energy output + 0.08 × MBW + body tissue gain) × 100],

where % to milk, maintenance, or body tissue is the percent of energy partitioned to milk production, maintenance requirement, or body tissue gain, respectively.

Statistical Analysis

Two cows were removed from analysis; one cow developed a displaced abomasum during the first period, and another cow developed mastitis during the second period. Therefore all results are from the 30 remaining cows. All data were analyzed using the fit model procedure of JMP (version 10; SAS Institute, Cary, NC). Data were analyzed using the following model:

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

where Y_{ijk} = dependent variable, μ = overall mean, C_i = random effect of cow (i = 1 to 30), P_j = fixed effect of period (j = 1 or 2), T_k = fixed effect of treatment (k = 1 or 2), pMilk = covariate of preliminary milk yield, and pMilk × T_k = interaction between preliminary milk yield and treatment. The interaction between period and treatment was initially included in the model and removed because it was not significant ($P > 0.20$). Likewise, the quadratic effect of preliminary milk yield and the interaction between treatment and the quadratic effect of preliminary milk yield were initially included in the model and removed because they were not significant ($P > 0.20$). The pMilk and pMilk × T_k terms were removed from the model when $P > 0.20$ for the interaction term. Main effects were declared significant at $P \leq 0.05$, and tendencies were declared at $P \leq 0.10$. Interactions were declared significant at $P \leq 0.10$, and tendencies were declared at $P \leq 0.15$. All data are expressed as least square means and standard error of the means, unless otherwise specified.

RESULTS

Treatment diets differed in NDF, FA, and starch concentration and to a lesser extent CP concentration (Table 5.1). The HFF diet contained 33% NDF, 20% forage NDF, 16% starch, and 5.4% FA, while the HS diet contained 25% NDF, 18% forage NDF, 33% starch, and 3.2% FA (Table 5.1). Treatments contained similar amounts of 18-carbon dietary FA however, HFF

contained an additional 2.2% DM of 16-carbon dietary FA from the palmitic acid-enriched fat supplement.

The HFF treatment reduced milk yield by 1.3 kg/d ($P < 0.05$; Table 5.2) with a trend for reduced DMI of 0.5 kg/d ($P = 0.10$) compared to HS. High producing cows had increased DMI on the HFF treatment while lower producing cows had reduced DMI on HFF compared with HS (interaction, $P < 0.05$). The HFF treatment increased 3.5% FCM ($P < 0.05$) because of a 7.7% increase in fat yield of ~ 130 g/d ($P < 0.001$). The HFF treatment increased milk fat concentration ($P < 0.001$) whereas, HFF decreased milk protein yield by ~ 100 g/d and concentration (both $P < 0.001$) compared to HS. The HFF treatment increased milk energy output measured the last 5 d of each period by 0.9 Mcal/d ($P < 0.01$). The HFF treatment increased milk:feed (ECM/DMI) by 3% ($P = 0.02$) compared with HS. Lower producing cows had increased milk:feed on HFF with similar feed efficiency for higher producing cows (interaction, $P = 0.06$).

The HFF treatment reduced BW ($P = 0.01$; Table 5.3) and reduced BCS ($P < 0.001$) compared with HS. The HFF treatment reduced change in BW by ~ 13 kg compared with HS, over the 28 d treatment periods. Cows on the HFF treatment maintained BCS over the 28-d treatment periods, whereas cows on the HS treatment increased BCS by ~ 0.25 points ($P < 0.001$). Change in BCS was further evaluated by measuring subcutaneous fat thickness over the rump and 12th intercostal space at the end of periods. HFF treatment reduced both measurements ($P < 0.05$) with a difference of 0.78 mm over the rump and 0.54 mm over the 12th intercostal space between the two treatments.

Using cow performance data to calculate energy density of treatment diets indicated similar NE_L concentrations were achieved for HFF vs. HS (1.78 vs. 1.79 Mcal/kg of DM; $P =$

0.64; Table 5.3) with similar daily apparent NE_L intakes ($P = 0.60$). Throughout treatment periods, the HFF treatment increased milk energy output ($P = 0.05$; Table 5.3) and decreased energy used for body tissue gain ($P < 0.001$) compared with HS. Therefore, the HFF treatment increased the percentage of energy partitioned towards milk (72.8 vs. 67.9 %; $P < 0.001$) and reduced the percentage of energy partitioned towards body tissue gain (4.03 vs. 10.1%; $P < 0.01$).

We detected no differences in plasma glucose concentrations between treatments ($P = 0.14$; Table 5.4). However, the HFF treatment reduced insulin concentration in plasma by 27% ($P < 0.001$). Higher producing cows on the HFF treatment had reduced plasma insulin concentration compared with HS with similar insulin concentrations for lower producing cows (interaction, $P = 0.06$; Figure 5.1). The HFF treatment increased the plasma concentrations of

The HFF treatment reduced DM digestibility ($P < 0.05$) with no differences in starch digestibility ($P = 0.39$, Table 5.5). The HFF treatment increased digestibility of NDF and CP (both, $P < 0.001$). However, the HFF treatment tended to reduce total FA ($P = 0.06$) and 16-carbon FA digestibility ($P = 0.07$), but increased 18-carbon FA digestibility ($P < 0.01$) compared to HS. No relationship was evident between total FA intake and FA digestibility ($R^2 = 0.001$; $P = 0.80$; Figure 5.2) between approximately 500 to 1900 g/d of total FA intake. However, we observed a positive linear relationship between total FA intake and total FA absorbed ($R^2 = 0.94$; $P < 0.001$; Figure 5.3) up to approximately 1900 g/d intake of total FA.

The concentration of FA in milk fat according to source (<16 carbon FA from de novo synthesis in the mammary gland, >16 carbon FA originating from extraction from plasma, and 16-carbon FA originating from mixed sources) are shown in Table 5.6. Concentrations and yields of selected individual FA are shown in Tables 5.8 and 5.9, respectively. Compared with

HS, the HFF treatment reduced the concentration of de novo synthesized and preformed milk FA (both $P < 0.001$) and increased milk FA derived from both sources (16-carbons in length; $P < 0.001$; Table 5.6) primarily through an increased concentration of C16:0 in milk ($P < 0.001$; Table 5.8). The large increase in C16:0 caused shifts in relative proportions of de novo and preformed milk FA. In particular, the HFF treatment increased C4:0 in milk fat ($P < 0.001$) and decreased milk FA from 6 to 14 carbons in length (all $P < 0.001$; Table 5.8). Compared with HS, the HFF treatment reduced the concentration of *trans*-10 C18:1 ($P = 0.01$; Table 5.9).

On a yield basis, we observed no effect of treatment on the yield of preformed milk FA ($P = 0.78$; Table 5.6). The HFF treatment decreased the yield of de novo synthesized milk FA by 58 g ($P < 0.001$). The reduction in C14:0 and *cis*-9 C14:1 accounted for almost half of the difference in de novo FA yield between the HFF and HS treatments (both $P < 0.001$; Table 5.9). The HFF treatment increased the yield of 16-carbon milk FA by 179 g ($P < 0.001$; Table 5.6) with ~ 175 g coming from C16:0 ($P < 0.001$; Table 5.9).

We used Pearson correlation coefficients to assess relationships between variables (Table 5.7). Milk fat yield was negatively correlated with insulin concentration ($P < 0.01$), glucose concentration ($P < 0.01$), and triglyceride concentration ($P < 0.05$). Milk fat concentration was negatively correlated to the concentration of *trans*-10 C18:1 in milk fat ($P < 0.001$). Change in BCS was positively correlated to plasma insulin concentration ($P < 0.01$), negatively correlated to plasma NEFA concentration ($P < 0.001$) and weakly positively correlated to the concentration of *trans*-10 C18:1 in milk fat ($P = 0.09$).

DISCUSSION

Feeding diets that differ in lipogenic and glucogenic nutrients has been shown to alter energy partitioning towards milk production or adipose tissue in early (van Knegsel et al., 2007a) and established lactation cows (Grum et al., 1996). Van Knegsel et al. (2007a) fed diets differing in FA and starch concentration and observed no effect on milk yield or milk protein yield. However cows fed the lipogenic diet increased milk fat concentration and yield, resulting in increased milk energy output with a trend for reduced energy retained as body fat. Grum et al. (1996) compared a high concentrate diet to a high forage diet with added fat, both diets were similar in predicted energy content. The high forage diet with added fat increased milk fat concentration and yield compared with the high concentrate diet. Cows in both studies on the more glucogenic diet had reduced milk energy output due to reduced milk fat yield, with the additional energy presumably stored as adipose tissue. In our study, we also examined the effect of a lipogenic, compared to an isocaloric glucogenic diet, on milk production and energy partitioning to evaluate an alternative to a high starch diet for high producing mid-lactation dairy cows. While we observed similar results with the lipogenic diet increasing milk fat yield and partitioning a greater proportion of energy to milk energy, we were also able to quantify the difference in body weight gain and subcutaneous fat thickness between the two treatments in 28-d periods.

We observed an increase in lactose yield on the high starch diet suggesting that this diet provided more glucose precursors for milk production. Increasing the starch concentration also may increase microbial protein production, thereby increasing milk protein yield, similar to results reported by Grum et al. (1996). Additionally, increases in insulin concentration are associated with increased milk protein yield and concentration (McGuire et al., 1995; Griinari et

al., 1997; and Mackle et al., 1999), perhaps due to the effects of insulin on the activation cascade for milk protein synthesis (Winkelman and Overton, 2013) or other unknown mechanisms. The high fat treatment increased milk fat yield through an increase in palmitic acid in milk fat similar to results observed by Lock et al. (2013) and Piantoni et al. (2013); these studies substituted a palmitic acid source for soyhulls and diets were not isocaloric. Nonetheless, a similar milk fat yield response was also observed when a palmitic acid source replaced stearic acid in isocaloric diets (Rico et al., 2014).

Similar apparent NE_L concentrations of the diet and minimal differences in DMI were observed between the HFF and HS treatments indicating that production differences and body composition differences were independent of apparent NE_L intake, which also did not differ between treatments. Unsaturated FA have been shown to reduce DMI, presumably through the effects of CCK reducing gastric emptying and slowing passage rate from the rumen (Allen, 2000; Relling and Reynolds, 2007). However, saturated fats have little to no effect on DMI (Allen, 2000). Although forage NDF, which is highly negatively correlated to rumen fill and therefore reduced DMI, was higher for HFF, the additional wheat straw in the HS may have minimized the effects of differences in forage NDF.

A primary objective of our study was to determine treatment effects on energy partitioning. The HS treatment reduced energy partitioning to milk and increased energy partitioning to body tissue gain, similar to what is commonly observed during diet-induced MFD (Griinari and Bauman, 2006). Changes in BCS may be insensitive in short-term studies and changes in BW may be confounded by differences in gut fill between diets (Harvatine et al., 2009). Therefore we used 28 d periods and ultrasound images to measure subcutaneous fat thickness between the hide and musculature over the rump and the 12^{th} intercostal space to

determine changes in back fat thickness throughout a period. A change in 1 mm of backfat thickness is estimated to equate to ~ 5 kg of body fat (Schröder and Staufenbiel, 2006). While a majority of work on adipose tissue deposits has been done in meat animals, the order of deposition is thought to be maintained in all species, with internal fat deposited first followed by subcutaneous, then inter and intramuscular fat (Allen, 1976). Our measurements in the present study only allowed us to quantify subcutaneous fat differences rather than internal fat deposits that could also be impacted by our dietary treatments. We found that cows fed the HS treatment increased BW and adipose tissue deposits compared to cows fed the HFF treatment.

The changes in plasma metabolites and hormones we observed are similar to previous studies that fed supplemental fat (Grummer, 1991; Chilliard, 1993; Grum et al., 1996; Piantoni et al., 2013). Due to incomplete uptake of NEFA after hydrolysis of plasma triglycerides by lipoprotein lipase, increases in plasma NEFA concentration when feeding supplemental fat are common (Drackley, 1999). The increases that we observed are relatively small compared with NEFA concentrations in early lactation, and the mammary gland would not take up additional NEFA at the concentrations observed in our study (Miller, 1991). Additionally, elevated insulin concentrations will reduce plasma NEFA through inhibiting lipolysis and/or increasing lipogenesis (Vernon, 2005). Increased concentrations of TAG could result from higher levels of dietary FA increasing the supply of TAG-rich lipoproteins available in circulation. We did not expect changes in glucose concentrations due to the regulatory role of insulin and the glucogenic role of the liver in dairy cattle (Aschenbach et al., 2010). However, insulin concentrations were significantly higher for the HS treatment, in agreement with studies that altered dietary starch concentration (Grum et al., 1996; van Knegsel et al. 2007b). The reduction in insulin concentration observed in higher producing cows on the HFF treatment, compared to HS

treatment might be due to differences in glucose precursors supplied by the two treatments. We would expect greater glucose clearance in high producing cows and therefore, reduced insulin concentrations compared to lower producing cows. However, the HS treatment presumably provided more glucose precursors and/or glucose thereby increasing insulin production to clear the additional glucose from circulation compared to when high producing cows were on the HFF treatment.

While *trans*-10 C18:1 concentrations are elevated in milk fat during MFD, abomasal infusions of this FA had no effect on milk fat yield (Lock et al., 2007). However, there is a robust relationship between production of *trans*-10 C18:1 in the rumen and milk fat synthesis (e.g. Loor et al., 2005; Shingfield, et al., 2009). Thus, *trans*-10 C18:1 can serve as a marker for the specific FA isomers that directly inhibit milk fat synthesis (Bauman et al., 2011). We observed that concentrations of *trans*-10 C18:1 in milk fat were negatively correlated to milk fat concentration, similar to previous observations (Loor et al., 2005; Shingfield et al., 2009). In our study, concentrations of *trans*-10, *cis*-12 C18:2 in milk fat were below the limits of detection in a majority of the samples; however, *trans*-10 C18:1 is present at much greater concentrations in milk fat, and treatment differences indicate alternative biohydrogenation for the HS treatment. Reduced rumen pH is a risk factor for MFD, with '*trans*-10' intermediate production increasing with lowered rumen pH (Abu-Ghazaleh et al., 2005). The reduction in pH possibly increases the lag phase of biohydrogenation and exacerbates the negative effects of PUFA on rumen bacteria (Maia et al., 2010). In our study, the increased concentration of starch in the HS treatment likely contributed to reduced rumen pH and favored alternative MFD-inducing biohydrogenation pathways. Harvatine et al. (2009) reported that during abomasal infusions of *trans*-10, *cis*-12 C18:2, there was a significant reduction in milk fat concentration and yield because of the down

regulation of lipogenic genes in mammary tissue. Conversely, there was a significant increase in the expression of lipogenic enzymes in adipose tissue (Harvatine et al., 2009). Therefore, increases in *trans*-10, *cis*-12 C18:2 may result in repartitioning of energy by reducing milk fat output and increasing body fat reserves. Interestingly, we observed a positive correlation between the concentration of *trans*-10 C18:1 in milk fat and change in BCS.

Corl et al. (2006) used a hyperinsulinemic-euglycemic clamp to increase insulin without changing glucose concentrations to assess the impact of increasing plasma insulin concentration on milk fat synthesis. Increasing insulin resulted in a marked reduction in preformed FA without any negative effects on de novo or mixed sources of milk FA, with a greater effect in early vs. established lactation animals. Elevated insulin concentrations could partition circulating TAG into adipose instead of uptake by the mammary gland and reduce lipolysis from adipose tissues. However, injections of long-acting insulin did not reduce milk fat concentration or yield and shifted the FA profile with an increase in de novo synthesized milk FA and a reduction in preformed milk FA compared to a control in established lactation dairy cattle (Winkelman and Overton, 2013). The reduction in preformed milk FA from these previous studies does not support our observations of increased insulin concentrations with the high starch diet yet no reduction in preformed milk FA compared to the high fat diet. However, we observed that insulin concentrations were negatively correlated with milk fat yield consistent with insulin potentially being a contributor to reduced milk fat with higher starch diets. Insulin was also positively correlated with change in BCS. Potentially, injections of insulin or the use of a hyperinsulemic-euglycemic clamp may not be indicative of the long-term effects of increases in insulin due to changes in dietary nutrients.

In our study we used relatively high producing cows with high requirements of glucose. We therefore anticipate that the dietary treatment effects on milk fat yield and energy partitioning would be greater in later lactation cows at lower milk yield with reduced glucose requirements. The oversupply of glucose precursors may further increase insulin, due to the increase in insulin and/or insulin sensitivity as lactation progresses. Additionally, lower producing cows may have increased concentrations of biohydrogenation intermediates associated with MFD as observed by Bradford and Allen (2004). Both of these factors could exacerbate the effects of a high starch diet compared to a high fiber and fat diet on later lactation dairy cows.

The reduction in de novo milk FA is typical of the trend we see when increasing palmitic acid in the diet with studies differing in their extent of response (Lock et al., 2013, Piantoni et al., 2013; Rico et al., 2014). Palmitic acid supplementation increases the yield of C4:0 while reducing the yield of C6:0 to *cis*-9 C14:1 with longer chain de novo synthesized FA having the largest reduction in FA yield (Rico et al., 2014).

Treatment had no effect on total-tract starch digestibility; however, we expect that differences in site of starch digestion might have occurred, with less ruminal starch digestibility and more intestinal starch digestibility with the HFF treatment (Firkins et al., 2001; Huntington et al., 2006). The HFF treatment increased NDF digestibility presumably because HFF contained soyhulls, an NDF source that is more highly digested than the NDF of the forages (especially wheat straw) in the HS diet. Even at high intakes of total FA we detected minimal treatment differences in FA digestibility and therefore observed a positive linear relationship between total FA absorbed and total FA intake. This is contrary to Piantoni et al. (2013), who reported total FA digestibility was reduced at higher intake of FA. Potentially these differences in FA digestibility are due to differences in size and relative solubility of the fat supplements or other dietary

differences. Results are supported by our recent meta-analysis in which we observed that C16:0 digestibility was not impacted by flow of C16:0 through the duodenum (Boerman et al., submitted).

Our aim was to evaluate the impact of isocaloric diets differing in lipogenic and glucogenic potential on energy partitioning. To achieve this, we were not able to simply replace one ingredient for another; thus, diets differed in NDF, starch, and FA with much of the starch in the HS diet being from high moisture corn. Based on how the diets were formulated, we expected to cause a greater reduction in milk fat yield. The HS treatment had what many would consider an acceptable milk fat concentration (3.6%), yet we still observed differences in milk fat output and subcutaneous fat accumulation between the two treatments. We anticipate that a greater reduction in milk fat synthesis would have exacerbated these differences. The causes of repartitioning could be linked to increased insulin concentrations and/or production of biohydrogenation intermediates. However, in our current study we were unable to separate the effects of insulin and MFD-inducing intermediates on partitioning; therefore the mode of action is still unknown and warrants further consideration.

CONCLUSIONS

Feeding diets differing in NDF, starch, and FA concentrations resulted in differences in energy partitioning in dairy cattle. A high forage diet, supplemented with palmitic acid, increased milk fat yield, whereas a high starch diet increased body fat gain. This repartitioning is likely from the combined effects of increasing plasma insulin concentrations and elevated ruminal biohydrogenation intermediates in the high starch diet, favoring storage of nutrients as adipose

over use for milk fat synthesis. The long-term implications of diets differing in their ability to partition energy warrant further investigation.

APPENDIX

APPENDIX

Table 5.1. Diet ingredients and nutrient composition of treatment diets[1].

	Treatments	
Ingredient, % DM	HFF	HS
Corn silage	24.0	24.0
Alfalfa silage	24.0	12.0
Wheat straw	2.00	4.00
Soybean meal	14.5	16.0
Soybean hulls	10.5	---
Corn grain, high moisture	10.0	20.0
Corn grain, dry ground	---	13.0
Cottonseed	9.00	7.50
C16:0-enriched fat supplement[2]	2.50	---
Vitamin & mineral mix[3]	2.00	2.00
Limestone	0.75	0.75
Sodium bicarbonate	0.75	0.75
Forage:Concentrate	50:50	40:60
Nutrient composition, % DM		
DM[4]	51.6	55.8
NDF	32.8	25.1
Forage NDF	20.2	17.6
CP	18.3	17.0
Starch	16.1	32.5
FA	5.36	3.19
16-Carbon FA	2.66	0.51
18-Carbon FA	2.39	2.46

[1] Experimental diets fed to 32 cows in a crossover design with 28-d periods.
[2] BergaFat F-100 (Berg + Schmidt America LLC, Libertyville, IL)
[3] Vitamin and mineral mix contained 34.1% dry ground shell corn, 25.6% white salt, 21.8% calcium carbonate, 9.1% Biofos, 3.9% magnesium oxide, 2% soybean oil, and < 1% of each of the following: manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, iodine, cobalt carbonate, vitamin E, vitamin A, vitamin D, and selenium.
[4] Expressed as percent of as fed.

Table 5.2. Dry matter intake, milk production, milk components and feed efficiency for cows fed treatment diets (n=30)[1].

	Treatments[2]			*P*-value[3]
	HFF	HS	SEM	TRT
DMI[a]	26.9	27.4	0.38	0.10
Milk Yield, kg/d				
Milk	45.8	47.1	1.44	0.02
3.5% FCM[4]	49.1	47.6	1.59	0.03
Milk Components				
Fat, kg/d	1.81	1.68	0.06	< 0.001
Fat, %	3.95	3.58	0.09	< 0.001
Protein, kg/d	1.34	1.44	0.04	< 0.001
Protein, %	2.93	3.07	0.03	< 0.001
Lactose, kg/d	2.22	2.31	0.06	0.001
Lactose, %	4.85	4.92	0.03	0.001
Milk energy, Mcal/d[5]	33.3	32.4	1.00	0.009
ECM[6]/DMI[a]	1.78	1.73	0.02	0.02

[1] Samples and data for production variables collected during the last 5 d of each treatment period (d 24 to 28).
[2] Treatments were either a high fiber and fat diet (HFF) containing a 50:50 ratio of forage to concentrate containing a C16:0-enriched fat supplement at 2.5% of diet DM or a high starch diet (HS) containing a 40:60 ratio of forage to concentrate containing a mixture of dry ground and high moisture corn.
[3] *P*-value associated with treatment differences (HFF vs. HS; Trt).
[4] Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].
[5] Milk energy = kg milk × [(0.0929 × milk fat %) + (0.0563 × milk protein %) + (0.0395 × milk lactose %)].
[6] Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)].
[a] Significant preliminary milk by treatment interaction; DMI (*P* = 0.04) and ECM/DMI (*P* = 0.06).

Table 5.3. Body weight, body condition score, and change in subcutaneous fat thickness measurements and calculated energy values for cows fed treatment diets (n = 30).

	Treatments[1]			*P*-value[2]
Variable	HFF	HS	SEM	TRT
BW	678	685	14.8	0.01
BCS	3.07	3.20	0.09	< 0.001
Change in BW, kg/d[3]	0.33	0.78	0.10	0.003
Change in BCS, pt/28 d	- 0.01	0.24	0.03	0.001
Change in Rump Fat, mm/28 d	- 0.41	0.37	0.22	0.04
Change in Rib Fat, mm/28 d	-0.08	0.46	0.14	0.04
Calculated energy values[4]				
Apparent NE_L of diet Mcal/kg	1.78	1.79	0.02	0.64
Apparent NE_L intake, Mcal/d	44.1	44.5	1.18	0.60
Milk, Mcal/d	33.6	33.0	1.05	0.05
Body Tissue Gain, Mcal/d	1.95	4.90	0.58	0.001
Maintenance, Mcal/d	10.6	10.6	0.17	0.02
Partitioning				
Milk, %	72.8	67.9	1.11	< 0.001
Body Tissue Gain, %	4.03	10.1	1.16	0.001
Maintenance, %	23.2	22.0	0.43	0.01

[1] Treatments were either a high fiber and fat diet (HFF) containing a 50:50 ratio of forage to concentrate containing a C16:0-enriched fat supplement at 2.5% of diet DM or a high starch diet (HS) containing a 40:60 ratio of forage to concentrate containing a mixture of dry ground and high moisture corn.
[2] *P*-value associated with treatment differences (HFF vs. HS; Trt).
[3] Determined by linear regression using BW measurements throughout 28 d periods.
[4] Determined from cow performance data collected throughout the 28 d periods.

Table 5.4. **Plasma concentrations of glucose, insulin, NEFA, and triglycerides for cows fed treatment diets (n=30)[1].**

	Treatments[2]			*P*-value[3]
Variable	HFF	HS	SEM	TRT
Glucose, mg/dL	63.3	64.8	0.73	0.14
Insulin, μg/L[a]	0.76	1.01	0.05	< 0.001
NEFA, μEq/L	135	96.7	3.80	< 0.001
TAG, mg/dL	11.2	10.1	0.29	< 0.001

[1] Samples for plasma measurements were collected during the last 5 d of each treatment period (d 24 to 28).

[2] Treatments were either a high fiber and fat diet (HFF) containing a 50:50 ratio of forage to concentrate containing a C16:0-enriched fat supplement at 2.5% of diet DM or a high starch diet (HS) containing a 40:60 ratio of forage to concentrate containing a mixture of dry ground and high moisture corn.

[3] *P*-value associated with treatment differences (HFF vs. HS; Trt).

[a] Significant linear preliminary milk by treatment interaction; $P = 0.06$.

Table 5.5. Apparent total tract digestibilies of nutrients for cows fed treatment diets (n=30)[1].

	Treatments[2]			*P*-value[3]
Nutrient	HFF	HS	SEM	TRT
DM	64.2	65.4	0.38	0.04
NDF	46.9	35.1	0.80	< 0.001
CP	69.2	66.5	0.44	< 0.001
Starch	94.8	95.0	0.23	0.39
FA	69.9	71.8	1.02	0.06
16 Carbon	63.6	65.8	0.99	0.07
18 Carbon	77.6	74.3	1.13	0.001

[1] Samples for digestibility measurements were collected during the last 5 d of each treatment period (d 24 to 28).
[2] Treatments were either a high fiber and fat diet (HFF) containing a 50:50 ratio of forage to concentrate containing a C16:0-enriched fat supplement at 2.5% of diet DM or a high starch diet (HS) containing a 40:60 ratio of forage to concentrate containing a mixture of dry ground and high moisture corn.
[3] *P*-value associated with treatment differences (HFF vs. HS; Trt).

Table 5.6. FA concentration and yield of milk FA by source for cows fed treatment diets (n=30)[1].

	Treatments[2]			*P*-value[3]
Summations of milk FA[4]	HFF	HS	SEM	TRT
FA concentration (g/100 g)				
De novo	21.5	26.8	0.34	< 0.001
Mixed	42.6	34.4	0.37	< 0.001
Preformed	35.9	38.9	0.48	< 0.001
FA yield (g/d)				< 0.001
De novo	367	425	18.66	< 0.001
Mixed	723	544	26.25	< 0.001
Preformed	603	606	18.29	0.78

[1] Samples for milk FA were collected during the last 5 d of each treatment period (d 24 to 28).
[2] Treatments were either a high fiber and fat diet (HFF) containing a 50:50 ratio of forage to concentrate containing a C16:0-enriched fat supplement at 2.5% of diet DM or a high starch diet (HS) containing a 40:60 ratio of forage to concentrate containing a mixture of dry ground and high moisture corn.
[3] *P*-value associated with treatment differences (HFF vs. HS; Trt).
[4] De novo = milk FA < 16 carbons in length; mixed = milk FA 16-carbons in length; preformed = milk FA > 16 carbons in length.

Table 5.7. Pearson correlation coefficients for cows fed treatment diets (n=30).

	Fat Yield	Fat %	Milk	Δ BCS	Insulin	NEFA	Glucose	TAG	C18:1 *trans*-10[1]
Fat Yield	1	0.443	0.782	-0.033	-0.352	0.077	-0.370	-0.294	-0.039
		0.0003	< 0.0001	0.80	0.006	0.56	0.004	0.02	0.76
Fat %		1	-0.203	-0.157	-0.104	0.183	-0.213	0.066	-0.460
			0.11	0.22	0.43	0.16	0.10	0.61	0.0002
Milk			1	0.079	-0.324	-0.069	-0.271	-0.392	0.278
				0.54	0.02	0.60	0.04	0.002	0.03
Δ BCS				1	0.337	-0.431	0.175	-0.100	0.218
					0.009	0.0006	0.18	0.45	0.09
Insulin					1	-0.242	0.288	0.322	-0.012
						0.06	0.03	0.01	0.93
NEFA						1	-0.086	0.311	-0.123
							0.51	0.02	0.35
Glucose							1	0.065	0.134
								0.62	0.31
TAG								1	-0.063
									0.63
C18:1 *trans*-10[1]									1

[1] Concentration of *trans*-10 C18:1 in milk fat.

Table 5.8. Milk FA concentrations of cows fed treatment diets (n=30)[1].

	Treatments[2]			*P*-value[3]
FA concentration (g/100 g)	HFF	HS	SEM	TRT
Selected individual FA[4]				
4:0	3.32	3.06	0.05	< 0.001
6:0	1.96	2.16	0.04	< 0.001
8:0	1.03	1.31	0.03	< 0.001
10:0	2.32	3.43	0.08	< 0.001
12:0	2.54	3.96	0.10	< 0.001
14:0	9.77	12.16	0.14	< 0.001
14:1 *cis*-9	0.54	0.70	0.03	< 0.001
16:0	41.29	33.06	0.36	< 0.001
16:1 *cis*-9	1.35	1.30	0.04	0.02
18:0	9.88	10.16	0.24	0.20
18:1 *trans*-4	0.01	0.02	0.0005	< 0.001
18:1 *trans*-5	0.011	0.012	0.0005	0.004
18:1 *trans*-6-8	0.22	0.26	0.01	< 0.001
18:1 *trans*-9	0.17	0.20	0.005	< 0.001
18:1 *trans*-10	0.32	0.53	0.06	0.01
18:1 *trans*-11	0.63	0.78	0.03	< 0.001
18:1 *trans*-12	0.34	0.42	0.01	< 0.001
18:1 *cis*-9	16.39	16.72	0.30	0.09
18:1 *cis*-11	0.35	0.51	0.01	< 0.001
18:2 *cis*-9, *cis*-12	1.99	2.50	0.04	< 0.001
18:2 *cis*-9, *trans*-11	0.26	0.35	0.02	< 0.001
18:2 *trans*-9, *cis*-11	< 0.001	0.002	0.001	0.19
18:2 *trans*-10, *cis*-12	< 0.001	0.001	0.001	0.19
18:3 *cis*-9, *cis*-12, *cis*-15	0.37	0.30	0.01	< 0.001

[1] Samples for milk FA were collected during the last 5 d of each treatment period (d 24 to 28).
[2] Treatments were either a high fiber and fat diet (HFF) containing a 50:50 ratio of forage to concentrate containing a C16:0-enriched fat supplement at 2.5% of diet DM or a high starch diet (HS) containing a 40:60 ratio of forage to concentrate containing a mixture of dry ground and high moisture corn.
[3] *P*-value associated with treatment differences (HFF vs. HS; Trt).
[4] A total of approximately 70 individual FA were quantified and used for calculations (summation by source). Only select FA are reported in the table.

Table 5.9. Milk FA yields of cows fed treatment diets (n=30)[1].

	Treatments[2]			*P*-value[3]
FA Yield (g/d)	HFF	HS	SEM	TRT
Selected individual FA[4]				
4:0	56.4	48.3	2.24	< 0.001
6:0	33.6	34.3	1.60	0.53
8:0	17.6	20.9	1.00	< 0.001
10:0	39.9	54.6	2.74	< 0.001
12:0	43.7	63.1	3.19	< 0.001
14:0	167	192	8.16	< 0.001
14:1 *cis*-9	9.20	11.03	0.63	< 0.001
16:0	700	524	25.42	< 0.001
16:1 *cis*-9	22.8	20.3	0.98	< 0.001
18:0	166	160	6.45	0.16
18:1 *trans*-4	0.23	0.24	0.01	0.20
18:1 *trans*-5	0.18	0.18	0.01	0.59
18:1 *trans*-6-8	3.67	4.08	0.14	< 0.001
18:1 *trans*-9	2.88	3.05	0.09	0.01
18:1 *trans*-10	5.28	7.61	0.54	0.002
18:1 *trans*-11	10.5	12.0	0.48	0.002
18:1 *trans*-12	5.72	6.49	0.22	< 0.001
18:1 *cis*-9	275	260	8.09	0.01
18:1 *cis*-11	5.85	7.93	0.27	< 0.001
18:2 *cis*-9, *cis*-12	33.4	38.8	1.17	< 0.001
18:2 *cis*-9, *trans*-11	4.35	5.26	0.22	< 0.001
18:2 *trans*-9, *cis*-11	< 0.01	0.02	0.01	0.17
18:2 *trans*-10, *cis*-12	< 0.01	0.01	0.01	0.17
18:3 *cis*-9, *cis*-12, *cis*-15	6.22	4.65	0.17	< 0.001

[1] Samples for milk FA were collected during the last 5 d of each treatment period (d 24 to 28).
[2] Treatments were either a high fiber and fat diet (HFF) containing a 50:50 ratio of forage to concentrate containing a C16:0-enriched fat supplement at 2.5% of diet DM or a high starch diet (HS) containing a 40:60 ratio of forage to concentrate containing a mixture of dry ground and high moisture corn.
[3] *P*-value associated with treatment differences (HFF vs. HS; Trt).
[4] A total of approximately 70 individual FA were quantified and used for calculations (summation by source). Only select FA are reported in the table.

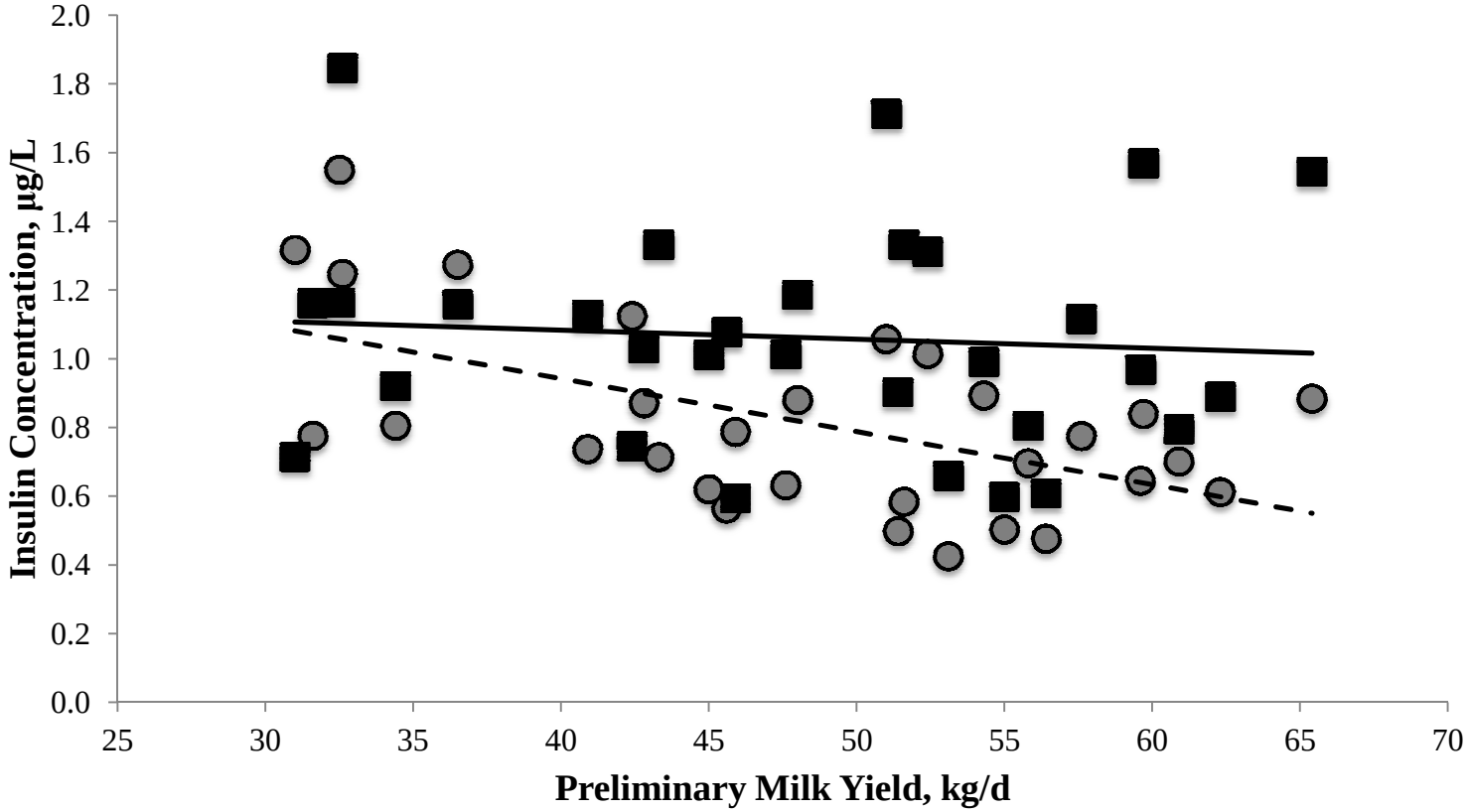

Figure 5.1. Relationship between preliminary milk yield (kg/d) and plasma insulin concentration (µg/L) of cows (n=30) fed treatment diets. Cows with black squares with a solid trend line were on the HS treatment (insulin concentration (µg/L) = 1.19 – 0.003 × (preliminary milk yield kg/d); $R^2 = 0.01$). Cows with grey circles with a dashed trend line were on the HFF treatment (insulin concentration (µg/L) = 1.56 – 0.015 × (preliminary milk yield kg/d); $R^2 = 0.31$). Linear preliminary milk yield by treatment interaction $P = 0.06$.

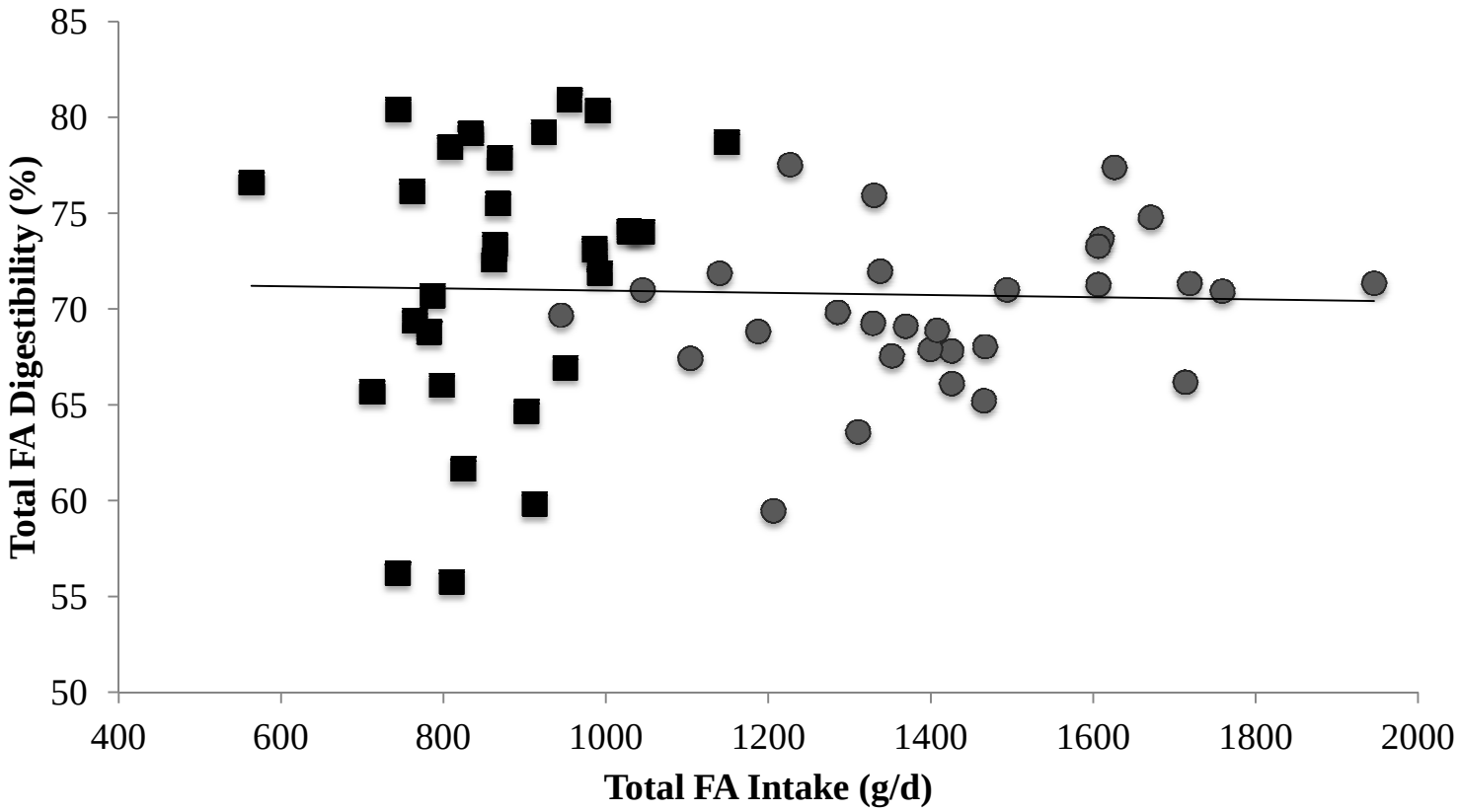

Figure 5.2. Relationship between total FA digestibility and total FA intake of cows fed treatment diets (total FA digestibility (%) = 71.5 – 0.001 × (total FA intake g/d); R^2=0.001; *P* = 0.81). Cows with black squares were on the HS treatment and cows with grey circles were on the HFF treatment (n=30).

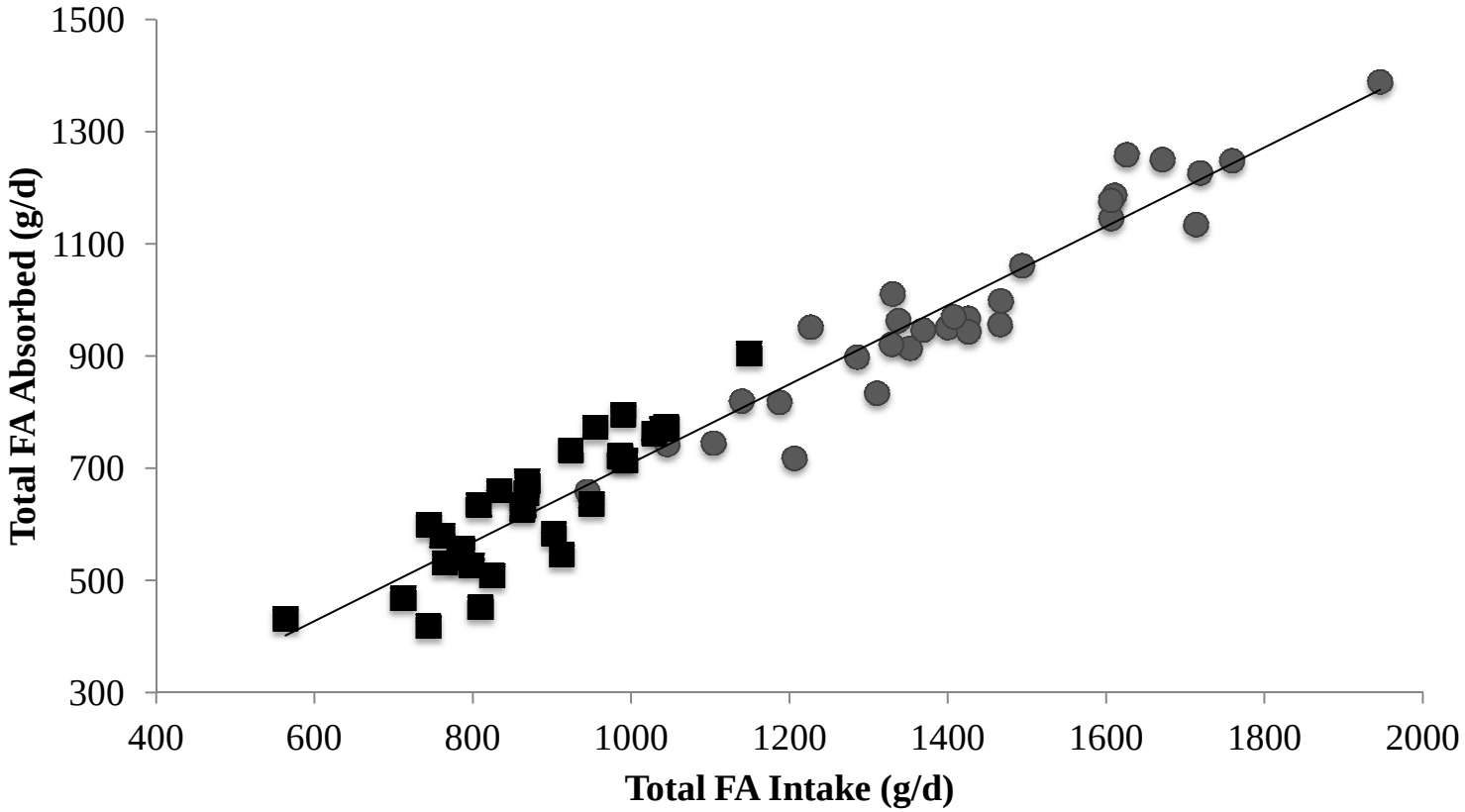

Figure 5.3. Relationship between total FA absorbed and total FA intake of cows fed treatment diets (total FA absorbed (g/d) = 5.55 + 0.70 × (total FA intake g/d); R^2=0.94; *P* < 0.0001). Cows with black squares were on the HS treatment; whereas cows with grey circles were on the HFF treatment (n=30).

REFERENCES

REFERENCES

Abu-Ghazaleh, A. A., M. B. Riley, E. J. Thies, and T. C. Jenkins. 2005. Dilution rate and pH effects on the conversion of oleic acid to trans C18:1 positional isomers in continuous culture. J. Dairy Sci. 88:4334-4341.

Allen, C. E. 1976. Cellularity of adipose tissue in meat animals. Fed. Proc. 35:2302-2307.

Allen, M.S. 2000. Effects of Diet on Short-Term Regulation of Feed Intake by Lactating Dairy Cattle. J. Dairy Sci. 83:1598–1624.

AOAC. 1990. Official Methods of Analysis. 15th ed. AOAC International, Arlington, VA.

Aschenbach, J. R., N. B. Kristensen, S. S. Donkin, H. M. Hammon, and G. B. Penner. 2010. Gluconeogenesis in dairy cows: the secret of making sweet milk from sour dough. IUBMB Life. 62(12):869-877.

Bauman, D. E. 2000. Regulation of nutrient partitioning during lactation: homeostasis and homeorhesis revisited. In Ruminant Physiology, Digestion, Metabolism, Growth and Reproduction. Proceedings of the 9th International Symposium on Ruminant Physiology, pp 311–328. Ed. PB Cronje. New York: CABI Publishing.

Bauman, D. E., K. J. Harvatine, and A. L. Lock. 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Ann. Rev. Nutr. 31: 299-319.

Boerman, J. P., J. L. Firkins, and A. L. Lock. Intestinal digestibility of long chain fatty acids in lactating dairy cows: a meta analysis. J. Dairy Sci. *Submitted*.

Bradford, B. J., and M. S. Allen. 2004. Milk fat responses to a change in diet fermentability vary by production level in dairy cattle. J. Dairy Sci. 87:3800-3807.

Chilliard, Y. 1993. Dietary fat and adipose tissue metabolism in ruminants, pigs, and rodents: a review. J. Dairy Sci. 76:3897-3931.

Cochran, R. C., D. C. Adams, J. D. Wallace, and M. L. Galyean. 1986. Predicting the digestibility of different diets with internal markers: Evaluation of four potential markers. J. Anim. Sci. 63:1476–1483.

Corl, B. A., S. T. Butler, W. R. Butler, and D. E. Bauman. 2006. Short communication: regulation of milk fat yield and fatty acid composition by insulin. J. Dairy Sci. 89:4172-4175.

Drackley, J. K. 1999. Biology of dairy cows during the transition period: the final frontier? J. Dairy Sci. 82:2259-2273.

Firkins, J. L., M. L. Eastridge, N. R. St-Pierre, and S. M. Noftsger. 2001. Effects of grain variability and processing on starch utilization by lactating dairy cattle. J. Anim. Sci. 79:E218-E238.
Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. USDA-ARS, Washington, DC.

Griinari, J. M., M. A. McGuire, D. A. Dwyer, D. E. Bauman, D. M. Barbano, and W. A. House. 1997. The role of insulin in the regulation of milk protein synthesis in dairy cows. J. Dairy Sci. 80:2361-2371.

Griinari, J.M., and D.E. Bauman. 2006. Milk fat depression: concepts, mechanisms and management applications. Ruminant physiology digestion, metabolism and impact of nutrition on gene expression, immunology and stress. Wageningen Academic Publ., Wageningen, The Netherlands. 389–409.

Grum, D. E., J. K. Drackley, L. R. Hansen, and J. D. Cremin, Jr. 1996. Production, digestion, and hepatic lipid metabolism of dairy cows fed increased energy from fat or concentrate. J. Dairy Sci. 79:1836-1849.

Grummer, R. R. 1991. Effect of feed on the composition of milk fat. J. Dairy Sci. 74:3244-3257.

Harvatine, K.J., J.W. Perfield, and D.E. Bauman. 2009. Expression of Enzymes and Key Regulators of Lipid Synthesis Is Upregulated in Adipose Tissue during CLA-Induced Milk Fat Depression in Dairy Cows. J. Nutr. 139:849–854.

Huntington, G. B., D. L. Harmon and C. J. Richards. 2006. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. J. Anim. Sci. 84:E14-E24.

Lock, A.L., C. Tyburczy, D.A. Dwyer, K.J. Harvatine, F. Destaillats, Z. Mouloungui, L. Candy, and D.E. Bauman. 2007. Trans-10 octadecenoic acid does not reduce milk fat synthesis in dairy cows. J. Nutr. 137:71–76.

Lock, A.L., C.L. Preseault, J.E. Rico, K.E. DeLand, and M.S. Allen. 2013. Feeding a C16:0-enriched fat supplement increased the yield of milk fat and improved conversion of feed to milk. J. Dairy Sci. 96:6650-6659.

Loor, J. J., A. Ferlay, A. Ollier, M. Doreau, and Y. Chilliard. 2005. Relationship among *trans* and conjugated fatty acids and bovine milk fat yield due to dietary concentrate and linseed oil. J. Dairy Sci. 88:726-740.

Kammes, K. L., and M. S. Allen. 2012. Nutrient demand interacts with grass maturity to affect milk fat concentration and digestion responses in dairy cows. J. Dairy Sci. 95:5133-5148.

Mackle, T. R., D. A. Dwyer, K. L. Ingvartsen, P. Y. Chouinard, J. M. Lynch, D. M. Barbano, and D. E. Bauman. 1999. Effects of insulin and amino acids on milk protein concentration and yield from dairy cows. J. Dairy Sci. 82:1512-1524.

Maia, M.R., L.C. Chaudhary, C.S. Bestwick, A.J. Richardson, N. McKain, T.R. Larson, I.A. Graham, and R.J. Wallace. 2010. Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, Butyrivibrio fibrisolvens. BMC Microbiology. 10:52.

McGuire, M. A., J. M. Griinari, D. A. Dwyer, and D. E. Bauman. 1995. Role of insulin in the regulation of mammary synthesis of fat and protein. J. Dairy Sci. 78:816-824.

Miller, P. S., B. L. Reis, C. C. Calvert, E. J. DePeters, and R. L. Baldwin. 1991. Relationship of early lactation and bovine somatotropin on nutrient uptake by cow mammary gland. J. Dairy Sci. 74:3800-3806.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Acad. Sci., Washington D.C.

Piantoni, P., A. L. Lock, and M. S. Allen. 2013. Palmitic acid increased yields of milk and milk fat and nutrient digestibility across production level of lactating cows. J. Dairy Sci. 96:7143-7154.

Relling, A. E., and C. K. Reynolds. 2007. Feeding rumen-inert fats differing in their degree of saturation decreases intake and increases plasma concentrations of gut peptides in lactating dairy cows. J. Dairy Sci. 90:1506-1515.

Rico, J.E., M.S. Allen, and A.L. Lock. 2014. Compared with stearic acid, palmitic acid increased the yield of milk fat and improved feed efficiency across production level of cows. J Dairy Sci. 97:1057–1066.

Roche, J. R., N. C. Figgens, J. K. Kay, M. W. Fisher, K. J. Stafford, and D. P. Berry. 2009. Invited review: body condition score and its association with dairy cow productivity, health, and welfare. J. Dairy Sci. 92:5769-5801.

Schröder, U. J., and R. Staufenbiel. 2006. Invited Review: Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. J. Dairy Sci. 89:1-14.

Shingfield, K. J., A. Sæbø, P.-C. Sæbø, V. Toivonen, and J. M. Griinari. 2009. Effect of abomasal infusions of a mixture of oxtadecenoic acids on milk fat synthesis in lactating cows. J. Dairy Sci. 92:4317-4329.

van Knegsel, A. T. M., H. van den Brand, J. Dijkstra, W. M. van Straalen, M. J. W. Heetkamp, S. Tamminga, and B. Kemp. 2007a. Dietary energy source in dairy cows in early lactation: energy partitioning and milk composition. J. Dairy Sci. 90:1467-1476.

van Knegsel, A. T. M., H. van den Brand, E. A. M. Graat, J. Dijkstra, R. Jorritsma, E. Decuypere, S. Tamminga and B. Kemp. 2007b. Dietary energy source in dairy cows in early lactation: metabolites and metabolic hormones. J. Dairy Sci. 90:1477-1485.

Van Soest, P. J. 1963. Ruminant fat metabolism with particular reference to factors affecting low milk fat and feed efficiency: a review. J. Dairy Sci. 46:204-216.

Vernon, R. G. 2005. Lipid metabolism during lactation: a review of adipose tissue-liver interactions and the development of fatty liver. J. Dairy Research. 72: 460-469.

Wildman, E. E., G. M. Jones, P. E. Wagner, and R. L. Bowman. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65: 495-501.

Winkelman, L. A., and T. R. Overton. 2013. Long-acting insulins alter milk composition and metabolism of lactating dairy cows. J. Dairy Sci. 96:7565-7577.

CHAPTER 6

IMPACT OF UNSATURATED FATTY ACIDS AND TRIGLYCERIDES FROM SOYBEANS ON MILK FAT SYNTHESIS AND BIOHYDROGENATION INTERMEDIATES IN DAIRY CATTLE

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INTRODUCTION

Feed ingredients vary in the amount and composition of FA that they contribute to dietary FA intake. In a recent study conducted in the Netherlands, ~100 samples of corn silage and grass silage were analyzed for FA concentration with both types of silages varying from approximately 1 to 3% total FA (DM basis; Khan et al., 2012). Grains and byproducts also vary in FA concentration depending on hybrid, processing, and growing conditions (Boufaeïd et al, 2003). Notably, distillers grains products vary considerably based on the amount of solubles added back to the grains, with the ratio of grains to solubles being a major contributor to the variation in FA content of the end product (Cao et al., 2009). There is limited information regarding the total concentration of free FA, and the proportion of total FA present as free FA, in dairy cow diets. However, it has been reported previously that harvesting and ensiling of forages can increase the proportion of free FA due to hydrolytic cleavage of esterified FA (Yang and Fujita, 1997, Elgersma et al., 2003, Vanhatalo et al., 2007, Halmemies-Beauchet-Filleau et al., 2013a), while suboptimal storage of byproducts in humid conditions can also increase the proportion of free FA (Cooke et al., 2007). Dietary ingredients, therefore not only vary in the amount of total FA that they contribute to the diet but also in their relative proportion of free to esterified FA.

Unsaturated FA are toxic to specific rumen bacteria because they alter cell integrity (Maia et al., 2007). Therefore rumen bacteria convert (biohydrogenate) unsaturated FA to saturated FA as a protection mechanism, and consequently saturated FA are often considered to be rumen inert. A free carboxyl group is required for the initial isomerase reaction of biohydrogenation to occur (Jenkins, 1993). However, the majority of dietary FA are esterified, typically present either as triglycerides or glycolipids in feed ingredients. Upon entering the rumen, esterified FA must first go through the process of hydrolysis, in which the ester bond connecting the FA to the glycerol backbone is cleaved exposing the carboxyl group allowing biohydrogenation to proceed. If FA enter the rumen as free FA, they can undergo biohydrogenation immediately without having to go through hydrolysis.

There is limited research examining if the amount or proportion of free to esterified FA has an impact on biohydrogenation rates and pathways and if this could have an effect on milk fat synthesis in the dairy cow. Cooke et al. (2007) reported that increasing the proportion of free FA in cottonseed, while keeping total FA concentration the same, decreased milk fat concentration when a greater proportion of free FA were fed. Additionally, in vitro work comparing free vs. esterified FA found an increase in the amount of unsaturated biohydrogenation intermediates remaining when free FA were added to the culture (Moore et al., 1969 and Noble et al., 1974) potentially due to an accumulation of FA that inhibit or change the pathways of biohydrogenation.

Altered rumen fermentation can result in shifts from normal biohydrogenation to alternative pathways, producing specific FA intermediates that reduce fat synthesis in the mammary gland resulting in milk fat depression (MFD; Bauman et al., 2011). It is well documented that increasing the amount of PUFA contained in the diet can reduce milk fat yield

through the production of specific biohydrogenation intermediates (e.g. Griinari et al., 1998; Leonardi et al., 2005). However, it is unclear if there is a difference in the risk for MFD depending on if the increased dietary unsaturated FA are esterified or free FA. Our objective therefore was to examine if altering the amount of unsaturated FA in the diet as esterified or free FA affected feed intake and production responses of dairy cows. We hypothesized that free FA fed to lactating dairy cows would be more detrimental than esterified FA for milk fat production because free FA are more rapidly available in the rumen and this could cause a greater shift in biohydrogenation toward pathways that produce FA intermediates that cause MFD.

MATERIALS AND METHODS

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University. Eighteen mid-lactation (132 ± 75 DIM) Holstein cows (6 primiparous, 12 multiparous) from the Michigan State University Dairy Field Laboratory were blocked by parity and 3.5% FCM then randomly assigned to treatment sequence in a replicated 3×3 Latin Square design experiment with 21 d periods.

Treatments consisted of a control diet (CON), or unsaturated FA treatments (UFA) supplemented with soybean oil (FA present as triglycerides; TAG) or soybean FA distillate (FA present as free FA; FFA). The TAG and FFA supplements were included in the diet at 2% DM rather than soyhulls in CON. Addition of supplements to diets was based upon weight of lipid, not total FA content, which were pre-mixed with dried ground corn prior to inclusion into TMR. The soybean oil (West Central Cooperative, Ralston, IA) contained approximately 84% FA of which 99% were present as triglycerides as the source of esterified FA and the soybean FA distillate (Arm & Hammer Animal Nutrition, Ewing, NJ) contained approximately 97% FA of

which 95% were present as free FA, as the source of free FA (Table 6.1). Although both supplements contained comparable concentrations of total unsaturated FA there were differences in the concentration of individual FA; soybean oil contained a higher concentration of *cis*-9 C18:1 (26.0 vs. 11.8 g/100 g FA) and a lower concentration of C16:0 (9.6 vs. 15.0 g/100 g FA) and *cis*-9, *cis*-12 C18:2 (50.5 vs. 59.1 g/100 g FA) than the soybean FA distillate (Table 6.1). The ingredient and nutrient composition of the diets fed as TMR are described in Table 6.2. All treatment diets contained equal amounts of forages in an approximate 2:1 ratio of corn silage to alfalfa silage and diets were formulated to contain 23% forage NDF and 17% CP, and mineral and vitamins were formulated according to NRC (2001) recommendations. DM concentration was determined twice weekly for forages and diets were adjusted when necessary.

All cows were housed in the same tie-stall throughout the experiment and milked twice daily (0400 and 1500 h). Access to feed was blocked from 0800 to 1000 h to allow for collection of orts and offering feed. Cows were fed 115% of expected intake at 1000 h daily. Water was available ad libitum in each stall and stalls were bedded with sawdust and cleaned twice daily.

Samples and data were collected during the last 4 d of each treatment period (d 18 to 21). Samples of all diet ingredients (0.5 kg) and orts from each cow (12.5%) were collected daily and composited by period for analysis. Milk yield was recorded and two milk samples were collected at each milking. One aliquot was collected in a sealed tube with preservative (bronopol tablet; D&F Control Systems, San Ramon, CA) and stored at 4^{o}C for milk component analysis. The second aliquot was stored without preservative at -20^{o}C until analyzed for FA composition. BW was measured on the last 3 d of each period after the morning milking. Three trained investigators determined BCS on a 5-point scale (in 0.25 point increments; Wildman et al., 1982) on the last day of each period.

Diet ingredients and orts were dried at 55°C in a forced-air oven for 72 h for DM determination. Dried samples were ground with a Wiley mill (1 mm screen; Arthur H. Thomas, Philadelphia, PA). Diet ingredients were analyzed for NDF with heat stable α-amylase and sodium sulfite (Van Soest et al., 1991), CP (AOAC 2000, method 990.03), and starch (Hall, 2009) by Cumberland Valley Analytical Services, Inc. (Hagerstown, MD). Total FA concentration of feed ingredients was determined using a modification of the one step transesterification method developed by Sukhija and Palmquist (1988) as described by Lock et al. (2013).

Additional FA analysis of feed ingredients determined the proportion of individual FA either in free or esterified fractions. One gram of dried, ground samples were combined with 1 mL of internal standard (1 mg/mL toluene). Internal standards used were *cis*-10 C17:1 for free FA and tritridecanoin for triglycerides, which were utilized to determine FA yields. Total lipids were extracted with chloroform and methanol using a modified method of Folch et al. (1957), dried under nitrogen gas, and reconstituted in 0.5 mL of chloroform. Solid phase extraction (SPE) of lipid fractions was performed using a modified method of Agren et al. (1992). A vacuum manifold fitted with aminopropyl (N2) SPE columns (1g/mL) was used to separate the free FA fraction, a combined triglyceride/cholesterol ester fraction, and phospholipid fraction of each sample. The column was loaded with sample and flushed with 4 mL chloroform:isoporpanol solution (2:1) to separate and remove the triglyceride fraction. Columns were subsequently flushed with 6 mL chloroform:methanol:acetic acid (100:2:2) to separate and remove the free FA fraction. To separate and remove the phospholipid fraction, columns were flushed with 6 mL methanol:chloroform:water solution (10:5:4). The phospholipid fraction was dried using 2% sodium chloride solution and dissolved in chloroform. The phospholipid and

triglyceride fractions were subsequently combined into a single esterified fraction and all fractions were dried under N_2 gas and reconstituted in 0.5 mL toluene prior to methylation.

Fatty acid methyl esters (FAME) for both fractions were prepared with a modified two-step transmethylation procedure as described by Jenkins (2010). In brief, 1.0 mL of sodium methoxide (0.5 M solution in methanol) was added to each sample, vortexed, and incubated at 50°C for 10 min. After the samples had cooled, 1.5 mL of a 5% methanolic HCl solution was added, samples vortexed, and incubated at 80°C for 10 min. Samples were cooled and neutralized with a 6% potassium carbonate solution. The FAME were extracted with hexane and filtered through charcoal and silica. Hexane was evaporated under nitrogen gas, FAME were weighed and then reconstituted in hexane to obtain a 1% solution.

Feed FAME were determined on a GC-2010 Plus gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a split injector (1:100 split ratio) and a FID using a CP8827 WCOT fused silica column (30 m × 0.32 mm i.d. × 0.25 μm film thickness; Varian Inc., Lake Forest, CA). Analytical conditions and FAME standards were the same as described previously for FAME analyses of feeds (Lock et al., 2013).

Individual milk samples were analyzed for fat, true protein, and lactose concentration by mid-infrared spectroscopy (AOAC, 2000, method 972.160) by the Michigan Herd Improvement Association (Universal Lab Services, Lansing MI). Yields of 3.5% FCM, ECM (NRC, 2001), and milk components were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each collection period. Milk samples used for analysis of FA composition were composited based on milk fat yield (d 18-21 of each period). Lipids were extracted, methylated, and FAME composition determined by GLC, according to the methods described by Lock et al. (2013). Quantification of FA composition

covered approximately 70 FA in the range C4:0 to C24:0. Yield of individual FA (g/d) in milk fat were calculated by using milk fat yield and FA concentration to determine yield on a mass basis using the molecular weight of each FA while correcting for glycerol content and other milk lipid classes (Piantoni et al., 2013).

All data were analyzed using the fit model procedure of JMP (version 8; SAS Institute, Cary, NC). Data were analyzed using the following model:

$Y_{ijk} = \mu + S_i + C(S)_{ij} + P_k + T_l + e_{ijkl}$

where Y_{ijk} = the dependent variable, μ = the overall mean, S_i = fixed effect of square (i = 1 to 6), $C(S)_{ij}$ = random effect of cow nested within square (j = 1 to 18), P_k = fixed effect of period (k = 1 to 3), T_l = fixed effect of treatment (l = 1 to 3), and e_{ijk} = the residual error.

The interaction between period and treatment was initially included in the model and removed because $P > 0.20$ for all variables. Two preplanned, orthogonal contrasts were used to evaluate the effects of added FA to diets (CON vs. UFA; ½ (FFA+TAG)) and the effects of free FA vs. esterified FA (FFA vs. TAG). Contrasts were declared significant at $P \leq 0.05$ and trends were declared at $P \leq 0.10$.

RESULTS

Dietary NDF, forage NDF, starch, and CP remained similar across all treatments, whereas total dietary FA were 2.6, 4.2, and 4.3% of diet DM for CON, FFA, and TAG, respectively (Table 6.2). The supplements used in this study provided a marked contrast as sources for free vs. esterified FA with both supplements having similar total unsaturated FA concentration although as noted earlier differences did exist between the supplements for individual FA concentrations (Table 6.1). Using these supplements we were able to achieve

differences in the proportion of FA in the treatment diets as free or esterified FA. As a proportion of total dietary FA, CON, FFA, and TAG contained 21, 51, and 16% free FA (Table 6.2). The majority of the 21% free FA in CON came from alfalfa and corn silages (data not shown). The FA composition of the diets when separated into free and esterified fractions was similar with a majority of the dietary FA being unsaturated 18-carbon FA for both fractions (Table 6.3). Due to the low concentration of *cis*-9 C18:1 in the soybean FA distillate (Table 6.1), the concentration of this FA was lower in the free vs. esterified FA fraction in the FFA treatment (Table 6.3). Total FA intake was increased by 57% for UFA treatments, with no difference between FFA and TAG ($P = 0.21$; Table 6.4). As expected, the intake of all individual FA increased for UFA treatments compared to CON (all $P = 0.0001$). There was no difference in the intake of C18:0, *cis*-9, *cis*-12 C18:2, and *cis*-9, *cis*-12, *cis*-15 C18:3 between FFA and TAG (all $P > 0.20$; Table 6.4). However, we observed a 24 g/d lower intake of C16:0 and a 64 g/d higher intake of *cis*-9 C18:1 for TAG compared with FFA (both $P < 0.0001$; Table 6.4).

We observed a decrease in DMI of 1 kg/d, for the UFA treatments compared to CON ($P = 0.02$, Table 6.5) with no difference between FFA and TAG ($P = 0.74$). Milk yield was 2.2 kg greater for UFA treatments than CON ($P = 0.004$) with no difference between FFA and TAG ($P = 0.47$). The yield of milk fat was not affected by treatment, however the concentration of fat decreased with UFA treatments ($P = 0.05$) with no difference between FFA and TAG ($P = 0.35$). Similarly, we observed no effect of treatments on the yield of milk protein but milk protein concentration was lower for the UFA treatments ($P < 0.001$). Further, milk protein concentration was lower for FFA compared with TAG ($P = 0.01$). Milk lactose yield and concentration were increased by the UFA treatments compared to CON ($P = 0.001$ and $P = 0.01$, respectively). We observed no effect of treatments on the yields of 3.5% FCM and ECM ($P =$

0.15 and $P = 0.14$, respectively). Because of the decrease in DMI with the UFA treatments we observed a 6% improvement in feed efficiency (ECM/DMI) compared with CON ($P = 0.01$) with no difference between FFA and TAG ($P = 0.16$). We did not observe any differences in BW or BCS across treatments ($P > 0.14$).

Milk FA are derived from 2 sources: < 16 carbon FA from de novo synthesis in the mammary gland and > 16 carbon FA originating from extraction from plasma. Mixed source FA (C16:0 and *cis*-9 C16:1) can originate from de novo synthesis in the mammary gland and extraction from plasma. The UFA treatments decreased the concentration of de novo synthesized milk FA (Figure 6.1A) through a reduction in the concentration of C8:0 to C14:0 (all $P < 0.01$; Table 6.6). The concentration of 16-carbon FA was also lower for the UFA treatments ($P < 0.0001$; Table 6.6). However, we observed an increase in the concentration of preformed FA with the UFA treatments compared with CON ($P < 0.001$; Figure 6.1A) because the concentrations of all 18-carbon FA were increased ($P < 0.10$; Table 6.6). Of note, we observed an increase in the concentration of *trans*-10 C18:1 ($P = 0.004$) and a trend for an increase in *trans*-10, *cis*-12 C18:2 ($P = 0.06$) and *trans*-9, *cis*-11 C18:2 ($P = 0.07$) for the UFA treatments compared with CON (Table 6.6).

Similar to the results for milk FA on a concentration basis, the yield of de novo synthesized and 16-carbon FA were reduced by the UFA treatments (40 and 86 g, respectively; both $P < 0.001$), with the yield of 16-carbon FA greater for FFA compared to TAG ($P = 0.03$; Figure 6.1B). Preformed FA were increased by the UFA treatments (134 g; $P < 0.001$) with no differences between FFA and TAG (Figure 6.1B). The UFA treatments increased the yield of all 18-carbon milk FA (Table 6.7). As a consequence of the shift in sources of milk FA there was no significant change in overall milk fat yield. However, individual FA associated with MFD were

affected by the UFA treatments as demonstrated by an increased yield of *trans*-10 C18:1 ($P < 0.001$) and *trans*-9, *cis*-11 C18:2 ($P = 0.05$) in milk fat, and a trend for an increased yield of *trans*-10, *cis*-12 C18:2 ($P = 0.07$; Table 6.7). We observed no differences in these biohydrogenation intermediates between FFA and TAG ($P > 0.10$), except for a trend for TAG to increase *trans*-9, *cis*-11 C18:2 compared with FFA ($P = 0.07$; Table 6.7).

DISCUSSION

The amount of FA in feed ingredients is influenced by several factors (e.g. species, growing conditions, and stage of harvest; Boufaeïd et al. 2003) with the proportion of free FA increased by the action of plant lipases during harvest and storage (Yang and Fujita, 1997, Elgersma et al., 2003, Vanhalto et al., 2007, Halmemies-Beauchet-Filleau et al., 2013a). For example, in fresh perennial ryegrass, 98% of the FA were present as esterified FA but after ensiling, 27 to 73% of the total FA consisted of free FA (Elgersma et al., 2003). The variation that exists in the relative proportion of free to esterified FA in feed ingredients highlights the need to determine if differences between the two sources of FA in diets impacts production parameters of dairy cattle. Unsaturated dietary free FA can undergo biohydrogenation immediately and have instant interaction with rumen microbial cells, whereas esterified FA must first undergo hydrolysis prior to entering the free FA pool. Previous research utilizing an in vitro model to examine rumen metabolism of esterified and free FA suggested that when free FA are introduced directly into rumen cultures, the initial high concentration of free C18:2 inhibits some steps in the biohydrogenation process (Moore et al., 1969) and/or reduces the rate of biohydrogenation (Noble et al., 1974) thereby increasing concentrations of biohydrogenation intermediates. In our current study we utilized soybean FA distillate and soybean oil as sources

of free and esterified FA, respectively, which allowed for dietary treatments with different proportions of free and esterified FA. While we based the supplementation of soybean FA distillate and soybean oil upon weight of lipid and not total FA content we were able meet our objective of providing similar amounts of FA either as free or esterified FA.

Although the concentration of total unsaturated FA was comparable in both supplements, the soybean oil contained a higher concentration of *cis*-9 C18:1 and lower concentrations of C16:0 and *cis*-9, *cis*-12 C18:2 compared with the soybean FA distillate. This resulted in similar daily intakes of dietary FA with the exception of a 24 g/d lower intake of C16:0 and a 64 g/d higher intake of *cis*-9 C18:1 for TAG compared with FFA. Importantly, daily intake of *cis*-9, *cis*-12 C18:2 was not different between FFA and TAG. Recent work comparing the effect of increasing dietary concentrations of *cis*-9 C18:1 and *cis*-9, *cis*-12 C18:2 on MFD indicated that *cis*-9, *cis*-12 C18:2 has a greater potential to reduce milk fat concentration and yield compared to *cis*-9 C18:1 (He et al., 2012). However, it remains to be determined whether maintaining *cis*-9 C18:1 between these treatments would have altered our results. The 64 g/d increased intake of *cis*-9 C18:1 for TAG was a result of the higher concentration of *cis*-9 C18:1 in the soybean oil vs. soybean distillate. Reasons for the differences in the FA profile of the soybean oil and soybean FA distillate are unclear, but could simply be attributed to differences in individual sources of soybeans used to make both supplements and/or incomplete hydrolysis of individual FA to produce the distillate. It is interesting to note, however that there is evidence from studies with fresh and ensiled perennial ryegrass (Elgersma et al., 2003), fresh, dried, or ensiled timothy and meadow fescue (Halmemies-Beauchet-Filleau et al., 2013a), grass and red clover silage (Halmemies-Beauchet-Filleau et al., 2013b), and corn silage (Lock et al., unpublished data) suggesting that the FA profile of esterified and free FA fractions from these plants may differ.

For example, in grass silage and red clover silage there were differences in the concentrations of unsaturated FA between free FA, phospholipid, and neutral lipid fractions (Halmemies-Beauchet-Filleau et al., 2013b). Further research is warranted to determine possible reasons for differences in these fractions across plants and factors that may alter the concentration and profile of free FA in feedstuffs. For experimental purposes the ability to blend other oils (or distillates) into treatments to correct imbalance in the FA profile of starting FA supplements should be considered for future studies.

The UFA treatments reduced DMI as has previously been reported for unsaturated FA supplements (Allen, 2000; Harvatine and Allen, 2005). The reduction in DMI has been attributed to the hypophagic effect of unsaturated FA causing satiety signals that decrease meal size, meal frequency, or a combination of the two (Allen, 2000). Abomasal infusions of soybean free FA reduced DMI to a greater extent than abomasal infusions of soybean oil triglycerides (Litherland et al., 2005). However, in our study because the unsaturated FA from the supplements in FFA and TAG were fed rather than bypassing the rumen, both supplements would primarily result in free FA reaching the duodenum, which likely explains why we saw no difference between FFA and TAG. The reduction in milk protein concentration with the UFA treatments could have been the result of lowered DMI. The reduction in DMI would suggest lowered MP available for milk protein synthesis (Weiss et al., 2011) or because yield did not change, this could also be due to dilution of milk protein thus changing concentration but not yield.

The observed reduction in DMI and increased yields of milk and milk lactose when cows received the UFA treatments resulted in increased feed efficiency (ECM/DMI). The reduction in DMI and increase in milk yield is similar to the results of a meta-analysis on fat supplements in lactating dairy cattle (Rabiee et al., 2012). The increase in yield of milk and lactose is possibly

due to the glucose-sparing effects of FA. The supplemental FA could have been used as an energy source for tissues as well as precursors for preformed FA in milk fat. The reduction in de novo synthesis would reduce the requirement of NADPH from the pentose phosphate pathway, which is produced by glucose oxidation (Bauman and Davis, 1975). Therefore, reducing de novo synthesis in the mammary gland has the potential to reduce the glucose demand for fat synthesis. If glucose supply were limiting, this 'spared' glucose could then be used by other tissues or for lactose synthesis in the mammary gland, the osmotic regulator of milk, resulting in increased milk yield.

Grummer (1991) reported that there is an inverse relationship between the amount of fat supplemented in the diet and the concentration of C4:0 to C14:0 in milk fat. As more dietary fat is added, the proportion of de novo synthesized milk FA decreases while the proportion of preformed milk FA increases. In a meta-analysis examining the relationship between milk FA yield and duodenal flows of FA, Glasser et al. (2008) reported that increasing the duodenal supply of 18-carbon FA causes a quadratic increase in 18-carbon milk FA yield with a subsequent linear reduction in de novo synthesized milk FA yield. However, the meta-analysis also indicated that a severe reduction in the synthesis of de novo milk FA could limit, or even prevent, any increase in 18-carbon milk FA yield (Glasser et al., 2008), as would typically be observed during severe MFD. On a FA yield basis, the substitution effect of preformed for de novo milk FA was recently reported by He and Armenetano (2011) and He et al. (2012) who noted that the reduction in yield of de novo milk FA was often compensated for by an increase in the yield of preformed milk FA when fat supplements were fed. Classically, MFD represents a depression of milk fat yield with no change in the yield of milk and other milk components (Bauman et al., 2011), and typically causes a reduction in both de novo and preformed milk FA

yield because of the coordinated regulation of key enzymes associated with lipid synthesis in the mammary gland (Peterson et al., 2003, Harvatine and Baumam, 2011).

In our study, we observed a reduction in the yield of de novo synthesized milk FA that was compensated for by an increase in the yield of milk FA originating from preformed sources. The increase in the yield and concentration of preformed FA in milk fat was presumably because of the increase in the amount of dietary long chain FA available to the mammary gland, and as discussed previously, the substitution effect of preformed for de novo milk FA is well established (e.g. Grummer, 1991; Glasser et al., 2008). When considering these results we believe there are two possible interpretations, both of which warrant consideration. One interpretation is that since we did not detect any change in overall milk fat yield, we did not observe what would typically be considered as 'diet-induced MFD'. An alternative interpretation focuses on the overall effect of the UFA treatments shifting biohydrogenation pathways and/or rates as evident by the increase in biohydrogenation intermediates associated with MFD. The UFA treatments increased the concentration and yield of *trans*-10 18:1 with a trend for increased concentrations and yields of *trans*-9, *cis*-11 C18:2 and *trans*-10, *cis*-12 C18:2 in milk fat. Although there was a reduction in milk fat concentration, the increased uptake of these biohydrogenation intermediates by the mammary gland (and incorporation into milk fat) did not result in an overall reduction in milk fat yield. However, the reduction in de novo synthesized milk FA could have been directly caused by increases in biohydrogenation intermediates associated with MFD, suggesting that under the dietary conditions tested perhaps we observed what could be referred to as "sub-clinical" MFD. It is possible that increases in specific biohydrogenation intermediates were sufficient to reduce de novo synthesis in the mammary gland, which is typically inhibited to a greater extent in normal MFD-situations than preformed

milk FA (Bauman et al., 2011). If the concentration of dietary unsaturated FA were increased further, we might have expected to see a greater reduction in de novo synthesized FA as well as a reduction in preformed milk FA, resulting in an overall reduction in milk fat yield.

As a result of the increase in biohydrogenation intermediates associated with MFD it appears that the negative effect of the increased dietary unsaturated FA with the UFA treatments was due to effects in the rumen. This leads us to question if we could remove or reduce the negative effect of unsaturated FA on rumen FA metabolism, would increasing unsaturated FA have positive effects on milk fat yield by increasing their use for milk fat synthesis, without detrimental effects on de novo FA synthesis? Previously, chemically treating oilseeds containing unsaturated FA has been used to reduce the negative effect of unsaturated FA on rumen fermentation. Formaldehyde treated oilseeds resulted in increases in milk fat yield (Ashes et al., 1992; Petit, 2003), mostly through an increase in 18-carbon unsaturated FA yield and minimal changes in de novo FA yield (Ashes et al., 1992), indicating that minimizing adverse effects on rumen fermentation may have positive effects on milk fat yield. However when unsaturated FA were infused in the abomasum of dairy cows, bypassing the effects of rumen fermentation, the proportion of unsaturated 18-carbon milk FA increased while the proportion of C16:0 was reduced without affecting total milk fat yield, indicating that there may be other mechanisms in the mammary gland that regulate milk FA synthesis. Though it should be noted that DMI and milk yield were also reduced in this study (Drackley et al., 1992). Interestingly, supplementing a C16:0-enriched fat supplement at 2% of diet DM, increased the secretion of 16-carbon FA into milk fat while maintaining both de novo and preformed milk FA secretion therefore increasing milk fat yield (Lock et al., 2013). Clearly further research is required to improve our

understanding of the impact of specific FA on the balance between the secretion of de novo and preformed FA in order to maximize milk fat synthesis.

Our study compared a mostly esterified FA (TAG) to a mostly free FA (FFA) supplement to evaluate the effects on rumen metabolism and production parameters. Contrary to previous research using cottonseed containing increasing amounts of free FA (Sullivan et al., 2004; Cooke et al., 2007), we observed no differences between the FFA and TAG supplements for milk fat concentration and yield, or the concentration of specific milk FA associated with MFD. Results from in vitro studies indicate that increased free FA results in an accumulation of biohydrogenation intermediates whereas esterified FA are more completely biohydrogenated to saturated FA (Moore et al., 1969; Noble et al., 1974). We expected to see a reduction in milk fat concentration and yield when feeding free FA compared to esterified FA due to a more rapid availability of unsaturated FA in the rumen leading to altered rumen biohydrogenation. However, we observed no indication that FFA caused an increase in biohydrogenation intermediates associated with MFD or reduced milk fat yield to a greater extent than TAG. However, since we utilized FA supplements as our source of free and esterified FA these results should not be extrapolated to conclude that other feeds with elevated concentrations of free FA will not cause MFD. Rather, our results suggest that when MFD occurs in these situations it probably is not the free FA per se, but may be any number of other issues that develop with poorly fermented and stored feeds.

Rico and Harvatine (2013) reported that by 11 d following the feeding of a milk fat depressing diet, milk fat yield from cows was not different from control. Thus, our 21 d periods with sampling starting at d 18 should have been sufficient time for the rumen to adjust to a diet change. The study was balanced for carryover effects and because there was no treatment by

period interactions, there were no detectable carryover effects between treatments. We used soybean-based fats because of the availability of both a free FA distillate and oil containing mostly esterified FA from soybeans. However, we recognize that corn is a more common source of FA for dairy cattle. The FA profile of corn and soybean oil is very similar, with a majority of FA being linoleic acid, therefore we would expect similar results if the trial was conducted with corn-based FA supplements. While there were some differences in the FA profile of the supplements, notably *cis*-9 C18:1, their use allowed us to substantially alter the proportion of FA present in the diet either as free or esterified FA. As a result of the low milk fat in CON, milk fat concentration for all treatments was lower than anticipated. A meta-analysis by Hollmann et al. (2011) indicated that the milk fat concentration of cows fed control diet impacts the response to supplementing distillers grains. Studies containing control cows with a milk fat concentration > 3.6% tended to respond more negatively to added distillers grains whereas studies containing control cows with a milk fat concentration < 3.6% tended to respond positively for milk fat concentration with added distillers grains (Hollmann et al., 2011). It remains to be determined if control cows starting at a higher concentration of milk fat would have responded similarly to the unsaturated FA supplements used in the current study.

CONCLUSIONS

Unsaturated FA supplements, fed as either free or esterified soybean FA reduced DMI, increased milk yield, but had no effect on yield of milk fat. Although there were indicators of an altered rumen environment with the UFA treatments, evidenced by the increased concentration and yield of biohydrogenation intermediates associated with MFD in milk fat, we did not observe a reduction in milk fat yield because the increase in preformed FA compensated for the

reduction in de novo and 16-carbon milk FA. There were no differences in yields of milk or components between FFA and TAG, specifically we observed no differences in the yield of biohydrogenation intermediates associated with MFD between the two UFA treatments, contrary to what has been observed in vitro and with byproducts varying in the proportion of free and esterified FA. It remains to be determined if testing with cows starting with a higher milk fat concentration and/or using diets that differ in their risk of MFD may have produced differences between free and esterified FA. Further research on the regulation of preformed and de novo synthesized milk FA in the mammary gland is needed in order to develop strategies to maximize potential income from milk components.

APPENDIX

APPENDIX

Table 6.1. Composition of FA supplements fed during the treatment periods[1].

	Soybean Free FA Distillate[2]	Soybean Oil[3]
Total FA content	97.0 ± 1.47	83.9 ± 0.47
Free FA, % of FA	95.3 ± 0.76	0.6 ± 0.13
Esterified FA, % of FA	4.7 ± 0.76	99.4 ± 0.13
FA (g/100 g of FA)		
C14:0	0.1 ± 0.003	0.1 ± 0.002
C16:0	15.0 ± 0.87	9.6 ± 0.26
C18:0	3.8 ± 0.17	5.3 ± 0.37
C18:1 *cis*-9	11.8 ± 0.15	26.0 ± 0.23
C18:2 *cis*-9, *cis*-12	59.1 ± 0.88	50.5 ± 0.55
C18:3 *cis*-9, *cis*-12, *cis*-15	6.8 ± 0.09	5.5 ± 0.10
Σ Others	3.3 ± 0.08	2.9 ± 0.14

[1]Average (± SD) composition of the supplements based on samples taken prior to the supplements being blended with corn grain at the start of each treatment period.
[2]Provided by Arm & Hammer Dairy Nutrition, Ewing, NJ.
[3]Provided by West Central Cooperative, Ralston, IA.

Table 6.2. Ingredients and nutrient composition of the treatment diets[1].

	Treatments[2]		
	CON	FFA	TAG
Ingredient, % of DM			
Corn silage	34.9	34.9	34.9
Alfalfa silage	17.9	17.9	17.9
Wheat straw	3.5	3.5	3.5
Ground corn	22.3	22.3	22.3
Soybean meal	15.8	15.8	15.8
Soyhulls	2.0	---	---
Soybean free FA distillate[3]	---	2.0	---
Soybean oil[4]	---	---	2.0
Vitamin & mineral mix[5]	2.2	2.2	2.2
Limestone	0.6	0.6	0.6
Sodium bicarbonate	0.7	0.7	0.7
Nutrient composition			
DM, %	55.3	55.3	55.4
NDF, % of DM	28.1	27.4	27.5
Forage NDF, % of DM	23.2	23.1	23.1
Starch, % of DM	28.7	29.6	29.3
CP, % of DM	17.2	17.0	17.0
Total FA, % of DM	2.6	4.2	4.3
16:0, % of DM	0.34	0.58	0.49
18:0, % of DM	0.07	0.13	0.12
18:1 *cis*-9, % of DM	0.48	0.55	0.89
18:2 *cis*-9, *cis*-12, % of DM	1.24	2.18	2.10
18:3 *cis*-9, *cis*-12, *cis*-15, % of DM	0.21	0.31	0.31
Free FA, % of FA	20.6	50.9	16.3
Esterified FA, % of FA	79.4	49.1	83.7

[1]Average composition of three periods fed to lactating dairy cows (n=18).
[2]Treatments contained 2% soyhulls (CON), 2% soybean free FA distillate (FFA), or 2% soybean oil (TAG).
[3]Provided by Arm & Hammer Dairy Nutrition, Ewing, NJ.
[4]Provided by West Central Cooperative, Ralston, IA.
[5]Vitamin and mineral mix contained 34.1% dry ground shell corn, 25.6% white salt, 21.8% calcium carbonate, 9.1% Biofos, 3.9% magnesium oxide, 2% soybean oil, and < 1% of each of the following: manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, iodine, cobalt carbonate, vitamin E, vitamin A, vitamin D, and selenium.

Table 6.3. FA composition of free and esterified fractions of treatment diets[1].

	Treatments[2]					
	CON		FFA		TAG	
FA (g/100 g of total FA)	Free	Esterified	Free	Esterified	Free	Esterified
14:0	0.4	0.4	0.2	0.4	0.2	0.2
16:0	19.4	13.7	18.1	14.0	16.4	11.9
18:0	2.8	2.6	3.9	2.7	3.5	3.8
18:1 *cis*-9	21.6	23.0	13.6	20.2	21.0	23.9
18:2 *cis*-9, *cis*-12	42.4	49.3	53.8	51.0	47.5	50.3
18:3 *cis*-9, *cis*-12, *cis*-15	8.4	5.6	7.1	6.1	7.4	5.6
22:0	0.3	0.3	0.4	0.3	0.3	0.3
24:0	0.2	0.2	0.2	0.2	0.2	0.1
Σ Others	3.8	4.7	2.6	4.8	3.2	3.6
Σ SFA	23.2	17.2	22.7	17.7	20.5	16.4
Σ RUFAL[3]	72.5	77.9	74.5	77.3	75.9	79.8

[1]Average composition of three periods fed to lactating dairy cows (n=18).
[2]Treatments contained 2% soyhulls (CON), 2% soybean free FA distillate (FFA), or 2% soybean oil (TAG).
[3]Rumen unsaturated fatty acid load – (18:1 *cis*-9 + 18:2 *cis*-9, *cis*-12 + 18:3 *cis*-9, *cis*-12, *cis*-15).

Table 6.4. Intake of FA (g/d) for cows fed treatment diets (n=18).

	Treatments[1]				*P*-values[2]	
FA Intake, g/d[3]	CON	FFA	TAG	SEM	CON vs. UFA	FFA vs. TAG
C16:0	93.9	154	130	4.15	< 0.0001	< 0.0001
C18:0	18.2	33.5	31.9	1.61	< 0.0001	0.34
C18:1 *cis*-9	129	172	236	5.49	< 0.0001	< 0.0001
C18:2 *cis*-9, *cis*-12	342	577	561	16.0	< 0.0001	0.20
C18:3 *cis*-9, *cis*-12, *cis*-15	56.6	83.0	82.5	2.36	< 0.0001	0.79
Total FA	716	1108	1136	31.4	< 0.0001	0.21

[1] Treatments contained 2% soyhulls (CON), 2% soybean free FA distillate (FFA), or 2% soybean oil (TAG).
[2] *P*-values associated with contrasts, CON vs. UFA – *P*-value associated with differences between control vs. average of FFA and TAG; FFA vs. TAG – *P*-value associated with differences between FFA vs. TAG.
[3] Calculated using DMI for individual cows and the FA composition of the respective diet during each treatment period.

Table 6.5. Dry matter intake, milk production and composition, ECM/DMI, BW, and BCS for cows fed treatment diets (n=18).

	Treatments[1]				*P*-values[2]	
Variable	CON	FFA	TAG	SEM	CON vs. UFA	FFA vs. TAG
DMI, kg/d	27.6	26.5	26.7	0.63	0.02	0.74
Milk Yield, kg/d						
Milk	39.5	42.0	41.4	0.94	0.004	0.47
3.5% FCM[3]	38.2	39.9	38.8	1.26	0.15	0.22
ECM[4]	38.7	40.2	39.4	1.14	0.14	0.32
Milk Composition						
Fat, kg/d	1.30	1.34	1.29	0.06	0.70	0.22
Fat, %	3.30	3.18	3.11	0.12	0.05	0.35
Protein, kg/d	1.25	1.28	1.29	0.03	0.17	0.83
Protein, %	3.16	3.06	3.12	0.04	< 0.001	0.01
Lactose, kg/d	1.88	2.03	2.00	0.06	0.001	0.45
Lactose, %	4.77	4.83	4.82	0.07	0.01	0.63
ECM/DMI	1.42	1.53	1.48	0.05	0.01	0.16
Body Weight	709	705	706	20.9	0.16	0.78
BCS	3.00	2.96	2.93	0.13	0.14	0.42

[1] Treatments contained 2% soyhulls (CON), 2% soybean free FA distillate (FFA), or 2% soybean oil (TAG).
[2] *P*-values associated with contrasts, CON vs. UFA – *P*-value associated with differences between control vs. average of FFA and TAG; FFA vs. TAG – *P*-value associated with differences between FFA vs. TAG.
[3] Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].
[4] Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)] (Tyrrell and Reid, 1965).

Table 6.6. Milk FA concentration of cows fed treatment diets (n=18).

	Treatments[1]				*P*-values[2]	
FA (g/100 g total FA)	CON	FFA	TAG	SEM	CON vs. UFA	FFA vs. TAG
Selected individual FA[3]						
4:0	2.89	3.06	2.99	0.08	0.06	0.34
6:0	2.13	2.04	2.02	0.07	0.09	0.81
8:0	1.35	1.21	1.22	0.05	0.001	0.79
10:0	3.68	2.96	3.02	0.14	< 0.0001	0.51
12:0	4.55	3.43	3.54	0.15	< 0.0001	0.23
14:0	13.2	11.4	11.5	0.26	< 0.001	0.49
14:1 *cis*-9	1.07	0.86	0.95	0.06	< 0.001	0.01
16:0	34.3	28.0	26.6	0.55	< 0.001	< 0.001
16:1 *cis*-9	1.62	1.27	1.29	0.06	< 0.001	0.51
18:0	7.29	10.24	10.18	0.26	< 0.001	0.76
18:1 *trans*-4	0.011	0.023	0.028	0.001	< 0.001	< 0.001
18:1 *trans*-5	0.008	0.019	0.023	0.001	< 0.001	< 0.001
18:1 *trans*-6-8	0.21	0.39	0.48	0.02	< 0.001	0.002
18:1 *trans*-9	0.17	0.32	0.36	0.01	< 0.001	0.001
18:1 *trans*-10	0.35	0.82	1.24	0.21	0.004	0.11
18:1 *trans*-11	0.62	1.54	1.70	0.07	< 0.001	0.04
18:1 *trans*-12	0.32	0.66	0.69	0.02	< 0.001	0.13
18:1 *cis*-9	16.3	20.2	20.6	0.55	< 0.001	0.29
18:1 *cis*-11	0.47	0.51	0.52	0.03	0.03	0.66
18:2 *cis*-9, *cis*-12	2.23	3.14	2.99	0.08	< 0.001	0.03
18:2 *cis*-9, *trans*-11	0.35	0.79	0.83	0.04	< 0.001	0.28
18:2 *trans*-9, *cis*-11	< 0.001	0.004	0.012	0.003	0.07	0.07
18:2 *trans*-10, *cis*-12	<0.001	0.003	0.005	0.002	0.06	0.44
18:3 *cis*-9, *cis*-12, *cis*-15	0.37	0.44	0.45	0.01	< 0.001	0.07

[1] Treatments contained 2% soyhulls (CON), 2% soybean free FA distillate (FFA), or 2% soybean oil (TAG).

[2] Two orthogonal contrasts; CON vs. UFA – *P*-value associated with differences between control vs. average of FFA and TAG; FFA vs. TAG – *P*-value associated with differences between FFA vs. TAG.

[3] A total of approximately 70 individual FA were quantified and used for calculations (summation by concentrations). Only select FA are reported in the table.

Table 6.7. Milk fatty acid yield of cows fed treatment diets (n=18).

	Treatments[1]				*P*-values[2]	
FA (g/d)[3]	CON	FFA	TAG	SEM	CON vs. UFA	FFA vs. TAG
Selected individual FA						
4:0	35.3	38.8	36.8	2.2	0.08	0.22
6:0	26.1	26.0	25.2	1.7	0.63	0.51
8:0	16.6	15.5	15.4	1.1	0.07	0.84
10:0	45.3	37.8	38.0	2.7	< 0.001	0.89
12:0	56.0	43.7	44.1	3.3	< 0.001	0.79
14:0	162	144	141	7.7	< 0.001	0.65
14:1 *cis*-9	13.1	10.8	11.1	0.7	< 0.001	0.33
16:0	421	353	325	20.1	< 0.001	0.02
16:1 *cis*-9	19.7	15.9	15.1	0.8	< 0.001	0.12
18:0	88.9	129	124	7.0	< 0.001	0.36
18:1 *trans*-4	0.14	0.29	0.34	0.02	< 0.001	0.003
18:1 *trans*-5	0.10	0.24	0.27	0.01	< 0.001	0.003
18:1 *trans*-6-8	2.52	4.82	5.51	0.16	< 0.001	< 0.001
18:1 *trans*-9	2.09	3.89	4.25	0.13	< 0.001	0.01
18:1 *trans*-10	4.24	9.57	12.29	1.47	< 0.001	0.14
18:1 *trans*-11	7.57	19.1	20.9	1.24	< 0.001	0.14
18:1 *trans*-12	3.84	8.13	8.17	0.29	< 0.001	0.92
18:1 *cis*-9	198	250	241	7.33	< 0.001	0.22
18:2 *cis*-9, *cis*-12	27.1	39.1	35.4	1.2	< 0.001	0.003
18:2 *cis*-9, *trans*-11	4.25	9.69	9.75	0.42	< 0.001	0.89
18:2 *trans*-9, *cis*-11	0.01	0.04	0.10	0.03	0.05	0.07
18:2 *trans*-10, *cis*-12	< 0.01	0.03	0.04	0.02	0.07	0.57
18:3 *cis*-9, *cis*-12, *cis*-15	4.52	5.42	5.34	0.18	< 0.001	0.67

[1] Treatments contained 2% soyhulls (CON), 2% soybean free FA distillate (FFA), or 2% soybean oil (TAG).
[2] Two orthogonal contrasts; CON vs. UFA – *P*-value associated with differences between control vs. average of FFA and TAG; FFA vs. TAG – *P*-value associated with differences between FFA vs. TAG.
[3] A total of approximately 70 individual FA were quantified and used for calculations (summation by yield). Only select FA are reported in the table.

Figure 6.1 A.

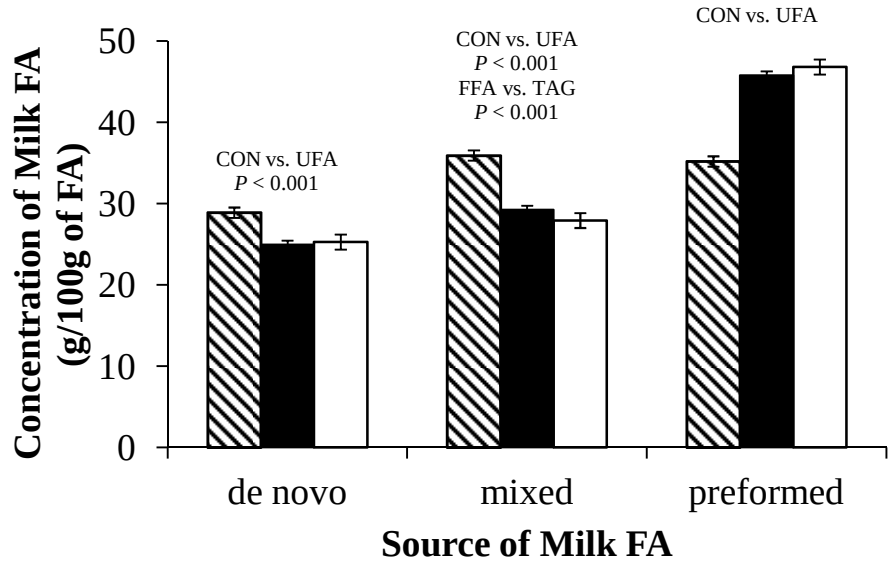

Figure 6.1 B.

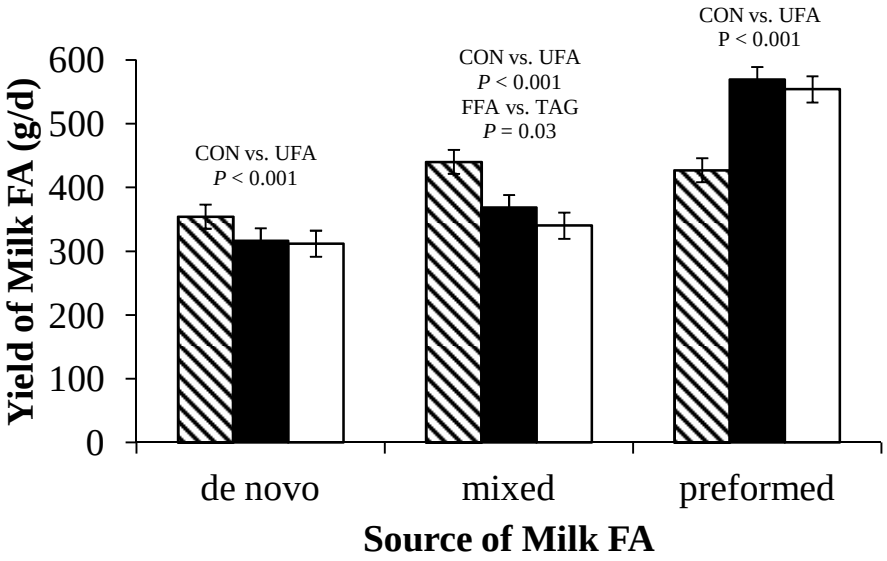

Figure 6.1. Concentration (Panel A) and yield (Panel B) of milk FA by source for cows (n=18) fed treatment diets. Hashed bar represents CON, solid bar represents FFA, and open bar represents TAG treatment. De novo = milk FA < 16 carbons in length; Mixed = milk FA 16-carbons in length; Preformed = milk FA > 16 carbons in length. *P*-values associated with contrasts, CON vs. UFA – *P*-value associated with differences between control vs. average of FFA and TAG; FFA vs. TAG – *P*-value associated with differences between FFA vs. TAG.

REFERENCES

REFERENCES

Agren, J. J., A. Julkunen, I. Penttila. 1992. Rapid separation of serum lipids for fatty acid analysis by a single aminopropyl column. J Lipid Res. 33(12): 1871-1876.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598-1624.

Ashes, J. R., P. V. Welch, S. K. Gulati, and T. W. Scott. 1992. Manipulation of the fatty acid composition of milk by feeding protected canola seeds. J. Dairy Sci. *75*: 1090–1096.

AOAC. 2000. Official Methods of Analysis. 17th ed. AOAC International, Arlington, VA.

Bauman, D. E., and C. L. Davis. 1975. in *Digestistion and Metabolism in the Ruminant* (McDonald, I. E. & Warner, A. C. I., eds.), pp. 296-509. The University of New England Publishing Unit, Armidale, N. S. W.

Bauman, D. E., K. J. Harvatine, and A. L. Lock. 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Ann. Rev. Nutr. 31: 299-319.

Boufaeïd, H., P. Y. Chouinard, G. F. Tremblay, H. V. Petit, R. Michaud, and G. Bèlanger. 2003. Fatty acids in forages. I. Factors affecting concentrations. Can. J. Anim. Sci. 83:501-511.

Cao, Z. J., J. L. Anderson, and K. F. Kalscheur. 2009. Ruminal degradation and intestinal digestibility of dried or wet distillers grains with increasing concentrations of condensed distillers solubles. J. Anim. Sci. 87:3013-3019.

Cooke, K. M., J. K. Bernard, C. D. Wildman, J. W. West and A. H. Parks. 2007. Performance and ruminal fermentation of dairy cows fed whole cottonseed with elevated concentrations of free fatty acids in the oil. J. Dairy Sci. 90:2329-2334.

Drackley, J. K., T. H. Klusmeyer, A. M. Trusk, and J. H. Clark. 1992. Infusion of long-chain fatty acids varying in saturation and chain length into the abomasum of lactating dairy cows. J. Dairy Sci. 75:1517-1526.

Elgersma, A., G. Ellen, H. van der Horst, B.G. Muuse, H. Boer, and S. Tamminga. 2003. Comparison of the fatty acid composition of fresh and ensiled perennial ryegrass (Lolium perenne L.), affected by cultivar and regrowth interval. Anim Feed Sci Tech. 108:191–205.

Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Glasser, F., A. Ferlay, M. Doreau, P Schmidely, D. Sauvant, and Y. Chilliard. 2008. Long-chain fatty acid metabolism in dairy cows: a meta-analysis of milk fatty acid yield in relation to duodenal flows and de novo synthesis. J. Dairy Sci. 91: 2771-2785.

Griinari, J.M., D.A. Dwyer, M.A. McGuire, D.E. Bauman, D.L. Palmquist, and K.V. Nurmela. 1998. Trans-octadecenoic acids and milk fat depression in lactating dairy cows. J Dairy Sci. 81:1251–1261.

Grummer, R.R. 1991. Effect of feed on the composition of milk fat. J. Dairy Sci. 74: 3244-3257.

Hall, M. B. 2009. Analysis of starch, including maltooligosacchardies, in animal feeds: A comparison of methods and a recommended method for AOAC collaborative study. J. AOAC Int. 92:42-49.

Halmemies-Beauchet-Filleau, A., P. Kairenius, S. Ahvenjärvi, L. K. Crosley, S. Muetzel, P. Huhtanen, A. Vanhatalo, V. Toivonen, R. J. Wallace, and K. J. Shingfield. 2013a. Effect of forage conservation method on ruminal lipid metabolism and microbial ecology in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. J. Dairy Sci. 96:2428-2447.

Halmemies-Beauchet-Filleau, A., A. Vanhatalo, V. Toivonen, T. Heikkil**i**, M. R. F. Lee, and K. J. Shingfield. 2013b. Effect of replacing grass silage with red clover silage on ruminal lipid metabolism in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. J. Dairy Sci. 96:5882-5900.

Harvatine, K. J., and M. S. Allen. 2005. The effect of production level on feed intake, milk yield, and endocrine responses to two fatty acid supplements in lactating cows. J. Dairy Sci. 88:4018-4027.

Harvatine, K.J., and D.E. Bauman. 2011. Characterization of the acute lactational response to trans-10, cis-12 conjugated linoleic acid. J Dairy Sci. 94:6047–6056.

He, M., and L. E. Armentano. 2011. Effect of fatty acid profile in vegetable oils and antioxidant supplementation on dairy cattle performance and milk fat depression. J. Dairy Sci. 94: 2481-2491.

He, M., K. L. Perfield, H. B. Green, and L. E. Armentano. 2012. Effect of dietary fat blend enriched in oleic or linoleic acid and monensin supplementation on dairy cattle performance, milk fatty acid profiles, and milk fat depression. J. Dairy Sci. 95: 1447-1461.

Hollmann, M., M. S. Allen, and D. K. Beede. 2011. Diet fermentability influences lactational performance responses to corn distillers grains: A meta-analysis. J. Dairy Sci. 94:2007-2021.

Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76: 3851-3863.

Jenkins, T.C. 2010. Technical note: common analytical errors yielding inaccurate results during analysis of fatty acids in feed and digesta samples. J Dairy Sci. 93:1170–1174.

Khan, N. A., J. W. Cone, V. Fievez, and W. H. Hendriks. 2012. Causes of variation in fatty acid content and composition in grass and maize silages. Anim. Feed Sci. Tech. 174:36–45.

Leonardi, C., S. Bertics, and L. E. Armentano. 2005. Effect of increasing oil from distillers grains or corn oil on lactational performance. J. Dairy Sci. 88:2829-2827.

Litherland, N.B., S. Thire, A. D. Beaulieu, C. K. Reynolds, J. A. Benson, and J. K. Drackley. 2005. Dry matter intake is decreased more by abomasal infusions of unsaturated free fatty acids than by unsaturated triglycerides. J. Dairy Sci. 88: 632-643.

Lock, A.L., C.L. Preseault, J.E. Rico, K.E. DeLand, and M.S. Allen. 2013. Feeding a C16:0-enriched fat supplement increased the yield of milk fat and improved conversion of feed to milk. J. Dairy Sci. 96:6650-6659.

Loor, J. J., K. Ueda, A. Ferlay, Y. Chilliard, and M. Doreau. 2005. Intestinal flow and digestibility of trans fatty acids and conjugated linoleic acids (CLA) in dairy cows fed a high-concentrate diet supplemented with fish oil, linseed oil, or sunflower oil. Anim. Feed Sci. and Tech. 119:203–225.

Maia, M. R. G., L. C. Chaudhary, L. Figueres, and R. J. Wallace. 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. Ant. van Leeuw. 91:303-314.

Moore, J. H., R. C. Noble, W. Steele, and J. W. Czerkawski. 1969. Differences in the metabolism of esterified and unesterified linoleic acid by rumen micro-organisms. Br. J. Nurt. 23: 869-878.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Acad. Sci., Washington D.C.

Noble, R.C., J. H. Moore, and C. G. Harfoot. 1974. Observations on the pattern of biohydrogenation of esterified and unesterified linoleic acid in the rumen. Br. J. Nutr. 31: 99-108.

Peterson, D. G., E. A. Matitashvili, and D. E. Bauman. 2003. Diet-induced milk fat depression in dairy cows results in increased *trans*-10, *cis*-12 CLA in milk fat and coordinate suppression of mRNA abundance for mammary enzymes involved in milk fat synthesis. J. Nutr. 133:3098-3102.

Petit, H. V. 2003. Digestion, milk production, milk composition, and blood composition of dairy cows fed formaldehyde treated flaxseed or sunflower seed. J. Dairy Sci. 86:2637–2646.

Piantoni, P., A. L. Lock, and M. S. Allen. 2013. Palmitic acid increased yields of milk and milk fat and nutrient digestibility across production level of lactating cows. J. Dairy Sci. 96:7143–7154.

Rabiee, A. R., K. Breinhild, W. Scott, H. M. Golder, E. Block, and I. J. Lean. 2012. Effect of fat additions to diets of dairy cattle on milk production and components: A meta-analysis and meta-regression. J. Dairy Sci. 95: 3225-2347.

Rico, D. E. and K. J. Harvatine. 2013. Induction of and recovery from milk fat depression occurs progressively in dairy cows switched between diets that differ in fiber and oil concentration. J. Dairy Sci. 96: 1-10.

Sukhija, P. S, and D. L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36: 1202-1206.

Sullivan, H. M., J. K. Bernard, H. E. Amos, and T. C. Jenkins. 2004. Performance of lactating dairy cows fed whole cottonseed with elevated concentrations of free fatty acids in the oil. J. Dairy Sci. 87: 655-671.

Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of the milk. J. Dairy Sci. 48: 1215-1223.

Vanhatalo, A., K. Kuoppala, V. Toivonen, and K.J. Shingfield. 2007. Effect of forage species and stage of maturity on bovine milk fatty acid composition. Eur. J. Lipid Sci. Technol. 109: 856-867.

Van Soest, P. J., J. B. Roberson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

Weiss, W. P., J. M. Pinos-Rodriguez, and D. J. Wyatt. 2011. The value of different fat supplements as sources of digestible energy for lactating dairy cows. J. Dairy Sci. 94: 931-939.

Wildman, E. E., G. M. Jones, P. E. Wagner, and R. L. Bowman. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65: 495-501.

Yang, U. M., and H. Fujita. 1997. Changes in grass lipid fractions and fatty acid composition attributed to hay making. Grassl. Sci. 42: 289-293.

CHAPTER 7

MILK PRODUCTION AND NUTRIENT DIGESTBILITY RESPONSES TO INCREASING LEVELS OF STEARIC ACID SUPPLEMENTATION IN DAIRY CATTLE

INTRODUCTION

Recent advances in the understanding of the effects of individual FA on production responses, digestibility, and metabolism have led to research on the biological effects of specific FA. Much of this focus has been on individual saturated FA, particularly C16:0 (Lock et al., 2013; Piantoni et al., 2013; Rico et al., 2014) with less focus with C18:0 (Piantoni et al., 2014). Due to biohydrogenation in the rumen, C18:0 is the major FA that is available for absorption in the small intestine. Since C18:0 is the major end product of biohydrogenation, and thus considered rumen inert, it is potentially an ideal FA to feed in a FA supplement for lactating dairy cattle. However, in recent studies evaluating the digestibility of C18:0 compared to other FA, C18:0 had reduced digestibility when supplemental FA were fed (Weisbjerg et al., 1992; Tice et al., 1994; Loor et al., 2002). Potential reasons for reduced digestibility include oversaturation of FA absorption sites, limited supply of emulsifiers such as lysolethecin in the small intestine, or a unique property of C18:0 that limits digestibility (Drackley, 2000).

Both C18:0 and *cis*-9 C18:1 are major FA in milk fat, with concentrations potentially limited by the requirement of the mammary gland to produce milk fat that is fluid at body temperature. The relatively high melting point of C18:0 requires the production of de novo synthesized FA or the conversion of C18:0 to *cis*-9 C18:1 in the mammary gland in order to maintain fluidity (Palmquist, 2006). In a meta-analysis analyzing milk FA yield in relation to duodenal flows of FA and de novo synthesis of FA in the mammary gland, Glasser et al. (2008) showed that desaturation of C18:0 in the mammary gland is positively linearly related to

mammary uptake of C18:0. While it is well established that specific rumen biohydrogenation intermediates inhibit de novo synthesis of milk FA in the mammary gland (Bauman et al., 2011) and there is an interdependence in milk FA yield between de novo and 18-carbon milk FA as described by Glasser et al (2008), we are uncertain if C18:0 itself will have an impact on de novo synthesized milk FA. Therefore, determining if there is a maximum amount of C18:0 that can be incorporated in milk fat while maintaining fluidity and not reducing de novo synthesis is of particular interest.

Supplementation of C18:0 has been shown to have variable responses for production parameters when fed to lactating dairy cattle. Feeding a relatively pure source of C18:0 at approximately 4% diet DM resulted in an increase in milk fat yield with no effect on milk yield (Steele and Moore, 1968). However, in a subsequent study with a similar C18:0 inclusion level an increase in milk yield was observed with no effect on milk fat concentration or yield (Steele, 1969). More recently, Piantoni et al. (2014) reported increased DMI, milk yield, and milk fat yield in one period of a crossover design with no effects in the other period with increases more evident in cows with higher milk yields, indicating that there was significant variation in response.

Therefore our objectives were to evaluate the dose-dependent effects of a C18:0-enriched fat supplement on feed intake, production responses, nutrient digestibility, and the incorporation of C18:0 into milk fat. Our hypothesis was that there is an optimal amount of C18:0 that can be supplemented to maximize production responses, specifically C18:0 incorporation into milk fat, without reducing C18:0 digestibility. Results from this experiment will further our understanding of the role of C18:0 in dairy cow nutrition and metabolism and help determine the optimal FA

profile of fat supplements fed to dairy cattle. Additionally, no studies to our knowledge have determined dose response effects to supplementing C18:0.

MATERIALS & METHODS

The Institutional Animal Care and Use Committee at Michigan State University approved experimental procedures. Thirty-two mid-lactation (147 ± 66 DIM; mean ± SD) multiparous Holstein cows from the Michigan State University Dairy Field Laboratory were blocked by milk yield (46.6 ± 9.6 kg/d [mean ± SD], range 29.6 - 70.4 kg/d) then randomly assigned to treatment sequence in a replicated 4 x 4 Latin Square design experiment with 21-d periods. All animals received a common diet with no fat supplementation during a 14 d covariate period to obtain baseline.

Treatments consisted of a control diet (CON) and three treatments supplemented with increasing doses of a C18:0-enriched fat supplement (SA) at 0.8% (L-SA), 1.5% (M-SA), and 2.3% SA (H-SA). The SA supplement was a FFA product, that is not commercially available, of high purity and contained approximately 6.6% C16:0, 93% C18:0, and, 0.6% C22:0 (Table 7.1). The ingredient and nutrient composition of the diets fed as TMR are described in Table 7.2. Diets contained approximately 16% forage NDF, and 21% CP on a DM basis, and mineral and vitamins were formulated according to NRC (2001) recommendations. The SA supplement replaced soyhulls in the diet. DM concentration was determined twice weekly for forages and diets were adjusted when necessary. All cows were housed in the same tie-stall throughout the entire experiment and milked twice daily (0430 and 1530 h). Access to feed was blocked from 1000 to 1200 h to allow for collection of orts and offering feed. Cows were fed 115% of

expected intake at 1200 h daily. Water was available ad libitum in each stall and stalls were bedded with sawdust and cleaned twice daily.

Samples and data for production results were collected during the last 4 d of each treatment period (d 18 to 21). Samples of all diet ingredients (0.5 kg) and orts from each cow (12.5%) were collected daily and composited by period for analysis. Milk yield was recorded and two milk samples were collected at each milking. One aliquot was collected in a sealed tube with preservative (bronopol tablet; D&F Control Systems, San Ramon, CA) and stored at 4^{o}C for milk component analysis. The second aliquot was stored without preservative at -20^{o}C until analyzed for FA composition.

For digestibility estimates, fecal samples (~400 g) were collected every 15 h during the last 5 d of each period resulting in 8 samples per cow per period, representing every 3 h of a 24-h period to account for diurnal variation. Feces were stored at -20^{o}C until dried and composited on equal DM basis for each cow period. BW was measured on the last 2 d of each period after the morning milking and four trained investigators determined BCS on a 5-point scale in 0.25 increments on the last day of each period (Wildman et al., 1982).

Diet ingredients, orts, and fecal samples were dried at 55°C in a forced-air oven for 72 h for DM determination. Dried samples were ground with a Wiley mill (1 mm screen; Arthur H. Thomas, Philadelphia, PA). Diet ingredients, orts, and fecal samples were analyzed for NDF with heat stable α-amylase and sodium sulfite (Van Soest et al., 1991), CP (AOAC 2000, method 990.03), and starch (Hall, 2009) by Cumberland Valley Analytical Services, Inc. (Hagerstown, MD.) The FA concentration of diet ingredients, orts, and fecal samples were determined using a as described by Lock et al. (2013). Indigestible NDF was used as an internal marker to estimate fecal mass to determine apparent total-tract digestibility of nutrients (Cochran et al., 1986).

Indigestible NDF was estimated as NDF after 240-h in vitro fermentation (Goering and Van Soest, 1970) using rumen fluid from a non-lactating mature cow fed dry hay; flasks were reinoculated at 120 h to ensure a viable microbial population.

Individual milk samples were analyzed for fat, true protein, and lactose concentration by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan Herd Improvement Association (Universal Lab Services, Lansing MI). Yields of 3.5% FCM, ECM, and milk components were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each collection period. Milk samples used for analysis of FA composition were composited based on milk fat yield (d 18-21 of each period). Milk lipids were extracted and FA methyl esters prepared according to our methods described previously (Lock et al., 2013). Yield of individual FA (g/d) in milk fat were calculated by using milk fat yield and FA concentration to determine yield on a mass basis using the molecular weight of each FA while correcting for glycerol content and other milk lipid classes (Piantoni et al., 2013). The FA yield response (FAYR) to additional FA intake was calculated for total FA and for C18:0 plus *cis*-9 C18:1 using the following equation:

FAYR (%) = [(FA yield for SA supplemented diet – FA yield for CON) / (FA intake for SA supplemented diet – FA intake for CON)] ×100

Energy balance was determined as energy intake minus maintenance and milk energy output using the following equations from NRC (2001):

Maintenance energy (Mcal/d) = $0.08 \times \text{kg BW}^{0.75}$

Milk energy (Mcal/d) = 0.0929 × Fat % + 0.0563 × True Protein % + 0.0395 × Lactose %

TDN = DMD + (FA × DMD × 1.25) With TDN adjusted to account for the additional energy value of fat.

DE (Mcal/kg) = 0.04409 × TDN (%)

ME (Mcal/kg) = 1.01 × DE (Mcal/kg) – 0.45

NE (Mcal/kg) = (0.703 × ME) – 0.19

Energy balance = (NE (Mcal/kg) × DMI) – (milk energy + maintenance energy)

All data were analyzed using the fit model procedure of JMP (version 10; SAS Institute, Cary, NC). Data were analyzed using the following model:

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

where Y_{ijk} = the dependent variable, μ = the overall mean, C_i = random effect of cow (i = 1 to 32), P_j = fixed effect of period (j = 1 - 4), T_k = fixed effect of treatment (k = 1 - 4), pMY = covariate of preliminary milk yield, $pMY \times T_k$ = interaction between preliminary milk yield and treatment, and e_{ijk} = residual error. The interaction between period and treatment was initially included in the model and removed because it was not significant ($P > 0.20$). Likewise, the quadratic effect of preliminary milk yield and the interaction between treatment and the quadratic effect of preliminary milk yield were initially included in the model and removed because they were not significant ($P > 0.20$). The preliminary milk yield by treatment interaction and preliminary milk covariate were removed from the model when $P > 0.20$ for the interaction term. Two preplanned contrasts were used to determine the linear and quadratic effects of increasing doses of C18:0. Treatment terms were declared significant at $P \leq 0.05$ and trends were declared at $P \leq 0.10$. All data are expressed as least square means and standard error of the means, unless otherwise specified.

RESULTS

As expected substituting SA for soyhulls increased dietary FA and reduced NDF with no differences in forage NDF, CP, or starch (Table 7.2). Dietary C18:0 content was 0.07, 0.78, 1.49, and 2.19% diet DM for CON, L-SA, M-SA, and H-SA treatments, respectively. A more modest increase was observed in C16:0 content of 0.34, 0.39, 0.44, and 0.48% diet DM for CON, L-SA, M-SA, and H-SA treatments, respectively. We observed linear increases in C16:0 intake (64 g/d) and C18:0 intake (658 g/d) when comparing CON to H-SA (both $P < 0.001$; Table 7.3). Increasing SA inclusion linearly increased DMI ($P = 0.02$) with no effect on milk yield ($P = 0.51$; Table 7.4). The L-SA and M-SA treatments tended to reduce milk fat concentration compared with CON and H-SA treatments (quadratic $P = 0.07$). However, increasing SA had no effect on milk fat yield or milk protein concentration or yield (all $P > 0.15$). The increase in DMI accompanied with no treatment effects on the yield of milk and milk components resulted in a linear reduction in ECM/DMI as inclusion of SA increased ($P < 0.01$). In our 21-d treatment periods, we detected no differences between treatments for BW or BCS (Table 7.4).

Increasing SA resulted in a numerically small but significant increase in starch and CP digestibility (both linear $P < 0.05$; Table 7.5). Increasing SA reduced total FA digestibility (quadratic, $P < 0.001$). This was most evident for 18-carbon FA digestibility which was reduced by ~30% units (quadratic, $P < 0.001$) when comparing CON vs. H-SA treatments, with 16-carbon FA digestibility reduced by ~13% units (quadratic, $P = 0.001$). We observed a reduction in total FA digestibility when total FA intake ranged from 425 to 1800 g/d ($R^2 = 0.50$; $P < 0.001$; Figure 7.1). However, total FA absorbed was positively linearly related to intake of total FA within this range ($R^2 = 0.64$; $P < 0.001$; Figure 2). Intake of 16-carbon and 18-carbon FA linearly increased with increasing SA (both $P < 0.001$; Table 7.6). The increase in 16-carbon FA

intake was ~ 55 g/d and the increase in 16-carbon FA absorbed was ~ 25 g/d when comparing CON vs. H-SA treatments. Whereas, the increase in 18-carbon FA intake was ~749 g/d and the increase in 18-carbon FA absorbed was ~227 g/d when comparing CON vs. H-SA treatments.

Milk FA are derived from 2 sources: < 16 carbon FA from de novo synthesis in the mammary gland and > 16 carbon FA originating from extraction from plasma. Mixed source FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis in the mammary gland and extraction from plasma. There were minimal effects of SA treatments on milk FA concentrations (Table 7.7). The concentration of de novo FA were elevated for L-SA and M-SA compared to CON and H-SA (quadratic, $P = 0.01$). We observed no differences in the concentration of FA from mixed sources ($P = 0.35$). Increasing SA increased the concentration of preformed FA (linear, $P = 0.01$) because of increased concentrations of C18:0 and *cis*-9 C18:1 (linear, $P < 0.001$). On a yield basis, increasing SA had no effect on the sources of FA in milk fat (all $P > 0.10$; Table 7.8). Although the yield of preformed FA was not affected by treatment, supplementation of SA increased the yield of C18:0 plus *cis*-9 C18:1 (linear, $P = 0.005$) resulting in a 16 g/d increase for H-SA vs. CON. However, increasing the amount of 18-carbon FA absorbed had a small impact on increasing 18-carbon FA in milk fat ($R^2 = 0.06$; P = 0.01; Figure 7.3).

The FAYR for C18:0 and *cis*-9 C18:1 to increasing SA was < 3% for all SA supplementation levels. Total FAYR for H-SA measured by total milk FA yield and total dietary FA intake compared to CON was not statistically different from 0%. Increasing total dietary FA from 2.4 to 4.1% had no effect on total FA in milk fat.

Using equations from NRC (2001) we estimated the energy content allocated towards maintenance and milk for incremental increases in SA (Table 7.9). Although increasing SA resulted in an increase in NE_L intake (linear, $P < 0.001$), we observed no difference in

maintenance or milk energy output (both $P > 0.27$). Calculating energy balance from NE_L intake minus maintenance and milk energy output resulted in an increase in energy balance from 12.6 to 18.4 Mcal/d for CON vs. H-SA (linear, $P < 0.001$).

DISCUSSION

Rather than simply supplying energy, research continues to highlight that individual FA have unique properties ranging from regulating gene transcription (Nakamura et al., 2014) to altering milk fat synthesis (Palmquist, 2006). C18:0 is the most prevalent FA available for absorption in the small intestine due to extensive biohydrogenation of dietary unsaturated 18-carbon FA in the rumen (Jenkins, 1993). Due to C18:0 being rumen inert, the potential benefit of supplementation of products high in C18:0 needs to be evaluated. Additionally, 18-carbon FA comprise approximately 1/3 of the FA in milk fat with the ratio highly dependent on nutrition. Therefore determining the maximum amount of FA that can be incorporated into milk fat from 18-carbon dietary FA will allow for precise feeding of 18-carbon FA to improve efficiency of 18-carbon FA transfer into milk fat.

Available literature is inconsistent on the impact of increasing dietary C18:0 on DMI. In our study, SA increased DMI similar to what was observed by the overall treatment effect of C18:0 supplementation reported by Piantoni et al. (2014). The increases in DMI observed by Piantoni et al. (2014) were driven by high producing cows, while we did not observe any interaction between DMI and level of preliminary milk yield. However, unlike the results of the Piantoni et al. (2014) study, we observed no effect on milk yield or milk fat yield in high producing cows. Steele and Moore (1968) reported an increase in milk fat yield with no effect on milk yield when comparing a C18:0 supplemented vs. a control diet. While a subsequent study

found no effect on milk fat yield but an increase in milk yield when C18:0 was supplemented (Steele, 1969). In a study comparing C16:0 and C18:0 supplementation, C18:0 had reduced milk fat yield compared to C16:0 (Rico et al., 2013).

A potential reason for the variation in response is the variability in digestibility of C18:0. Digestibility of C18:0 may be negatively impacted by increased C18:0 available for absorption in the small intestine (Weisbjerg et al., 1992). In a meta-analysis evaluating the digestibility of individual FA using duodenually cannulated cows, we determined that as C18:0 reaching the duodenum increased the digestibility of C18:0 linearly decreased across diets containing a wide range of fat supplements and in diets containing no fat supplementation encompassing a wide range of C18:0 duodenal flow rates (Boerman et al., submitted). Because we did not use duodenally cannulated cows in this study, we are unable to estimate C18:0 digestibility per se due to rumen biohydrogenation. Rather, utilizing feed to feces estimates measurements we combined all 18-carbon FA into a single digestibility estimate. We observed a reduction of approximately 30% units in 18-carbon FA digestibility as C18:0 supplementation increased when comparing H-SA to CON. Similarly, using a 98% C18:0 supplement at approximately 2% diet DM reduced 18-carbon FA digestibility by approximately 24 percentage units (Piantoni et al., 2014). Our fat supplement had mean particle size of approximately 660 μm, which is smaller than the supplement used by Piantoni et al. (2014) with a mean particle size of 980 μm. However, we cannot eliminate the possibility of the physical characteristics of the fat supplement impacting digestibility and therefore production results. While the exact mechanisms for the reduction in C18:0 / 18-carbon FA digestibility are unknown, potential causes have been suggested and they include limits in lysolecithin and/or competition for absorption sites of C18:0 (Drackley, 2000).

Lysolecithin acts as an amphiphile for C18:0 aiding with FA solubilization that is required for absorption (Freeman, 1969). If absorption sites are limited or there is increased competition of binding sites, FA digestibility may be reduced. Previous studies have fed SFA as either saturated triglycerides (Pantoja et al., 1995) and relatively pure sources of C16:0 (Piantoni et al., 2013) and C18:0 (Piantoni et al., 2014) and observed reductions in FA digestibility. Because the reductions in digestibility are for both 16 and 18-carbon FA, it may be a combination of effects that reduce FA digestibility. However, our recent meta-analysis indicated that up to 500 g/d duodenal flow of C16:0 there was no reduction in C16:0 digestibility. Whereas, increasing the duodenal flow of C18:0 up to 500 g/d for non-fat supplemented diets reduced C18:0 digestibility (Boerman et al., submitted). Our current study would have resulted in less than 500 g/d duodenal flow of C16:0 however, we observed reductions in 16-carbon FA digestibility, potentially indicating the profile of FA reaching the duodenum impacts individual FA digestibility.

Using infusions of C16:0 and C18:0, Enjalbert et al. (1998) reported that C16:0 had increased transfer efficiency into milk fat compared with C18:0. In recent studies feeding relatively pure sources of C16:0, transfer efficiency ranged from 16.5 to 24 % (Piantoni et al., 2013, Preseault et al., 2014). We calculated FAYR to determined the efficiency of utilization of the SA supplement (increased dietary C18:0) for milk fat yield. Both C18:0 and *cis*-9 C18:1 are included in the transfer efficiency calculations to account for changes that occur in the mammary gland via Δ^9 desaturase. Increasing dietary C18:0 and *cis*-9 C18:0 by 100 g/d for the H-SA treatment resulted in a 2.1 g/d increase in C18:0 plus *cis*-9 C18:0 in milk fat, or a FAYR of 2.1%. The H-SA treatment had the highest FAYR, with L-SA and M-SA both < 1% FAYR. Piantoni et al., (2014) reported 8.2% FAYR in transfer of C18:0 plus *cis*-9 C18:0 from the diet

into milk fat. Similarly, when evaluating C16:0 transfer efficiency, both C16:0 and *cis*-9 C16:0 are included. Compared to our results for FAYR for supplemental C18:0 (< 3% FAYR for all treatments), the transfer efficiency from dietary to milk FA was greater for diets supplemented with a C16:0 enriched FA (16.5 - 20% FAYR; Lock et al., 2013; Piantoni et al., 2013; Preseault et al., 2014).

Piantoni et al. (2014) reported overall improvements in milk fat yield with an increase in preformed FA and 18:0 in milk fat when C18:0 was supplemented at 2% DM. While they observed an increase of 44 g/d in preformed FA in milk fat, we observed no effect on preformed milk FA when supplementing C18:0. The increase in preformed milk FA that Piantoni et al. (2014) reported came from a combination of C18:0 (20 g/d) and *cis*-9 C18:1 (21 g/d) while we observed a 16 g/d increase in C18:0 and *cis*-9 C18:1. Feeding a mostly saturated FA from prilled hydrogenated free FA from tallow (mixture of C16:0 and C18:0) resulted in an increase in milk fat yield of 177 g/d compared to a control diet (Relling and Reynolds, 2007). Comparing our results of no increase in milk FA to studies that supplemented C16:0, the increase in milk FA, calculated from g/d of sources of milk FA reported, ranged from 73 to 88 g/d increase in total milk FA when C16:0-enriched FA supplement was fed (Lock et al., 2013; Piantoni et al., 2013). Rico et al. (2013) compared a C16:0 to a C18:0-enriched FA supplement and observed a 90 g/d increase in total milk FA with the C16:0-enriched supplement due to a 117 g/d increase in 16-carbon FA with small reductions in both de novo and preformed milk FA.

A potential reason for the discrepancy between C16:0 and C18:0 transfer into milk fat besides differences in digestibility are differences in the incorporation of these individual FA into triglycerides in the mammary gland. Fairly uniform distribution is observed for C16:0 on the *sn*-1 and *sn*-2 positions of the triglyceride with more modest concentrations found on *sn*-3.

Comparatively, C18:0 is preferentially located on the *sn*-1 position and *cis*-9 C18:1 while found at all positions, is preferentially located on either the *sn*-1 or *sn*-3 position of the triglyceride (Jensen, 2002). Palmitoyl-CoA when used as the initial acylation to glycerol 3-phosphate resulted in maximized triglyceride synthesis (Kinsella and Gross, 1973). This is thought to be due to palmitoyl-CoA accelerating the incorporation of de novo synthesized FA to the other positions of the triglyceride. As C18:0 uptake by the mammary gland increases, desaturation of C18:0 to *cis*-9 C18:1 increases in order to maintain fluidity (Enjalbert et al., 1998). Oleic acid reduces total de novo synthesis potentially reducing total milk FA yield by competing with butyryl-CoA for attachment at the *sn*-3 position of the triglyceride (Hanson and Knudsen, 1987). However, in our study we observed no effect of supplemental SA on de novo synthesized milk FA probably due to the modest increases in C18:0 and *cis*-9 C18:1 in milk fat.

Increasing SA supplementation increased both the energy density of diets and DMI. As a result we observed a predicted increase in energy balance with increasing SA supplementation of 5.8 MCal/d for H-SA vs. CON. Based upon the BW and BCS of the cows used in our study approximately 500 Mcal would be required to increase one BCS (NRC, 2001). Therefore, in our 21-d periods we would have expected an approximately 0.25 pt. increase in BCS when comparing CON to H-SA. Because we observed no difference in either BW or BCS, we are uncertain if our short periods and experimental design impacted our ability to detect differences.

Due to a formulation error, unfortunately our diets did not contain the desired content of NDF, CP, and starch. Even with these atypical diets, the production, digestibility, and milk FA are within a normal range and the additional SA was effectively evaluated. However, from this study we are unable to determine the effects of additional C18:0 on more convention diets that are typical in the Midwest.

CONCLUSIONS

Increasing C18:0 supplementation increased DMI but had no effect on the yields of milk or milk components, and therefore reduced ECM/DMI. Increasing C18:0 supplementation reduced total, 18-carbon, and 16-carbon FA digestibilities. Regardless of the modest increase in C18:0 and *cis*-9 C18:1 FA in milk fat, the increase was not enough to positively impact milk fat yield.

APPENDIX

APPENDIX

Table 7.1. Composition of the C18:0-enriched fat supplement fed during treatment periods.

FA Profile, g/100 g of FA	Mean[1]	Standard Deviation
16:0	6.59	0.04
17:0	0.14	0.00
18:0	92.6	0.04
18:1 *cis*-9	0.08	0.00
22:0	0.59	0.01
Particle Size, μm	659	1.5

[1] Average and standard deviation from composited samples taken during each period (n=4).

Table 7.2. Diet ingredients and nutrient composition of experimental diets[1].

	Treatments[2]			
Ingredient, % DM	CON	L-SA	M-SA	H-SA
Corn silage	17.4	17.3	17.3	17.3
Alfalfa silage	12.4	12.4	12.4	12.4
Corn grain, dry ground	34.3	34.3	34.3	34.3
Soybean meal	26.1	26.1	26.1	26.1
Soybean hulls	2.3	1.5	0.7	---
C18:0-enriched fat supplement	---	0.8	1.5	2.3
Wheat straw	2.6	2.6	2.6	2.6
MSU vitamin & mineral mix[3]	3.4	3.4	3.4	3.4
Limestone	0.8	0.8	0.8	0.8
Sodium bicarbonate	0.8	0.8	0.8	0.8
Nutrient composition, % DM				
DM[4]	57.4	57.4	57.5	57.5
NDF	23.3	22.8	22.3	21.8
Forage NDF	15.9	15.9	15.9	15.9
CP	21.0	20.9	20.8	20.7
Starch	31.6	31.6	31.6	31.6
FA	2.44	3.20	3.95	4.70
16:0	0.34	0.39	0.44	0.48
18:0	0.07	0.78	1.49	2.19
18:1	0.49	0.48	0.48	0.48
18:2	1.25	1.25	1.24	1.24
18:3	0.16	0.16	0.16	0.16

[1] Experimental diets fed to 32 cows in a 4 x 4 Latin square design with 21-d periods.
[2] Treatments contained 0.0% (CON), 0.8% (L-SA), 1.5% (M-SA), or 2.3% (H-SA) C18:0-enriched fat supplement.
[3] Vitamin and mineral mix contained 34.1% dry ground shell corn, 25.6% white salt, 21.8% calcium carbonate, 9.1% Biofos, 3.9% magnesium oxide, 2% soybean oil, and < 1% of each of the following: manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, iodine, cobalt carbonate, vitamin E, vitamin A, vitamin D, and selenium.
[4]Expressed as percent of as fed.

Table 7.3. Intake of FA (g/d) for cows fed experimental diets (n=30).

	Treatments[1]						Contrasts[3]	
FA Intake, g/d	CON	L-SA	M-SA	H-SA	SEM	TRT[2]	Linear	Quadratic
C16:0	88.0	114	130	152	2.80	< 0.001	< 0.001	0.41
C18:0	15.5	221	428	674	9.28	< 0.001	< 0.001	0.02
C18:1 cis-9	127	143	146	153	3.37	< 0.001	< 0.001	0.08
C18:2 cis-9, cis-12	350	377	384	397	8.31	< 0.001	< 0.001	0.27
C18:3 cis-9, cis-12, cis-15	45.3	48.4	49.2	50.1	1.01	< 0.001	< 0.001	0.18
Total FA	662	945	1181	1472	24.0	< 0.001	< 0.001	0.84

[1]Treatments contained 0.0% (CON), 0.8% (L-SA), 1.5% (M-SA), or 2.3% (H-SA) C18:0-enriched fat supplement (SA).
[2] TRT is the *P*-value associated with treatment effect.
[3] Contrasts correspond to the linear effect of increasing SA and the quadratic effect of increasing SA.

Table 7.4. Production responses for cows fed experimental diets fed increasing amounts of stearic acid (n=30).

	Treatments[1]						Contrasts	
Variable	CON	L-SA	M-SA	H-SA	SEM	TRT	Linear	Quadratic
DMI, kg/d	28.5	29.1	29.6	30.0	0.61	0.13	0.02	0.80
Milk Yield, kg/d								
Milk	38.3	38.6	38.2	37.8	1.65	0.51	0.26	0.36
3.5% FCM, kg/d[4]	39.8	39.4	39.3	39.3	1.4	0.77	0.34	0.64
ECM, kg/d[5]	40.6	40.4	40.1	40.0	1.4	0.74	0.27	0.86
Milk Components								
Fat Yield, kg/d	1.43	1.40	1.40	1.42	0.04	0.61	0.54	0.24
Fat, %	3.79	3.72	3.74	3.82	0.08	0.29	0.59	0.07
Protein Yield, kg/d	1.33	1.33	1.32	1.30	0.05	0.49	0.19	0.47
Protein, %	3.49	3.50	3.48	3.49	0.05	0.91	0.83	0.84
BW, kg	738	739	735	737	12	0.58	0.36	0.84
BCS	3.44	3.40	3.39	3.42	0.08	0.37	0.45	0.11
ECM/DMI	1.43	1.39	1.35	1.33	0.04	0.03	0.003	0.80

[1] Treatments contained 0.0% (CON), 0.8% (L-SA), 1.5% (M-SA), or 2.3% (H-SA) C18:0-enriched fat supplement.
[2] TRT is the *P*-value associated with treatment effect.
[3] Contrasts correspond to the linear effect of increasing SA and the quadratic effect of increasing SA.
[4] Fat-corrected milk; 3.5% FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].
[5] Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)] (Tyrell and Reid, 1965).

Table 7.5. Apparent total tract digestibility for cows fed experimental diets containing increasing amounts of stearic acid (n=30).

	Treatments[1]						Contrasts[3]	
Variable	CON	L-SA	M-SA	H-SA	SEM	TRT[2]	Linear	Quadratic
DM	72.0	72.2	71.9	72.8	0.41	0.10	0.08	0.19
NDF	41.1	42.0	41.6	41.9	0.77	0.58	0.36	0.57
CP	74.6	74.6	75.2	76.5	0.45	< 0.001	< 0.001	0.06
Starch	96.7	96.7	96.7	97.2	0.23	0.04	0.01	0.15
Total FA[a]	77.9	63.7	55.1	52.2	1.11	< 0.001	< 0.001	< 0.001
16-carbon	78.4	71.2	66.3	63.7	0.73	< 0.001	< 0.001	0.001
18-carbon[a]	82.6	63.8	54.3	49.3	1.11	< 0.001	< 0.001	< 0.001

[1] Treatments contained 0.0% (CON), 0.8% (L-SA), 1.5% (M-SA), or 2.3% (H-SA) C18:0-enriched fat supplement.
[2] TRT is the *P*-value associated with treatment effect.
[3] Contrasts correspond to the linear effect of increasing SA and the quadratic effect of increasing SA.
[a] Significant preliminary milk by treatment interaction.

Table 7.6. Intake of FA (g/d) and absorbed FA (FA) for cows fed experimental diets (n=30).

	Treatments[1]						Contrasts[3]	
Variable	CON	L-SA	M-SA	H-SA	SEM	TRT[2]	Linear	Quadratic
Total FA intake, g/d	662	945	1181	1472	24.0	< 0.001	< 0.001	0.84
16-carbon intake, g/d	97.2	117	133	152	2.68	< 0.001	< 0.001	0.96
18-carbon intake, g/d	533	795	1013	1282	20.8	< 0.001	< 0.001	0.81
Total FA absorbed, g/d	517	603	650	771	19.5	< 0.001	< 0.001	0.34
16-carbon FA absorbed, g/d	73.8	83.5	88.4	98.6	2.07	< 0.001	< 0.001	0.51
18-carbon FA absorbed, g/d	435	513	552	662	17.1	< 0.001	< 0.001	0.34

[1] Treatments contained 0.0% (CON), 0.8% (L-SA), 1.5% (M-SA), or 2.3% (H-SA) C18:0-enriched fat supplement.
[2] TRT is the *P*-value associated with treatment effect.
[3] Contrasts correspond to the linear effect of increasing SA and the quadratic effect of increasing SA.

Table 7.7. Milk FA concentration for cows fed experimental diets with increasing amounts of stearic acid (n=30).

	Treatments[1]						Contrasts[3]	
	CON	L-SA	M-SA	H-SA	SEM	TRT[2]	Linear	Quadratic
Summations, g/100g of FA[4]								
De novo	29.7	30.0	30.0	29.4	0.31	0.03	0.13	0.01
Mixed	36.0	35.7	35.5	35.6	0.45	0.35	0.15	0.29
Preformed	34.3	34.3	34.5	35.0	0.41	0.02	0.01	0.16
C18:0 + C18:1 9c	22.1	22.2	22.8	23.6	0.37	< 0.001	< 0.001	0.05
Individual FA, g/100 g of FA[5]								
C 4:0	2.74	2.73	2.79	2.82	0.05	0.01	0.001	0.60
C 6:0	2.11	2.12	2.17	2.16	0.03	0.04	0.02	0.45
C 8:0	1.41	1.42	1.45	1.42	0.02	0.16	0.34	0.10
C 10:0	4.12	4.16	4.19	4.05	0.08	0.07	0.29	0.02
C 12:0	5.24	5.32	5.27	5.06	0.12	0.002	0.01	0.00
C 14:0	13.1	13.2	13.1	12.9	0.15	0.01	0.01	0.01
C 16:0	34.2	34.0	33.9	34.0	0.44	0.45	0.30	0.22
C 16:1 9c	1.71	1.73	1.64	1.62	0.05	0.01	0.003	0.58
C 18:0	6.53	6.50	6.99	7.35	0.18	< 0.001	< 0.001	0.07
C 18:1 9t	0.14	0.14	0.14	0.13	0.003	0.02	0.01	0.43
C 18:1 10t	0.35	0.38	0.33	0.30	0.02	0.003	0.01	0.02
C 18:1 11t	0.46	0.45	0.44	0.43	0.02	0.04	0.004	0.95
C 18:1 12t	0.23	0.23	0.22	0.21	0.01	< 0.001	< 0.001	0.45
C 18:1 9c	15.6	15.7	15.8	16.2	0.26	< 0.001	< 0.001	0.14
C 18:1 11c	0.54	0.53	0.50	0.51	0.02	0.01	0.005	0.39
C 18:2 9c, 12c	2.96	2.98	2.89	2.77	0.05	< 0.001	< 0.001	0.01
C 18:3 9c, 12c, 15c	0.38	0.39	0.37	0.36	0.01	< 0.001	< 0.001	0.05
CLA 9c, 11t	0.30	0.29	0.27	0.25	0.01	< 0.001	< 0.001	0.26

[1] Treatments contained 0.0% (CON), 0.8% (L-SA), 1.5% (M-SA), or 2.3% (H-SA) C18:0-enriched fat supplement (SA).
[2] TRT is the *P*-value associated with treatment effect.
[3] Contrasts correspond to the linear effect of increasing SA and the quadratic effect of increasing SA.
[4] Summations; de novo = milk FA < 16 carbons in length; mixed = milk FA 16-carbons in length; preformed = milk FA > 16 carbons in length; C18:0 + C18:1 c9 = the sum of C18:0 and *cis*-9 C18:1.
[5] A total of approximately 70 individual FA were quantified and used for calculations (summation by concentrations). Only select FA are reported in the table.

Table 7.8. Milk FA yield for cows fed experimental diets with increasing amounts of stearic acid (n=30).

	Treatments[1]						Contrasts[3]	
	CON	L-SA	M-SA	H-SA	SEM	TRT[2]	Linear	Quadratic
Summations, g/d[4]								
De novo	398	394	394	390	13.4	0.72	0.29	0.99
Both	484	471	469	476	17.0	0.33	0.32	0.12
Preformed	453	448	452	458	12.8	0.69	0.45	0.35
C18:0 + C18:1 9c	291	289	297	307	8.26	0.02	0.005	0.19
Individual FA, g/d[5]								
C 4:0	36.7	36.1	36.9	37.5	1.37	0.42	0.23	0.96
C 6:0	28.4	27.9	28.7	28.6	1.02	0.65	0.45	0.66
C 8:0	18.9	18.7	19.1	18.8	0.66	0.81	0.88	0.94
C 10:0	55.1	54.5	55.2	53.6	2.06	0.57	0.32	0.59
C 12:0	70.1	69.6	69.2	66.9	2.65	0.15	0.04	0.38
C 14:0	175	173	172	171	6.05	0.60	0.19	0.75
C 16:0	461	448	448	454	16.4	0.34	0.40	0.11
C 16:1 9c	23.0	22.7	21.6	21.6	0.97	0.07	0.01	0.80
C 18:0	85.8	84.7	91.5	95.6	3.25	0.00	< 0.001	0.16
C 18:1 9t	1.84	1.84	1.77	1.75	0.06	0.13	0.02	0.83
C 18:1 10t	4.62	4.99	4.37	4.01	0.32	0.00	< 0.001	0.03
C 18:1 11t	6.17	5.92	5.82	5.62	0.32	0.06	0.01	0.87
C 18:1 12t	3.09	3.01	2.87	2.75	0.13	0.00	< 0.001	0.79
C 18:1 9c	205	204	206	212	5.65	0.30	0.11	0.29
C 18:1 11c	7.12	7.02	6.61	6.76	0.32	0.07	0.03	0.39
C 18:2 9c, 12c	39.2	39.2	37.9	36.6	1.41	0.00	< 0.001	0.21
C 18:3 9c, 12c, 15c	5.03	5.08	4.88	4.79	0.18	0.02	0.006	0.35
CLA 9c, 11t	3.91	3.78	3.55	3.30	0.17	< 0.0001	< 0.001	0.56

[1] Treatments contained 0.0% (CON), 0.8% (L-SA), 1.5% (M-SA), or 2.3% (H-SA) C18:0-enriched fat supplement.
[2] TRT is the *P*-value associated with treatment effect.
[3] Contrasts correspond to the linear effect of increasing SA and the quadratic effect of increasing SA.
[4] Summations; de novos = milk FA < 16 carbons in length; mixed = milk FA 16-carbons in length; preformed = milk FA > 16 carbons in length; C18:0 + C18:1 c9 = the sum of C18:0 and *cis*-9 C18:1.
[5] A total of approximately 70 individual FA were quantified and used for calculations (summation by concentrations). Only select FA are reported in the table.

Table 7.9. Energy balance estimates[1] for cows (n=30) fed experimental diets using milk composition and BW measurements.

	Treatments[2]						Contrasts[4]	
Variable	CON	L-SA	M-SA	H-SA	SEM	TRT[3]	Linear	Quadratic
Maintenance (Mcal/d)	11.3	11.3	11.3	11.3	0.14	0.67	0.51	0.98
Milk (Mcal/d)	27.8	27.7	27.5	27.5	0.96	0.72	0.28	0.78
Digestible Energy (Mcal/kg)	3.27	3.31	3.33	3.40	0.02	< 0.001	< 0.001	0.19
Metabolizable Energy (Mcal/kg)	2.85	2.89	2.91	2.98	0.02	< 0.001	< 0.001	0.20
Net Energy (Mcal/kg)	1.82	1.84	1.86	1.91	0.01	< 0.001	< 0.001	0.18
Net Energy Intake (Mcal/d)	51.8	53.5	55.0	57.1	1.13	< 0.001	< 0.001	0.83
Energy Balance (Mcal/d)	12.6	14.5	16.2	18.4	1.04	< 0.001	< 0.001	0.91

[1] Energy balance estimates based on equations from the NRC (2001).
[2] Treatments contained 0.0% (CON), 0.8% (L-SA), 1.5% (M-SA), or 2.3% (H-SA) C18:0-enriched fat supplement.
[3] TRT is the *P*-value associated with treatment effect.
[4] Contrasts correspond to the linear effect of increasing SA and the quadratic effect of increasing SA.

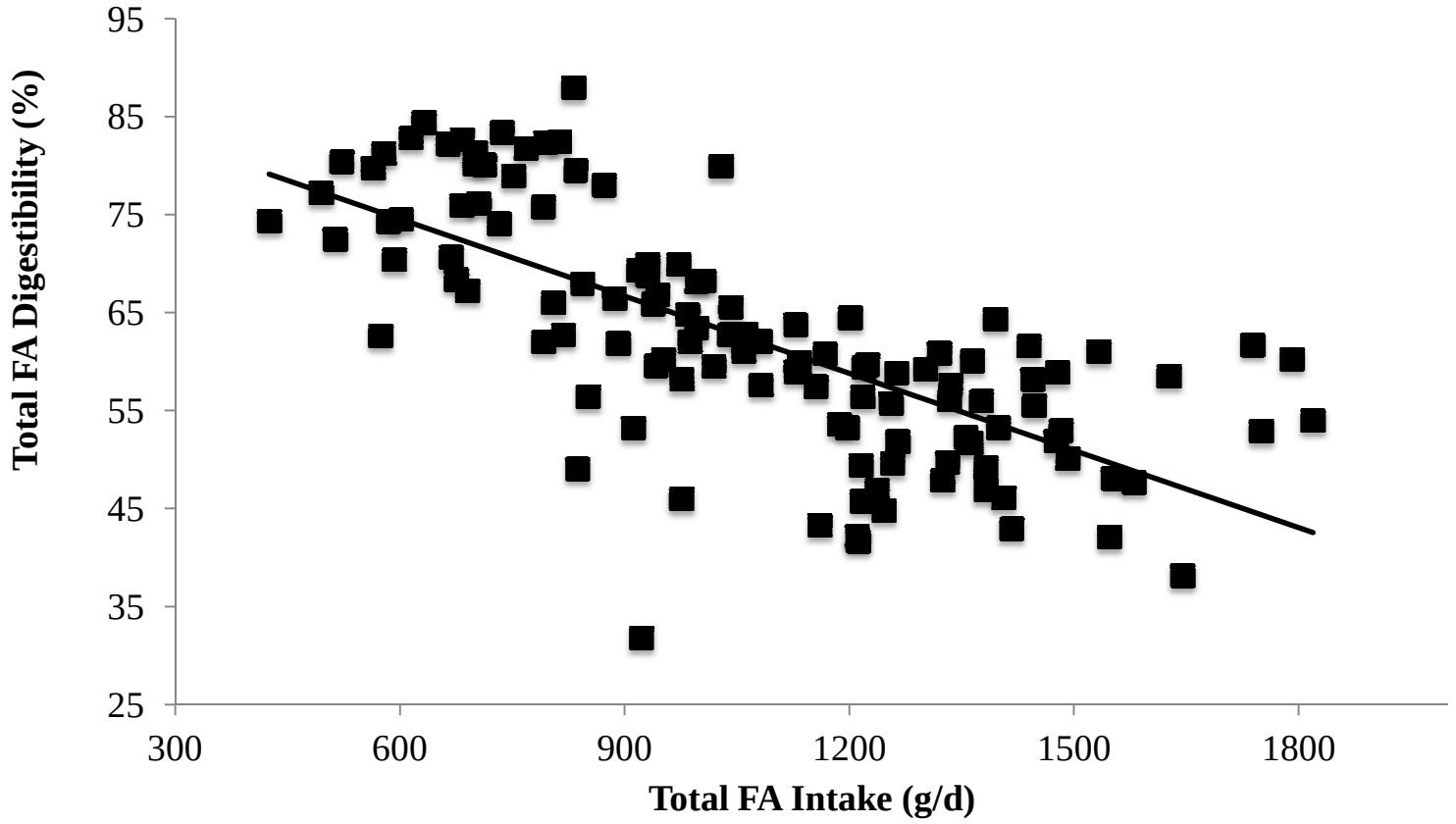

Figure 7.1. Total FA digestibility by total FA intake for cows (n=30) fed treatment diets. Total FA digestibility (%) = 90.32 - 0.026 × total FA intake (g/d); ($P < 0.001$; $R^2 = 0.50$).

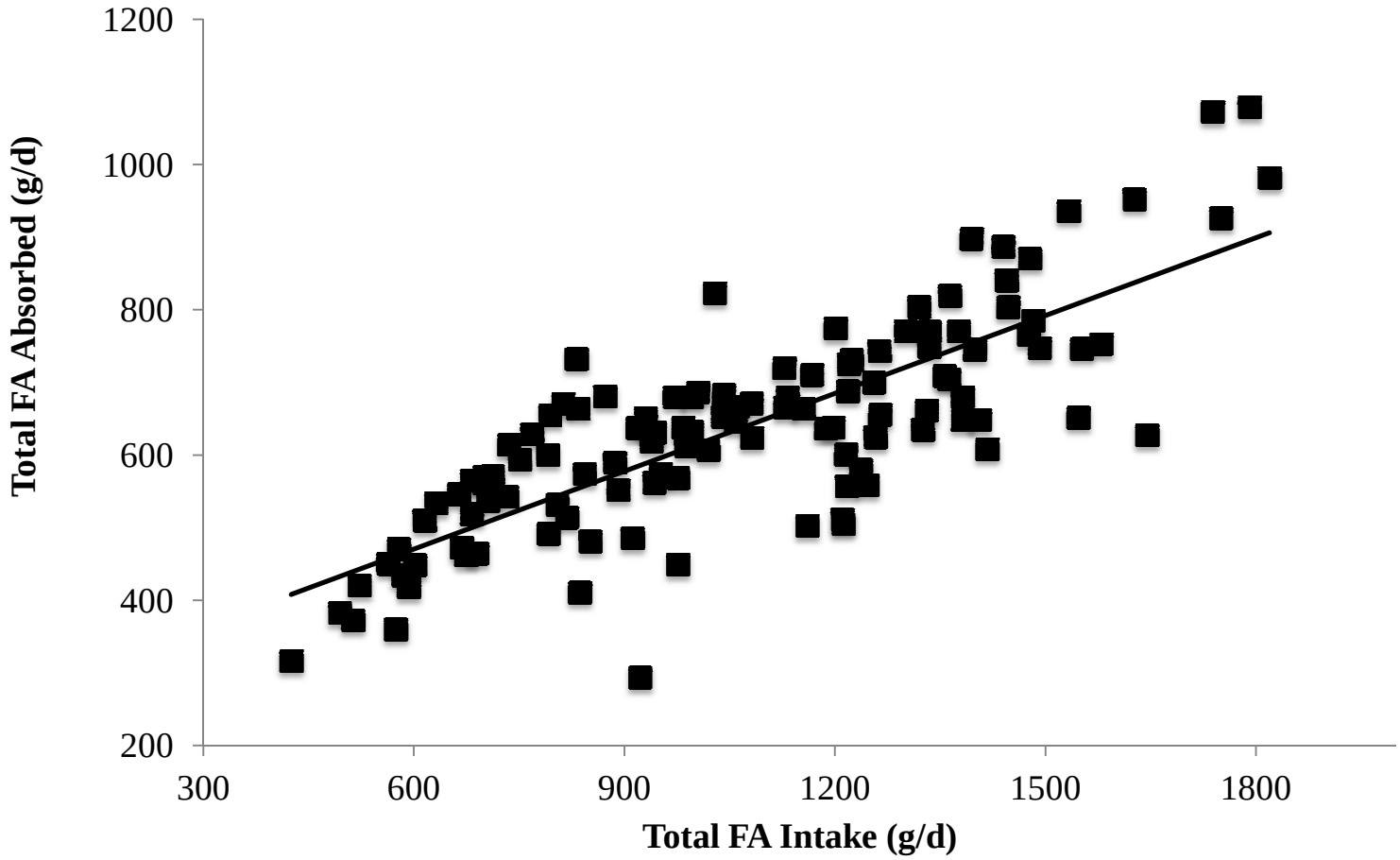

Figure 7.2. Total FA absorbed by total FA intake for cows (n=30) fed treatment diets. Total FA absorbed (g/d) = 256 + 0.36 × total FA intake (g/d); ($P < 0.0001$; $R^2 = 0.64$).

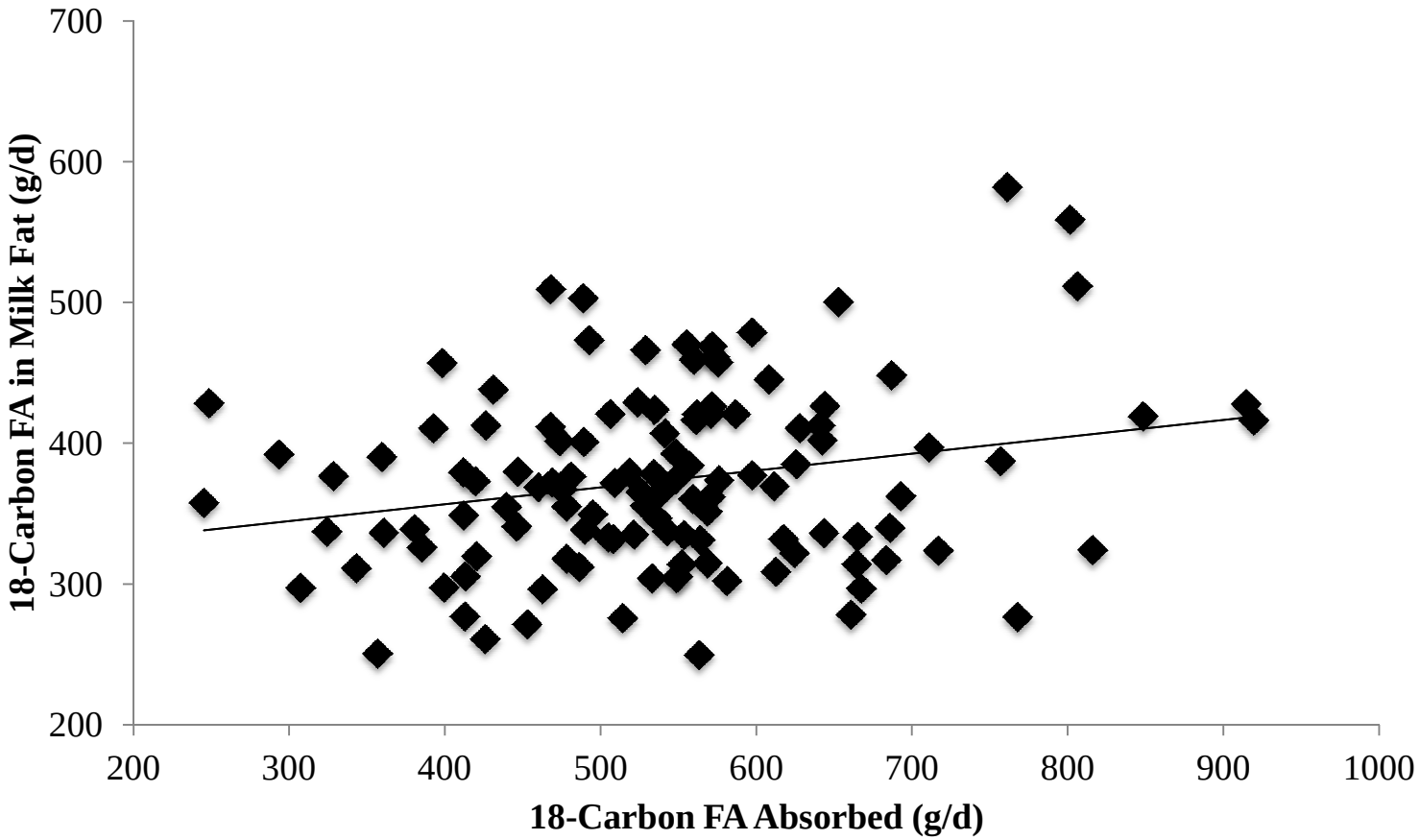

Figure 7.3. 18-carbon FA in milk fat (g/d) by 18-carbon FA absorbed for cows (n=30) fed treatment diets. 18-carbon FA in milk fat (g/d) = 309 + 0.12 × 18-carbon FA absorbed (g/d); (P = 0.009; R^2 = 0.06).

REFERENCES

REFERENCES

AOAC. 1990. Official Methods of Analysis. 15th ed. AOAC International, Arlington, VA.

AOAC. 2000. Official Methods of Analysis. 17th ed. AOAC International, Arlington, VA.

Bauman, D. E., K. J. Harvatine, and A. L. Lock. 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Ann. Rev. Nutr. 31: 299-319.

Boerman, J. P., J. L. Firkins, and A. L. Lock. Intestinal digestibility of long chain fatty acids in lactating dairy cows: a meta analysis. J. Dairy Sci. *Submitted*.

Cochran, R. C., D. C. Adams, J. D. Wallace, and M. L. Galyean. 1986. Predicting the digestibility of different diets with internal markers: Evaluation of four potential markers. J. Anim. Sci. 63:1476–1483.

Drackley, J. K. 2000. Lipid Metabolism. Pp. 97-119 *in* Farm Animal Metabolism and Nutrition. (ed. J. P. F. D'Mello). CABI Publishing, New York, NY.

Enjalbert, F., M. C. Nicot, C. Bayourthe, and R. Moncoulon. 1998. Duodenal infusions of palmitic, stearic, or oleic acids differently affect mammary gland metabolism of fatty acids in lactating dairy cows. J. Nutr. 128:1525-1532.

Freeman, C. P. 1969. Properties of fatty acids in dispersions of emulsified lipid and bile salt and the significance of these properties in fat absorption in the pig and the sheep. British J. Nutr. 23:249-263.

Glasser, F., A. Ferlay, M. Doreau, P. Schmidely, D. Sauvant, and Y. Chilliard. 2008. Long-chain fatty acid yield in relation to duodenal flows and de novo synthesis. J. Dairy Sci. 91: 2771-2785.

Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. USDA-ARS, Washington, DC.

Hall, M. B. 2009. Analysis of starch, including maltooligosacchardies, in animal feeds: A comparison of methods and a recommended method for AOAC collaborative study. J. AOAC Int. 92:42-49.

Hansen, H. O., and J. Knudsen. 1987. Effect of exogenous long-chain fatty acids on lipid biosynthesis in dispersed ruminant mammary gland epithelial cells: esterification of long-chain exogenous fatty acids. J. Dairy Sci. 70:1344-1349.

Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76: 3851-3863.

Jensen, R. G. 2002. Invited review: the composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 85:295-350.

Lock, A.L., C.L. Preseault, J.E. Rico, K.E. DeLand, and M.S. Allen. 2013. Feeding a C16:0-enriched fat supplement increased the yield of milk fat and improved conversion of feed to milk. J. Dairy Sci. 96:6650-6659.

Loor, J. J., J. H. Herbein, and T. C. Jenkins . 2002. Nutrient digestion, biohydrogenation, and fatty acid profiles in blood plasma and milk fat from lactating Holstein cows fed canola oil or canolamide. Anim. Feed Sci. Techno. 97:65-82.

Kinsella, J. E., and M. Gross. 1973. Palmitic acid and initiation of mammary glyceride synthesis via phosphatidic acid. Biochem. Biophy. Acta. 316:109-113.

Nakamura, M. T., B. E. Yudell, and J. J. Loor. 2014. Regulation of energy metabolism by long-chain fatty acids. Prog. Lipid. Res. 53:124-144.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Acad. Sci., Washington D.C.

Palmquist, D. L., 2006. Milk Fat: Origin of Fatty Acids and Influence of Nutritional Factors Thereon (Ed. P.F. Fox and P. L. H. McSweeney) *in* Advanced Dairy Chemistry, Volume 2: Lipids, 3rd edition. Springer, New York.

Pantoja, J., J. L. Firkins, M. L. Eastridge. 1995. Site of digestion and milk production by cows fed fats differing in saturation, esterification, and chain length. J. Dairy Sci. 78:2247-2258.

Piantoni, P., A. L. Lock, and M. S. Allen. 2013. Palmitic acid increased yields of milk and milk fat and nutrient digestibility across production level of lactating cows. J. Dairy Sci. 96:7143-7154.

Piantoni, P., A. L. Lock, and M. S. Allen. 2014. Milk production responses to dietary stearic acid vary by production in dairy cattle. J. Dairy Sci. *Submitted*.

Preseault, C. L., J. P. Boerman**,** and A. L. Lock. 2014. Lactational responses to palmitic acid supplementation when substituted for soyhulls or corn grain. J. Dairy Sci. *Abstract*.

Relling, A. E., and C. K. Reynolds. 2007. Feeding rumen-inert fats differing in their degree of saturation decreases intake and increases plasma concentrations of gut peptides in lactating dairy cows. J. Dairy Sci. 90:1506-1515.

Rico, D. E., Y. Ying, and K. J. Harvatine. 2014. Comparison of enriched palmitic acid and calcium salts of palm fatty acids distillate fat supplements on milk production and metabolic profiles of high-producing dairy cows. J. Dairy Sci. 97:5637-5644.

Rico, J. E., M. S. Allen, and A. L. Lock. 2013. Compared with stearic acid, palmitic acid increased the yield of milk fat and improved feed efficiency across production level of cows. J. Dairy Sci. 97:1057-1066.

Steele, W. J. 1969. The effects of dietary palmitic and stearic acids on milk yield and composition in the cow. J. Dairy Res. 36:369-373.

Steele, W., and J. H. Moore. 1968. The effects of a series of saturated fatty acids in the diet on milk fat secretion in the cow. J. Dairy Res. 35:361-370.

Tice, E.M., M.L. Eastridge, and J.L. Firkins. 1994. Raw Soybeans and Roasted Soybeans of Different Particle Sizes. 2. Fatty Acid Utilization by Lactating Cows. J. Dairy Sci. 77:166–180.

Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of the milk. J. Dairy Sci. 48: 1215-1223

Van Soest, P. J., J. B. Roberson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

Weisbjerg, M. R., C. F. Borsting, and T. Hvelplund. 1992. Fatty acid metabolism in the digestive tract of lactating cows fed tallow in increasing amounts at two feed levels. Acta. Agric. Scand., Sect. A, Animal Sci. 42:106-114.

Wildman, E. E., G. M. Jones, P. E. Wagner, and R. L. Bowman. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65: 495-501.

CHAPTER 8

CONCLUSIONS

Results reported in this dissertation have examined the digestion and metabolism of FA and use of FA supplements in lactating dairy cattle using multiple *in vivo* cow studies and previously published research trials results. Differences in FA biohydrogenation in the rumen, FA digestibility in the small intestine, and FA uptake by adipose all play a role in the amount and profile of FA available for uptake by the mammary gland as preformed FA. Our meta-analysis (Chapter 3) analyzed the digestibility of individual FA. Due to rumen biohydrogenation, unsaturated FA are mostly transformed in the rumen to saturated FA, limiting the number of studies available due to the requirement of determining duodenal flows of individual FA. Of the available studies, we found that overall there are only small differences in digestibility of individual FA regardless of whether this was determined from the duodenum to ileum or duodenum to feces. However, C18:0 digestibility was reduced as the flow of C18:0 reaching the duodenum increased. As C18:0 is the major dietary FA available for absorption in the small intestine, a common FA contained in fat supplements and, a major FA found in milk fat these findings have a direct effect on dietary recommendations and the development of new fat supplements. Future research on the cause(s) for reduced C18:0 digestibility in the small intestine is needed.

In support of the findings in our meta-analysis, feeding increasing doses of C18:0 (Chapter 7) had little effect on the yield of milk or milk components. This was at least partly due to reductions in C18:0 digestibility as the amount in the diet increased. Feeding up to 674 g of supplemental C18:0 resulted in only a 16 g increase in C18:0 and *cis*-9 C18:1 in milk fat. This low transfer efficiency from the diet to milk fat indicates that for the C18:0-enriched FA

supplement used in this study with mid-lactation dairy cattle did not effectively improve milk fat yield nor was there any detectable differences observed in BCS or BW change with supplementation. A portion of these results can be explained by the 30 percentage unit reduction in 18-carbon FA digestibility as C18:0 was supplemented in the diet resulting in only modest increases in 18-carbon FA absorbed by the cow. We are uncertain if the results would be consistent with all C18:0-enriched FA supplements or in cows at different levels and/or stages of production.

We did observe differences in milk fat yield, BCS, and BW when a C16:0-enriched FA supplement was fed in a high fiber diet compared with a high starch diet (Chapter 5). The high fiber and fat diet had an increased proportion of energy that was partitioned to milk production through increased milk fat yield with a smaller proportion of energy allocated to body tissue gain. Both BCS and BW change was reduced for the high fiber and fat diet compared with the high starch diet in a 28-d period. Supporting these results ultrasound scans of subcutaneous fat thickness showed that the high fiber and fat diets had reduced deposition of subcutaneous fat throughout the treatment period compared with the high starch diet. Diets were similar in energy content however, the composition of the diets led to changes in both plasma insulin concentrations and *trans*-10 C18:1 in milk fat. Insulin concentrations were increased for the high starch diet with no effect of level of preliminary milk yield on insulin concentration. However, for the high fiber and fat diet insulin concentrations were reduced as preliminary milk yield increased probably due to differences in glucose precursors provided by the treatments. The regulatory role of insulin on lipogenesis and lipolysis is well established and supports our findings that cows on the high starch diet with increased plasma insulin concentrations had increased BW gain and BCS. Additionally, the role of MFD- inducing intermediates, specifically *trans*-10, *cis*-12 C18:2,

on the gene expression of lipogenic enzymes in adipose tissue is another potential cause for the differences in BW and BCS that we observed. While the gene expression of several key lipogenic enzymes is down regulated in the mammary gland during MFD, the opposite has been reported for genes associated with lipogenesis in adipose tissue during MFD. This would result in reduced milk fat yield and increased BCS. However, from our results and from previous research, we are unable to separate out the effects of insulin and MFD-inducing intermediates on energy partitioning during MFD. Future research should consider novel ways to separate the effects of biohydrogenation intermediates and insulin to determine the mechanisms responsible for partitioning of energy in lactating dairy cows. We did effectively alter energy partitioning by increasing milk fat yield with the high fiber and fat diet and increasing BW and subcutaneous fat with the high starch diet. The potential for use of high fiber and fat diets to maintain BCS in mid- and late-lactation cows to reduce excessive over conditioning, prevent metabolic diseases, and reduce the risk of culling and death in the subsequent lactation requires further examination.

The combination of high fiber and fat used in the previous study was required in order to keep energy content similar between the two diets. In Chapter 4, we evaluated the effects of a high starch vs. a high fiber diet in cows with a wide range of milk production. Overall, the high starch diet, increased yields of milk, protein, and fat compared with the high fiber diet. However, the response to the treatments varied depending on level of preliminary milk yield. Higher producing cows, with greater requirements for glucose, performed better on the high starch diet. Whereas, lower producing cows were able to maintain milk production on the high fiber diet which contained just 12% starch. Results from this study support the value of grouping cows based on their glucose requirements in order to maximize the effectiveness of high starch ingredients and utilization of lower cost byproducts. An on farm application of this research is to

group cows based on BCS, as cows increase BCS in mid-lactation they are being fed in excess of their requirements and can be switched to a lower starch diet without sacrificing milk yield. The differences between the two diets in our study were extreme; however, we still observed that cows below approximately 35 kg/d of preliminary milk yield had similar responses on both treatments for yields of milk and milk components.

Finally, soybean oil and soybean free FA distillate were evaluated to determine if differences existed between the two for production of biohydrogenation intermediates in the rumen and if this had an effect on milk fat yield (Chapter 6). Soybean based FA contain large amounts of unsaturated FA that must be biohydrogenated in the rumen in order to reduce the toxic effects to certain rumen microbes. The FA in TAG must be hydrolyzed from the glycerol prior to undergoing biohydrogenation, whereas free FA are readily available for biohydrogenation. We hypothesized that free FA would overwhelm biohydrogenation pathways leading to an accumulation of MFD-inducing intermediates. However, we observed no differences between the two treatments for milk fat yield nor intermediates associated with MFD. Compared with the control treatment, both soybean-based FA treatments resulted in increased milk yield and preformed milk FA yield with a reduction in the yield of de novo synthesized milk FA. In this case we observed a coordinated regulation of milk fat synthesis; as preformed milk FA increased de novo FA were reduced, resulting in no difference in milk fat yield.

The five research chapters presented in this dissertation show the complex role that FA play in dairy cattle nutrition. The interaction between feed components, rumen microbial populations, rumen pH, and the type and form of the FA may alter biohydrogenation rates and pathways. In chapter 3, we consider the effect of the rumen to alter the FA profile reaching the duodenum and available for absorption. Chapter 6, attempts to determine if altering the form that the fat is in,

either as free FA or triglycerides, alters rumen biohydrogenation and therefore milk fat synthesis in the mammary gland. In chapter 4, we anticipated causing lowered milk fat yield with our high starch diet yet milk fat concentration was similar between the two treatments and yield was greater on the high starch diet compared to high fiber diet. We were able to illustrate the differences in glucose (starch) requirements for cows at different levels of milk production. Chapter 5, used a high fiber and fat diet to partition more energy towards milk production compared to a high starch diet that partitioned more energy towards body tissue gain. With similar energy intake between the two diets, the high fiber and fat diet reduced change in BW, BCS, and subcutaneous fat thickness while increasing milk fat yield. Although we were unable to separate out the effects of insulin and biohydrogenation intermediates associated with MFD on changes in energy partitioning, we believe that both contributed to the effects that we observed in adipose tissue and milk fat synthesis. Lastly chapter 7, studied the effect of adding a C18:0-enriched fat supplement to increase milk fat yield. This FA is the most abundant FA available to the dairy cow under normal feeding conditions due to large amounts of unsaturated 18-carbon FA being converted to C18:0 in the rumen. However, supplementing C18:0 had little effect on milk fat yield and reduced the digestibility of 18-carbon FA. In combination, these studies increase our understanding of FA metabolism in the rumen, small intestine, adipose tissue, and mammary gland.