EFFECT OF TWO TYPES OF FAT SUPPLEMENTS DIFFERING IN SATURATION ON PRODUCTION PERFORMANCE AND ENERGY PARTITIONING

By

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ABSTRACT

EFFECT OF TWO TYPES OF FAT SUPPLEMENTS DIFFERING IN SATURATION ON PRODUCTION PERFORMANCE AND ENERGY PARTITIONING

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Our objective was to examine the effect of *trans*-10*, cis*-12 conjugated linoleic acid (CLA) on production performance and energy partitioning. Holstein cows (n=32; 93±35 DIM) were randomly assigned to treatment sequence in a crossover design experiment and fed iso-energetic diets containing fat supplements differing in saturation. Treatment diets contained 2.5% palmitic acid-enriched triglyceride (BergaFat T-300, SAT) or 2.5% soybean oil (UNSAT), with 25% NDF, 32% starch, 18% CP, and 4.6% FA (DM basis). Treatment periods were 28 d in length with the final 5 d used for sample and data collection. The statistical model included the random effect of cow and fixed effects of treatment and period. Compared to the SAT treatment, UNSAT did not alter dry matter intake (DMI), energy intake, or milk yield but decreased milk fat concentration and yield, with reduced de novo fatty acid (FA) and 16-carbon FA yield. UNSAT also decreased fat-corrected milk (FCM) and energy-corrected milk (ECM). UNSAT increased body weight (BW) gain but did not alter body condition score (BCS) or fat thickness over the rump and rib. UNSAT tended to reduce NDF digestibility and increased FA digestibility in period 1 but not in period 2. UNSAT increased plasma insulin, NEFA, and triglyceride concentrations, with increased milk *trans*-10 C18:1 and *trans*-10, *cis*-12 C18:2. In conclusion, with similar NE_L intake, the SAT diet containing the palmitic acid-enriched triglyceride partitioned more energy toward milk, while the UNSAT diet containing soybean oil partitioned more energy toward body gain.

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KEY TO ABBREVIATIONS

ΔBCS = BCS change

ΔBodyE = Body energy change

ΔBW = BW change

BCS = Body condition score

BW = Body weight

CCK = Cholecystokinin

CLA = Conjugated Linoleic Acid

CP = Crude protein

DD = Digestible discount

DE = Digestible energy

DM = Dry matter

DMI = Dry matter intake

ECM = energy corrected milk

EE = Ether extract

FA = Fatty Acids

FCM = fat corrected milk

GE = Gross energy

GLP-1 = Glucagon-like peptide 1

HFF = High fiber/ high fat diet

HS = High starch diet

iNDF = indigestible NDF

MBW = Metabolic BW

ME = Metabolizable energy

MFD = Milk fat depression

MilkE = Milk energy output

MM = Multiple of maintenance

NE = Net energy

NEFA = non-esterified fatty acids

NFC = Non-fibrous carbohydrate

NDF = Neutral detergent fiber

SAT = Saturated fat supplements

SCC = Somatic cell count

TAG = Triglyceride

TDN = Total digestible nutrients

UNSAT = Unsaturated fat supplements

VFA = Volatile fatty acid

CHAPTER 1

INTRODUCTION

Milk fat depression (MFD) is the phenomenon when the yield of fat in milk decreases even though milk yield is unimpaired (Griinari and Bauman, 2006). This was first reported by a French scientist in 1845 (Van Soest, 1994), and has been explored in greater detail in the last two decades. One kind of milk fat depression (MFD), commonly seen and explored by scientists, is diet-induced MFD, which can be caused by a high energy diet, especially a high starch diet (Griinari and Bauman, 2006). To increase milk yield to make more profit, farmers often feed diets with high starch levels to cows. Milk yield is determined by the yield of lactose, which is synthesized from glucose, and glucose supply is highly correlated to the amount of starch that a cow consumes (Allen and Piantoni, 2014). However, high starch diets sometimes cause MFD, which not only decreases the sale price of milk, but also decreases production efficiency. As the most energy-dense component in milk, milk fat is also the highest energy investment for cows. Milk fat represents approximate 50% of the total milk energy (NRC 2001, Equation 2-15). During MFD, more nutrients are stored in adipose tissue but fewer are utilized for milk production (Van Soest, 1963). Boerman et al. (2015) proposed that the spared nutrient due to the reduction of milk fat could be repartitioned to the gain in body condition score (BCS) and body weight (BW). So for cows suffering MFD, energy is less efficiently utilized for milk synthesis but more deposited as body tissue gain. This is not only a waste of money for producers (decreased milk income over feed cost), but also increases the likelihood that a cow will become fat later in the lactation cycle. Over conditioned cows are more likely to suffer metabolic disorders and reproductive disorders in the next lactation. This causes dairy producers higher culling rates, which raises cost to run their farms (NRC 2001; Roche et al., 2009).

The mechanism for diet-induced MFD has been studied for decades and now is considered to involve the biohydrogenation of unsaturated fatty acids in the rumen. In this "biohydrogenation theory", conjugated linoleic acid (CLA) is produced from the biohydrogenation of unsaturated fatty acids in the rumen (Bauman and Griinari, 2001). Among all the CLA isomers, *trans-9, cis-11* CLA, *cis-10, trans*-12 CLA and *trans*-10, *cis*-12 CLA are three CLAs that are known for the inhibitory effect on milk fat synthesis in the mammary gland. Of these, *trans*-10, *cis*-12 CLA inhibits milk fat synthesis more than *trans*-9, *cis*-11 and *cis*-10, *trans-12* CLA, so in this thesis, when I discuss the effects of CLA, I am referring to *trans*-10, *cis*-12 CLA. During CLA-induced MFD, the yields of both de novo and preformed fatty acids are depressed (Griinari and Bauman, 2006), and the depression is dose-dependent (De Veth et al., 2004). During mild CLA-induced MFD, de novo fatty acid synthesis and incorporation of preformed fatty acids into milk fat are inhibited equally. De novo fatty acid synthesis is inhibited to a greater degree than is the incorporation of preformed fatty acid in severe MFD (Griinari and Bauman, 2006). The maximum MFD is a 50% drop in milk fat content (Griinari and Bauman, 2006).

Although the effects of CLA on milk fat are well known, the effects of CLA isomers on adipose tissue are not clear. In monogastric animals, many studies have shown that CLA inhibits fat deposition (Núria et al. 2013; Susana et al, 2011; Intarapichet et al, 2008; Park et al, 2007), but the effects of CLA in ruminants are not consistent and vary by physiological state and lactation stage. CLA inhibits subcutaneous fat accretion in steers (Gassman et al., 2002) and it down-regulates the genes involved in lipogenesis in growing beef cattle (Kadegowda et al., 2013). In fact, the effect of CLA on reducing body fat mass and enhancing lean body mass seems commonly agreed upon in the beef industry (Park and Pariza, 2007). However, the antilipolytic

effect of CLA was proposed by Harvatine et al. (2009), who observed that CLA up-regulated genes involved in lipogenesis in adipose tissue of mid-lactation dairy cows suffering milk fat depression. Furthermore, Von Soosten et al. (2011; 2012) found that CLA inhibited body fat mobilization in dairy cows during early lactation. In contrast, in ewes during early lactation, CLA did not affect body fat mobilization (Sinclair et al., 2010). No consistent results were achieved for the effect of CLA isomers on body fat mobilization for the last decade.

Another potential mechanism for diet-induced MFD is that high starch diets enhance the secretion of insulin, which in turn suppresses milk fat synthesis. This “glucogenic- insulin theory" for MFD was once thought to be the major mediator of MFD because insulin reduces the release of non-esterified fatty acids from adipose tissue, and hence the supply of milk fat precursors (McClymont and Vallance, 1962; Bauman and Griinari, 2000). This theory was questioned in later studies because fatty acids from adipose tissue account for less than 10% of milk fat during all stages of lactation except the first month (Grinari and Bauman, 2006), so it’s not likely that insulin could cause a 50% reduction in severe MFD. Moreover, during insulin-induced MFD, most of the reduction in milk fat is from long-chain fatty acids (Palmquist and Mattos, 1978; Pullen et al., 1989), which is the opposite from that usually seen in diet-induced MFD. In diet-induced MFD, de novo fatty acids are the major depressed proportion of reduced milk fat (Bauman and Griinari, 2000).

Even though most scientists currently seem to support the “biohydrogenation theory” for the cause of MFD, this single theory does not seem sufficient to explain all that occurs during diet-induced MFD. For example, postruminal infusion of glucose depresses milk fat synthesis (Hurtaud et al.,1998; 2000; Rigout et al., 2002; 2003;Lemosquet et al.,1997; Léonard and Block,1997; Oldick et al.,1997). In these studies, the production of CLA isomers likely was not

altered because CLA is produced in the rumen and glucose was infused postruminally. So the increased insulin, but not CLA isomers, seems the more likely explanatory mechanism for the reduced milk fat yield. Also, insulin infusion, with or without euglycemic clamp, depressed the fat content of milk (Palmquist and Mattos, 1978; Pullen et al., 1989 Grinari et al., 1997; Corl et al., 2006). Finally, using a meta-analysis of 22 studies, Schmidt and Lock (2015) found that post-ruminal infusions of glucose and propionate caused MFD, and greater infusion doses linearly increased the severity of MFD. This also could not be explained by the "biohydrogenation theory". Therefore both theories seem important to fully explain MFD.

Davis et al. (1970) divided MFD into two categories: 1) MFD caused by feeding copious amounts of rapidly digested carbohydrates along with inadequate fiber, and 2) MFD caused by feeding diets with excess polyunsaturated fatty acids. Later studies and reviews debunked this categorization and pointed toward only one main dietary cause of MFD, the feeding of excess rapidly digested carbohydrates when sufficient amounts of polyunsaturated fat is present (Griinari et al., 1998; Griinari and Bauman, 2006). We know that CLA mediates MFD, at least partially. We also know that insulin inhibits lipid mobilization and stimulates lipid synthesis in adipose tissue, and thus reduces the yield of preformed milk fatty acids (Griinari and Bauman, 2006). However, the relationship and interaction between CLA and insulin are not understood. As one of the two key factors promoting CLA production in diet-induced MFD, unsaturated fat could also induce the release of insulin. Opara et al. (1994) observed that the effect of fatty acids on insulin release depended on chain length and also the degree of unsaturation in mice. In rats, polyunsaturated fatty acids can enhance insulin action (Storlien et al., 2000; 1991). Khorasani et al. (1998) showed that plasma insulin concentration increased linearly after feeding unsaturated fat to dairy cows. Based on what has been observed above, we can propose that both CLA

isomers and insulin production will be enhanced when both high starch and sufficient unsaturated fat are present. Therefore, the effects of CLA and insulin are confounded during MFD, and scientists may have under-estimated the effect of either CLA or insulin as they gave attention to the other.

Boerman et al. (2015) fed cows diets differing in starch, forage, and fat concentrations and found that the high starch diet increased insulin secretion but rarely increased CLA daily yield, and caused MFD compared to a high saturated fat/high fiber diet. Along with increased insulin, they observed an increase in BCS and BW, and partitioning of nutrients toward body tissues in cows fed high the starch diet (Boerman et al., 2015). Whether the increase in partitioning toward body tissues was caused directly by insulin, indirectly by CLA through insulin or direct by CLA is not clear. Perhaps many of the inconsistencies observed during diet-induced MFD could be explained if the interaction between CLA and insulin in ruminants was understood.

One possible way to separate the effects of insulin and CLA on milk fat synthesis and energy partitioning is to feed isocaloric isoglucogenic diets with different fat saturations. In the work of Boerman et al (2015), a high starch diet, compared to a high saturated fat/ high fiber diet, decreased milk fat yield by 7% (1.81 vs. 1.68 kg/d; $P< 0.001$), increased plasma insulin concentration by 33% (1.01 vs. 0.76μg/L; $P<0.001$), increased *trans*-10 C18:1 daily yield by 44% (5.28 vs. 7.61 g/d; $P<0.01$) but did not alter *trans*-10, *cis*-12 CLA production (<0.01 vs. 0.01 g/d; $P=0.17$). Hence, changes in insulin and also CLA isomers can account for the results in the study by Boerman et al. (2015), including increased body weight, increased body condition score and higher energy partitioning to body tissue gain. However, the change in the insulin account for most of the results since the *trans*-10*, cis*-12 C18:2 was not altered.

In the current study, our goal was to manipulate CLA production with minimal change in insulin secretion. We thought that we might achieve this goal by feeding diets with the same level of starch but with fat supplements differing in saturation, to make the isocaloric isoglucogenic diets. We postulated that an unsaturated FA diet, compared to saturated FA diet, would enhance production of CLA with less effect on insulin secretion than would high starch, and therefore enable us to determine if a large increase of CLA with limited change in insulin could also alter energy partitioning. Thus, the objective of our study was to determine the effect of an unsaturated fat supplement, compared to a saturated fat supplement, on milk production and energy partitioning. The hypothesis for our study is that feeding an unsaturated fat supplement to mid lactation cows will increase CLA isomer production much in the rumen while increasing plasma limited insulin level, decrease synthesis of milk fat in the mammary glands, and partition more energy toward body gain.

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CHAPTER 2

MATERIALS AND METHODS

Cows, Experimental Design, and Diets

Experimental procedures were approved by the Institutional Animal Care and Use Committee of Michigan State University. Thirty-two mid lactation Holstein cows (14 primiparous and 18 multiparous) were fed diets that differed in types of fat supplements. Mean DIM, BW, and milk yield for all cows (mean± SD) were 93 ± 35 d, 668 ± 61 kg, and 46 ± 11 kg/d, respectively, at the start of the experiment. Cows were grouped to 2 cohorts based on milk yield and BW and randomly assigned to a treatment sequence. Cows were fed each diet for a 28-d period in a cross-over design with half the cows fed the diet containing saturated fat supplements and half fed the diet containing unsaturated fat supplements during period 1, and then groups were fed the opposite diet in period 2. All cows were housed in individual tie-stalls and milked twice daily (0400 and 1530 h). Water was available ad libitum and feed was offered once daily at 1200 h at 115% of expected intake based on intake of the previous day. Tie-stalls were equipped with a double-cupped water system to prevent contamination of feed with water and with side panels and a front gate to prevent other cows from stealing feed during cow movements.

During experimental periods, cows were fed similar diets containing either a saturated fat supplement (2.5% DM palmitic acid-enriched triglyceride [BergaFat T-300], SAT) or a polyunsaturated fat supplement (2.5% DM soybean oil, UNSAT) (Table 1). Diets contained corn silage and alfalfa silage as forage sources and contained 25% NDF, 18% forage NDF, 32% starch, 18% CP, and 4.6% FA. Diets were adjusted for changes in forage DM concentration twice weekly.

Sample Collection and Analysis

Cows were fed once per day and orts were removed and weighed daily prior to feeding. Milk yield was recorded electronically at each milking. Milk samples obtained from 4 consecutive milkings per week (d 4, 5, 11, 12, 18, 19, 25, 26 of each period) were used for energy partitioning calculation, and 10 consecutive milking samples during the last 5 days of each period were collected for production performance analysis. Milk samples were analyzed for fat, protein, lactose, somatic cell count, and milk urea nitrogen (MUN) with infrared spectroscopy by Michigan DHIA (East Lansing). Body weight for each cow was recorded three days per week immediately following the morning milking. Body condition score (BCS) for each cow was recorded on a 5-point scale, where 1 is thin and 5 is fat, at the beginning and end of each period and determined by the calculated average of the scores reported by three trained investigators. On the last day of the preliminary period and last day 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). The National Centralized Ultrasound Processing Lab (Ames, IA) analyzed the ultrasound images, and the change in subcutaneous fat thickness was calculated as the difference between one measurement and the previous measurement.

During the last 5 d of the experimental periods, samples of feed ingredients were collected daily to determine the nutrient profile of the diets. Samples of feces were collected every 15 h (1200 h on d 1, 0300 h and 1800 h on d 2, 0900 h and 2400 h on d 3, 1500 h on d 4, and 0600 h and 2100 h on d 5) to procure 8 samples per cow to represent 3-h intervals during a 24-h period. Samples of orts (12.5%) from each cow per day and diet ingredients (~0.5 kg) were

collected each day during the collection periods. All samples were stored at -20°C after collection until analysis.

Samples were composited to obtain one sample per period and dried using a forced air oven (55°C for 84 h) before grinding through a Wiley mill (2-mm screen for cotton seed, 1-mm screen for other ingredients, orts and fecal sample; Arthur H. Thomas Co., Philadephia, PA). Feces collected from each cow during each period (8 samples per cow) were composited on an equal DM basis.

Samples of feed, orts and feces were analyzed for neutral detergent fiber (NDF), indigestible NDF, and fatty acids (FA) in lab. Feed was also analyzed for crude protein (CP) and starch. NDF and indigestible NDF were determined according to Mertens (2002) and Goering and Van Soest (1970). Indigestible NDF was used as an internal marker to estimate fecal output and nutrient digestibility (Cochran et al., 1986). Flasks were reinoculated at the 120^{th} h to ensure a viable microbial population. Ruminal fluid for the in vitro incubations was equally collected and composited from 3 peak-lactation cows fed a high grain diet. The concentration of FA in the feed ingredients, orts, feces were determined as described by Lock et al. (2013). CP (AOAC International, 2000; Method 990.03) and starch (Hall, 2009) were analyzed by Cumberland Valley Analytical Services Inc (Hagerstown, MD). Concentrations of all nutrients are expressed as a percent of diet DM.

A single composited milk sample per period for each cow was used for analysis of FA composition. Milk samples were composited based on milk fat yield over the last 5 days of each period (d 24-28). Milk lipids were extracted and FA-methyl esters were prepared and quantified using GLC 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).

Samples of blood were collected every 15 h, at the same time as fecal samples, during the last 5 d of each period to acquire samples that collectively represented every 3-h interval of a 24-h period. Blood was sampled via coccygeal venipuncture into 3 evacuated tubes (6 mL each), two that contained potassium EDTA and one that contained potassium oxalate with sodium fluoride as a glycolytic inhibitor. Immediately after collection, samples were centrifuged at 2,000 $\times g$ for 15 min, and plasma was separated and stored at -20°C.

Individual plasma samples were composited to form one sample per cow per period and were analyzed for concentrations of non-esterified fatty acids (NEFA), insulin, triglycerides (TAG) and glucose. Concentration of plasma NEFA was determined by an enzymatic colorimetric method (NEFA-HR (2) kit; Wako Chemicals, Richmond, VA). Insulin concentration was determined by an ELISA kit (Bovine Insulin ELISA; Mecodia, Uppsala, Sweden). TAG was determined by an enzymatic colorimetric method (L-Type triglyceride M kit; Wako Chemicals, Richmond, VA). Plasma glucose concentration was analyzed using a glucose oxidase method that combined 10 uL of plasma with 250 uL of AB solution (Sigma Chemical Co.) and absorbance was measured with a micro-plate reader (SpectraMax 190; Molecular Devices Corp., Sunnyvale, CA). Samples were analyzed in duplicate; if coefficient of variance (CV) between duplicates was > 5% for NEFA, glucose and TAG, or >10% for insulin, then samples were reanalyzed until a CV< 5% for NEFA, glucose and TAG or <10% for insulin between two analyses was achieved.

Calculations

Energy partitioning was determined during treatment periods using weekly milk samples taken from four consecutive milkings and analyzed for fat, protein, and lactose concentrations (University Lab Service, Lansing MI). BW was measured three times per week following morning milking. BCS was 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. Data were used to calculated milk energy output, metabolic BW and body tissue gain throughout treatment periods.

Milk energy output (MilkE; Mcal/d) for a cow was estimated by the following equation (NRC, 2001; from Equation 2-15):

MilkE=[9.29 x fat (kg) + 5.63 x true protein (kg) + 3.95 x lactose (kg)]

where each component is based on the average output of a cow during a 28-d period.

Metabolic BW for a cow (MBW; $kg^{0.75}$) was estimated as $BW^{0.75}$, where BW was the mean BW for the cow during the 28-d period. Mean daily BW change (ΔBW; kg) was calculated for each cow within a period by linear regression after one iteration of outlier removal. After the first regression, records > 3.5 SD were removed before the second regression was performed before determining ΔBW, which was the slope from the second regression. Energy expended for body tissue gain (Δ BodyE; Mcal/d) was estimated by an equation derived from NRC (2001; Table 2-5):

ΔBodyE=[[2.88+1.036xBCS] x ΔBW]

where BCS is the average BCS for a cow during a 28-d period. Energy partitioning was predicted based on observed performance:

% to milk= MilkE / (MilkE+ 0.08 x MBW + ΔBodyE) x 100

where % to milk is the percent of apparent net energy partitioned to milk production

% to maintenance= 0.08 x MBW / (MilkE+ 0.08 x MBW + ΔBodyE) x 100

where % to maintenance is the percent of apparent net energy partitioned to maintenance

% to body tissue= BodyE / (MilkE+ 0.08 x MBW + ΔBodyE) x 100

where % to body tissue is the percent of apparent net energy partitioned to body tissue gain

The milk to feed ratio for a cow during a period was determined as the average daily energy-corrected milk yield (ECM; ECM= [0.327 x milk(kg) + 12.95 x fat (kg) +7.20 x protein (kg)]; Tyrell and Reid, 1965) over the average daily DMI. Apparent diet energy content ($DietNE_L$; Mcal/kg) was calculated for each diet as the average NE_L required by each cow divided by her average daily intake for the diet:

Energy concentration of the diet was calculated for individual cows for each treatment:

$DietNE_l$ = [(MilkE+ 0.08 x MBW + ΔBodyE) / DMI]

where DMI is the average DMI for a cow when she was fed the diet.

Statistical Analysis

Production, efficiency, FA profile, hormone and blood metabolites, and digestibility responses to SAT and UNSAT diets were analyzed using a mixed model in SAS (SAS, 9.4 version) that included the fixed effects of diets, period, and two-way interaction of fixed effects, and the random effect of cow.

Main effects were considered significant at $P< 0.05$ and trends at $P< 0.1$. Interactions were considered significant at $P< 0.1$ and trends at $P< 0.15$. All results are expressed as least square means and standard error of the means, unless otherwise specified.

APPENDIX

Table 2-1: Ingredients and Nutrient Composition of Experimental Diets[1,2,3]

	Treatments[2]	
Ingredient, % DM	SAT	UNSAT
Corn silage	29.0	29.1
Alfalfa silage	14.1	14.1
Cottonseed, whole	5.3	5.3
Corn, ground	10.5	10.5
Corn, High Moisture	18.5	18.5
Soybean meal	16.7	16.7
C16:0-enriched fat supplement[4]	2.5	----
Vegetable oil - soybean oil	----	2.5
Vitamin & mineral premix[5]	2.0	2.0
Limestone	0.7	0.7
Sodium bicarbonate	0.7	0.7
Forage: Concentrate	43:57	43:57
Nutrient composition, % DM		
DM	57.1	57.1
NDF	25.0	25.1
Forage NDF	18.1	18.2
CP	18.2	17.9
Starch	31.6	31.9
FA	4.79	4.40
16-Carbon FA	2.19	0.64
18-Carbon FA	2.48	3.65
Apparent NE_l, Mcal.kg[6]	1.64	1.66

[1]Experimental diets fed to 32 cows in a crossover design within 28-d periods

[2]Treatments contained 2.5% added palmitic acids-enriched triglyceride (SAT) or soybean oil (UNSAT) on a DM basis

[3]Nutrient composition was determined from feed ingredients sampled during the last 5 d of each 28-d experimental period.

[4]BergaFat T-300 (Berg+ Schmidt America LLC, Libertyville, IL)

[5]The vitamin and mineral premix was designed to meet the mineral and vitamin requirements of lactating cows as set forth by NRC (2001). The premix 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.

[6] Mean apparent net energy concentration of diets, based on average cow performance. For each diet, Diet NE_L= the average of (MilkE+0.08 x MBW+**Δ**BodyE)/ DMI for all cows on the diet.

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REFERENCES

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CHAPTER 3

RESULTS

Production Performance

Compared with SAT, the UNSAT treatment did not alter milk yield (P=0.58) or DMI (P=0.65; Table 3-1). However, UNSAT decreased milk fat concentration by 0.65 units (2.42% vs. 3.07%; P<0.01) and yield by ~ 240 g/ d (1110 vs. 1350 g/d; P<0.01). UNSAT increased milk protein concentration by 0.07 units (3.12% vs. 3.05%; P<0.01) and yield by ~ 40 g/d (1440 vs. 1400 g/d; P=0.04). UNSAT decreased 3.5%- FCM (38.1 vs. 41.9 kg/d; P<0.01) and ECM (39.8 vs. 42.6 kg/d; P<0.01) compared with SAT. The amount of ECM per unit of DMI was lower in UNSAT treatment than SAT treatment by 9% (1.53 vs. 1.67 kg/kg; P<0.01).

Body Composition

UNSAT tended to increase BW (681 vs. 677 kg; P=0.09) but did not alter BCS (3.29 vs. 3.33; P=0.13) compared with the SAT treatment (Table 3-2). UNSAT increased BW gain by 0.27 kg/d compared with SAT. Cows maintained BCS over 28-d treatment period, and treatment did not alter BCS (gain of 0.1 units in 28 d for either group, P=0.8). Change in body fat was further measured by measuring subcutaneous fat thickness over the rump and 12th intercostal space at the end of periods. Treatment did not alter fat thickness over the rump (0.20 vs. 0.06 mm/28 d for SAT and UNSAT; P=0.7) or rib (0.33 vs. 0.57 mm; P=0.5).

Calculated Energy Values and Partitioning

Compared with SAT, the UNSAT treatment decreased milk energy output (27.1 vs. 29.1 Mcal/ d; P< 0.01; Table 3-2) and increased energy deposited in body tissue gain, as determined by ΔBW (3.33 vs. 1.40 Mcal/ d; P< 0.01) throughout the treatment periods. UNSAT decreased

calculated milk energy as a fraction of NE_L use (66% vs. 71%, $P< 0.01$) and increased calculated body energy gain as a fraction of NE_L use (8% vs. 3%, $P< 0.01$) compared with SAT. Based on cow performance, apparent dietary NE_L values were similar for the two diets (1.64 vs. 1.66 Mcal/kg; $P= 0.51$).

Plasma Metabolites and Hormones

UNSAT increased plasma glucose concentration in period 1 but did not alter plasma glucose concentration in period 2 (P=0.25; Table 3-3). However, UNSAT increased plasma insulin concentration by 12% (1.34 vs. 1.18 ug/L; P=0.025) and increased the plasma concentrations of NEFA and TAG (10% and 8%, respectively; P<0.001 and P=0.05, respectively), compared with SAT.

Digestibility

Compared with SAT, the UNSAT treatment did not alter DM digestibility (P=0.34; Table 3-4) but tended to reduce NDF digestibility (29% vs. 26%; P=0.09). Interactions between treatment and period for total FA digestibility and also 18-carbon FA digestibility were detected. UNSAT increased FA digestibility in period 1 but not in period 2; in contrast, UNSAT decreased 18-carbon FA digestibility in period 2 but not in period 1. UNSAT increased 16-carbon FA digestibility (52% vs. 69%; P<0.001) across two treatment periods.

Pearson Correlation Coefficients

Person correlation coefficients are calculated (Table 3-5). Daily milk fat yield was positively correlated with daily milk fat % (P<0.01) and daily milk protein yield (P<0.01), but negatively correlated with daily milk protein % (P<0.01), ΔBW (P<0.05), plasma glucose concentration (P<0.01), insulin concentration (P<0.01), and plasma NEFA concentration

(P<0.01). Daily milk fat % was negatively correlated with plasma glucose concentration (P<0.01), insulin concentration (P<0.01) and NEFA concentration (P<0.01). Daily milk protein yield was negatively correlated with plasma glucose concentration (P<0.01) while daily protein % was positively correlated with ΔBW (P<0.05), plasma glucose concentration (P<0.01) and plasma triglyceride concentration (P<0.01). *Trans*-10 C18:1 was negatively correlated with daily milk fat yield, milk fat percentage, and positively correlated with insulin, NEFA and CLA (all P<0.01).

Milk Fatty Acids

Milk FA (concentration basis) are shown in Table 3-6 by metabolic source (<16 carbon FA are from de novo synthesis in the mammary glands, >16 carbon FA originate from extraction from plasma, and 16 carbon FA are from mixed sources). Compared with SAT, the UNSAT treatment reduced de novo synthesized and mixed source FA, but increased preformed milk FA (all P< 0.01). UNSAT reduced most of the de novo synthesized milk FAs (all P< 0.01) except C14:0 (P=0.13). The decreased mixed source milk FA (16 carbon in length) in UNSAT occurred mainly because of a lower concentration of C16:0 (P< 0.01). UNSAT increased the concentration of preformed milk FA (all P< 0.01).

Selected milk FA are shown in the table 3-7 on a yield basis. Compared with SAT, the UNSAT treatment reduced de novo synthesized milk FA (P<0.01) and mixed source milk FA (P<0.001), but had no effect on preformed milk FA (P=0.27). Almost half of the reduced de novo synthesized milk FA were from reduced C14:0, which accounted for 28 of the 65 g/d decrease. UNSAT reduced mixed source milk FA by 178 g/d, while C16:0 accounted for almost all of this.

APPENDIX

Table 3-1: Dry matter intake, milk production, milk components and feed efficiency for cows fed treatment diets (n=32).

	Treatments[1]			P-value[2,3]
	SAT	UNSAT	SEM	TRT
DMI	25.0	24.9	0.63	0.65
NDF	6.29	6.20	0.16	0.25
FA	1.22	1.11	0.03	< 0.01
Milk Yield, kg/d				
Milk	46.1	46.5	1.75	0.58
ECM[4]	42.6	39.8	1.56	< 0.01
3.5% FCM[5]	41.9	38.1	1.60	< 0.01
Milk Components				
Fat, kg/d	1.35	1.11	0.05	< 0.01
Fat, %	3.07	2.42	0.13	< 0.01
Protein, kg/d	1.40	1.44	0.05	0.04
Protein, %	3.05	3.12	0.03	< 0.01
Lactose, kg/d	2.21	2.24	0.08	0.42
Lactose, %	4.81	4.83	0.03	0.2
ECM/DMI[6]	1.67	1.53	0.04	< 0.01

[1] Treatments contained 2.5% added palmitic acid enriched triglyceride (SAT) or 2.5% soybean oil (UNSAT) on a DM basis.
[2] *P*-value associated with treatment differences (SAT vs. UNSAT; Trt).
[3] All P value for period*treatment are over 0.6
[4] Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)] (Tyrrell and Reid, 1965).
[5] Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].
[6] Milk: feed ratio =ECM/DMI

Table 3-2: Body weight, body condition score and change in subcutaneous fat thickness measurements and calculated energy values for cows fed experimental diets (n = 32).

	Treatments[1]			P-value[2,3]
Variable	SAT	UNSAT	SEM	TRT
BW	677	681	10.9	0.09
BCS	3.29	3.33	0.07	0.13
Change in BW, kg/d[4]	0.19	0.46	2.37	0.04
Change in BCS, pt/28 d	0.11	0.12	0.03	0.81
Change in Rump Fat, mm/28 d	0.20	0.06	0.19	0.67
Change in Rib Fat, mm/28 d	0.33	0.57	0.18	0.45
Calculated energy values[5]				
Apparent NE_L of diet Mcal/kg	1.64	1.66	0.03	0.51
Milk, Mcal/d	29.1	27.1	1.12	< 0.01
Body Tissue Gain, Mcal/d	1.40	3.33	0.55	< 0.01
Maintenance, Mcal/d	10.6	10.7	0.13	0.17
Partitioning[6]				
Milk, %	71	66	1.4	< 0.01
Body Tissue Gain, %	3	8	1.5	< 0.01
Maintenance, %	26	26	0.6	0.79

[1] Treatments contained 2.5% added palmitic acid enriched triglyceride (SAT) or 2.5% soybean oil (UNSAT) on a DM basis.
[2] *P*-value associated with treatment differences (SAT vs. UNSAT; TRT).
[3] All *P* value for Period* Treatment are over 0.5
[4] Determined by linear regression using BW measurements throughout the period.
[5] Milk (MilkE)=[9.29 x fat (kg) + 5.63 x true protein (kg) + 3.95 x lactose (kg)]. Body tissue gain (ΔBodyE) =[(2.88+1.036xBCS) x ΔBW], Maintenance=0.08x MBW
[6] % to milk, maintenance, or body tissue= [MilkE, 0.08x MBW, or ΔBodyE/ (MilkE+ 0.08x MBW + ΔBodyE) x 100].

Table 3-3: Plasma concentrations of glucose, insulin, NEFA, and triglycerides for cows fed experimental diets (n=32).

	Treatments[1]			P-value[2,3]	
Variable	SAT	UNSAT	SEM	TRT	PER*TRT
Glucose, mg/dL	59.6	60.1	0.42	0.25	0.06[4]
Insulin, μg/L	1.18	1.34	0.05	0.025	0.71
NEFA, μEq/L	122	137	5.3	< 0.01	0.94
TAG, mg/dL	7.9	8.5	0.29	0.05	0.89

[1] Treatments contained 2.5% added palmitic acid enriched triglyceride (SAT) or Soybean oil (UNSAT) on a DM basis.
[2] *P*-value associated with treatment differences (SAT vs. UNSAT; TRT).
[3] All P value for treatment*period are over 0.7 except for Glucose, which is 0.06
[4]UNSAT increased plasma glucose concentration in period 1 but not in period 2

Table 3-4: Apparent total tract digestibility for nutrients for cows fed experimental diets (n=32).

	Treatments[1]			P-value[2]	
Nutrient	SAT	UNSAT	SEM	TRT	PER*TRT[3]
DM	64.9	64.2	0.55	0.34	0.24
NDF	29.1	26.4	1.47	0.09	0.31
FA	62.2	68.1	1.26	< 0.01	0.04
16 Carbon	52.4	68.5	1.51	< 0.01	0.01
18 Carbon	72.9	69.9	1.34	0.04	0.04

[1] Treatments contained 2.5% added palmitic acid (SAT) or soybean oil (UNSAT) on a DM basis.
[2] *P*-value associated with treatment differences (SAT vs. UNSAT; TRT).
[3] Interaction exists in total FA and 18-carbon FA. In period 1, total FA was increased by UNSAT while 18-carbon FA was not; in period 2, 18-carbon FA was reduced by UNSAT while total FA was not.

Table 3-5: Pearson correlation coefficients between production variables, plasma insulin and metabolites for cows fed treatment diets (n=32).

	Milk Fat Yield	Milk Fat %	Milk Protein Yield	Milk Protein %	Daily ΔBW	Glucose	TAG	Insulin	NEFA	C18: 1 *trans*-10	C18: 2 *trans*-10, *cis*-12
Milk Fat Yield	1	0.70 *P*<0.01	0.60 *P*<0.01	-0.43 *P*<0.01	-0.30 *P<0.05*	-0.55 *P*<0.01	-0.19 *P*=0.1	-0.36 *P*<0.01	-0.35 *P*<0.01	-0.41 *P*<0.01	-0.30 *P<0.05*
Milk Fat %		1	-0.13 *P*=0.3	-0.21 *P*=0.1	-0.16 *P*=0.2	-0.36 *P*<0.01	-0.07 *P*=0.5	-0.35 *P*<0.01	-0.59 *P*<0.01	-0.68 *P*<0.01	-0.62 *P*<0.01
Milk Protein Yield			1	-0.23 *P*=0.1	-0.23 *P*=0.1	-0.36 *P*<0.01	-0.19 *P*=0.1	-0.10 *P*=0.5	0.21 *P*=0.1	0.23 *P*=0.1	0.31 *P<0.05*
Milk Protein %				1	0.31 *P<0.05*	0.36 *P*<0.01	0.40 *P*<0.01	0.17 *P*=0.1	0.31 *P<0.05*	0.14 *P*=0.3	0.10 *P*=0.5
Daily ΔBW					1	0.01 *P*=0.9	0.21 *P*=0.1	0.11 *P*=0.4	0.03 *P*=0.9	0.09 *P*=0.5	0.09 *P*=0.5
Glucose						1	0.16 *P*=0.2	0.30 *P<0.05*	0.21 *P*=0.1	0.25 *P<0.05*	0.10 *P*=0.5
TAG							1	0.15 *P*=0.3	0.30 *P<0.05*	-0.11 *P*=0.4	-0.15 *P*=0.3
Insulin								1	0.17 *P*=0.2	0.40 *P*<0.01	0.23 *P*=0.1
NEFA									1	0.474 *P*<0.01	0.397 *P*<0.01
C18: 1 trans-10										1	0.857 *P*<0.01

Table 3-6: Milk FA concentrations of cows fed treatment diets (n=32).[1]

	Treatments[2]			*P*-value[3,4]
FA concentration (g/100 g)	SAT	UNSAT	SEM	TRT
De novo[5]	22.3	20.9	0.43	< 0.01
Mixed	39.2	30.5	0.67	< 0.01
Preformed	38.5	48.5	0.88	< 0.01
Selected individual FA[6]				
4:0	2.56	2.34	0.09	< 0.05
6:0	1.62	1.41	0.06	< 0.01
8:0	0.92	0.78	0.04	< 0.01
10:0	2.39	2.03	0.09	< 0.01
12:0	2.91	2.61	0.09	< 0.01
14:0	11.1	10.8	0.17	0.13
14:1 *cis*-9	0.84	0.98	0.03	< 0.01
16:0	37.5	28.9	0.64	< 0.01
16:1 *cis*-9	1.72	1.63	0.08	0.26
18:0	8.02	9.96	0.32	< 0.01
18:1 *trans*-4	0.02	0.02	0.002	< 0.01
18:1 *trans*-5	0.01	0.02	0.001	< 0.01
18:1 *trans*-6-8	0.41	0.75	0.04	< 0.01
18:1 *trans*-9	0.25	0.44	0.02	< 0.01
18:1 *trans*-10	2.50	4.55	0.5	< 0.01
18:1 *trans*-11	0.64	1.01	0.08	< 0.01
18:1 *trans*-12	0.40	0.67	0.03	< 0.01
18:1 *cis*-9	17.3	20.3	0.38	< 0.01
18:1 *cis*-11	0.67	0.77	0.03	< 0.01
18:2 *cis*-9, *cis*-12	2.30	2.86	0.07	< 0.01
18:2 *cis*-9, *trans*-11	0.37	0.57	0.03	< 0.01
18:2 *trans*-9, *cis*-11	0.02	0.05	0.005	< 0.01
18:2 *trans*-10, *cis*-12	0.01	0.03	0.003	< 0.01
18:3 *cis*-9, *cis*-12, cis-15	0.26	0.34	0.008	< 0.01

[1] Samples for milk FA were collected during the last 5 d of each treatment period (d 24 to 28).
[2]Treatments contained 2.5% added palmitic acid enriched triglyceride (SAT) or Soybean oil (UNSAT) on a DM basis.
[3] *P*-value associated with treatment differences (SAT vs. UNSAT; TRT).
[4]All *P* value for treatment*period are over 0.5.
[5]De novo = milk FA < 16 carbons in length; mixed = milk FA 16-carbons in length; preformed = Milk FA >16 carbons in length
[6] A total of approximately 70 individual FA were quantified and used for calculations (summation by source). Only selected FA are reported in the table.

Table 3-7: Milk FA yields of cows fed treatment diets (n=32).[1]

	Treatments[2]			*P*-value[3,4]
FA Yield (g/d)	SAT	UNSAT	SEM	TRT
De novo[5]	292	227	11.2	< 0.01
Mixed	493	315	14.1	< 0.01
Preformed	479	499	17.5	0.27
Selected individual FA[6]				
4:0	40.0	48.3	1.73	< 0.01
6:0	21.8	16.0	1.99	< 0.01
8:0	12.4	9.0	1.20	< 0.01
10:0	32.3	23.1	3.11	< 0.01
12:0	38.8	29.0	1.74	< 0.01
14:0	143	115	4.71	< 0.01
14:1 *cis*-9	10.4	9.7	0.33	< 0.01
16:0	472	299	13.9	< 0.01
16:1 *cis*-9	20.7	16.1	0.73	< 0.01
18:0	103	106	5.25	0.52
18:1 *trans*-4	0.19	0.25	0.02	< 0.01
18:1 *trans*-5	0.17	0.21	0.01	< 0.01
18:1 *trans*-6-8	4.77	7.24	0.44	< 0.01
18:1 *trans*-9	2.96	4.33	0.24	< 0.01
18:1 *trans*-10	26.6	41.5	4.62	< 0.01
18:1 *trans*-11	8.29	11.4	1.4.0	< 0.05
18:1 *trans*-12	4.94	7.02	0.45	< 0.01
18:1 *cis*-9	217	209	7.43	0.34
18:1 *cis*-11	8.09	7.71	0.35	0.28
18:2 *cis*-9, *cis*-12	28.8	29.3	1.04	0.63
18:2 *cis*-9, *trans*-11	4.53	6.01	0.48	< 0.01
18:2 *trans*-9, *cis*-11	0.26	0.41	0.04	< 0.01
18:2 *trans*-10, *cis*-12	0.16	0.27	0.03	< 0.01
18:3 *cis*-9, *cis*-12, cis-15	3.23	3.44	0.13	0.11

[1] Samples for milk FA were collected during the last 5 d of each treatment period (d 24 to 28).
[2]Treatments contained 2.5% added palmitic acid enriched triglyceride (SAT) or Soybean oil (UNSAT) on a DM basis.
[3] *P*-value associated with treatment differences (SAT vs. UNSAT; TRT).
[4] All *P* value for treatment*period are over 0.5.
[5]De novo = milk FA < 16 carbons in length; mixed = milk FA 16-carbons in length; preformed = Milk FA >16 carbons in length
[6]A total of approximately 70 individual FA were quantified and used for calculations (summation by source). Only selected FA are reported in the table

CHAPTER 4

GENERAL DISCUSSIONS

Production Performance

In our study, feeding diets with fat supplements differing in saturation did not alter DMI. Our results are similar to several other studies showing that feeding unsaturated fat did not alter DMI (Avila et al., 2000; Schauff et al., 1992; Kargar et al, 2010) but contradict other studies showing that DMI was decreased by unsaturated fat (Harvatine and Allen, 2005; Harvatine and Allen, 2006; Pantoja et al., 1996). Considering that dietary supplemented unsaturated fat produces CLA isomers, CLA isomers have been fed directly to cows in early lactation to measure their effect on DMI. However, the effect of feeding CLA on DMI in these studies was also not consistent, with studies finding no effects (von Soosten et al., 2011), inhibitory effects (Pappritz et al., 2011), or stimulatory effects (Shingfield et al., 2004). For those studies observing depressed DMI, one hypothesis is that depressed DMI was caused by an inhibitory effect of unsaturated fat on fiber fermentation (Patra, 2013; Hristov et al, 2009; Yang et al, 2009), which in turn, increased rumen fullness and limited DMI. Additionally, Allen (2000) proposed that unsaturated fat supplements reduced DMI through postruminal effects regulating meal size or/and meal frequency. Secretion of cholecystokinin (CCK) is increased during a meal, and CCK regulates satiety, and thus meal size, and is more sensitive to unsaturated fatty acids than saturated fatty acids, leading to a greater depression in DMI with unsaturated fat (Bradford et al., 2008). Consistent with a direct hypophagic effect of unsaturated FA, post-ruminal infusions of unsaturated fat consistently depressed DMI (Relling and Reynolds, 2007; Bradford et al., 2008; Drackley et al. 1992). However, Avila (2000) suggested that ~2% unsaturated fat supplement could not influence the rumen environment nor depress DMI because cellulolytic bacteria are not

affected by this dosage level of unsaturated fat. As such, our treatment level at 2.5% fat supplementation perhaps was not enough to decrease DMI.

In our study, we found no difference in milk yield between the two treatment groups. A survey of the literature reveals that supplemental unsaturated fat decreased (Boerman et al., 2014; Relling and Reynolds, 2007), increased (Rabiee et al, 2012; Boerman and Lock, 2014), or had no effect on milk yield (Schauff et al, 1992; Harvatine and Allen, 2005; Avila et al., 2000). Boerman and Lock (2014) argued that increased milk yield might be expected because of a glucose sparing effect from decreased milk fat synthesis. We proposed that acetate was also spared in cows fed UNSAT because of the decreased de novo FA synthesis in mammary gland. Thus more acetate would be more available to adipose tissue to make FA and to muscle as fuel, which in turn, spared more glucose. During diet induced-MFD, depressed de novo synthesis of FA reduces the requirement of NADPH, half of which is from glucose oxidation (pentose phosphate pathway) (Bauman and Davis, 1975). Therefore, decreased de novo fatty acid synthesis should increase glucose available for lactose synthesis, with a concomitant increase in milk yield. As ~70% of whole body glucose flux is used by mammary glands (Baldwin and Smith, 1979), and ~70% of mammary glucose uptake is used for lactose synthesis (Palmquist, 2006; Katz and Wals, 1972), about half of the whole body glucose flux is used for lactose synthesis in lactating dairy cows. The average lactose yield in this study was ~2.2 kg/d, so whole body glucose flux would have been ~4.4 kg/d and mammary glucose uptake ~3.1 kg/d. Palmquist (2006) concluded that ~26% of glucose uptake is oxidized in pentose phosphate pathway in mammary gland to generate NADPH for de novo fatty acid synthesis, which means ~18% of daily whole body glucose flux is oxidized in the Pentose phosphate pathway (PPP). Given that we observed a 20% reduction in de novo fatty acids with feeding UNSAT, compared

with SAT (292 vs. 227 g/d), then we could expect a 20% reduction in daily whole body glucose used for oxidation in PPP to generate NADPH (assuming half of the NADPH was from glucose) (Mellenberger et al., 1973; Mellenberger and Bauman, 1974). Based on the results and assumptions above, ~0.16 kg/d (4.4 kg/d x 18% x 20%= 0.16 kg/d) glucose likely was spared in UNSAT-treated cows, and this glucose could be used instead to make lactose. The average lactose concentration was 4.8% in this study, so the 0.16 kg of glucose spared daily would provide 3.3 kg of milk per day. However, UNSAT only increased milk by ~0.4 kg/d and lactose yield by 0.03 kg/d. The UNSAT response in this study was lower than the theoretical calculation for several reasons, including 1) possible non-detected decreases in DMI and fiber digestion (Avila et al., 2000), 2) energy costs for synthesizing extra protein in the UNSAT cows (Bionaz et al., 2012), 3) glucose availability was not limiting the lactose synthesis (Lemosquet et al., 1996) and 4) uptake of extra glucose by other tissues (e.g. adipose tissues) in the UNSAT cows. Overall, we propose that milk yield in this study did not increase because of these mechanisms combined and interacting with each other (i.e. DMI & fiber digestion along with glucose sparing and nutrient repartitioning).

In our study, cows fed the diet with UNSAT supplements had lower milk fat concentration and yield, resulting in decreased milk energy output. These results are consistent with some previous studies (Firkins and Eastridge, 1994; Pantoja et al., 1996; Relling et al, 2007), but not with others (Harvatin and Allen, 2005; Boerman and Lock, 2014). The effect of unsaturated fat on milk fat synthesis can be explained by the "biohydrogenation theory" (Griinari and Bauman, 2006). According to this theory, diets high in unsaturated fat result in *trans*-10*, cis*-12 CLA production in the rumen, which in turn, reduces milk fat synthesis (Bauman et al, 2011; Griinari and Bauman, 2006). Based on previous studies where CLA was directly added to the

diet or infused post-ruminally (Baumgard et al, 2001; Loor and Herbein, 2003; Perfield et al, 2002), CLA reduces milk fat synthesis and causes MFD. Moreover, De Veth et al (2004) described an exponential decay model to describe the relationship between milk fat and CLA dose: as dose of CLA increases, milk fat decreases. Even though in our study milk fat yield was reduced as found in other studies, milk fat yield and milk fat percentage were lower than expected, even in the SAT diet. Reasons for the low fat are not clear, but the 50% drop in milk fat was within a reasonable range for MFD.and was less than the maximal drop proposed by Griinari and Bauman (2006). In our study, we formulated basal diets that put cows at high risk of MFD by increasing starch level to 32% and lowering NDF to 25%. No health issues or metabolic disorders were detected during the study. Thus, the low milk fat percentage and milk fat yield in both treatment groups likely were the result of this high starch - low NDF diet (Griinari and Bauman, 2006). In the UNSAT group, milk fat content dropped to 2.4%, which likely was the result of the high starch-low NDF diet in combination with unsaturated fat. Future studies should include rumen pH measurements to examine this theory.

We observed an increase in protein concentration and yield in cows fed the UNSAT treatment. In previous studies, unsaturated fat supplements had inconsistent effects on milk protein yield, but generally protein yield or concentration were decreased (Harvatine and Allen, 2005; Firkins and Eastridge, 1994; Pantoja et al., 1996) or not altered (Boerman and Lock, 2014; Avila et al., 2000). In those studies with decreased protein concentration and yield, the decrease was mostly ascribed to depressed DMI and/or fatty acid -mediated inhibition of microbial protein production. In those studies with increased milk protein synthesis, the increase was ascribed to increased available nutrients from reduced milk fat synthesis (Griinari and Bauman, 2006). Both dietary energy and protein content in a ration are known to alter milk protein yield and

concentration (Broderick, 2003). The energy and protein content of the diets in our study were similar between the two treatments. As such, protein repartitioning towards milk must account for the higher milk protein yield in UNSAT cows. This might be expected because we also observed an 8% greater insulin concentration in the UNSAT compared to SAT treatment. Insulin increases extraction of AA by the mammary gland when concentrations of AA in the blood and blood-flow are adequate (Mackle et al. 2000; Metcalf et al., 1991). Insulin also has been shown to activate the cascade for milk protein synthesis (Winkelman and Overton, 2013). This positive relationship between plasma insulin and milk protein yield and concentration has been confirmed by the study of supplementing fat to dairy cows (Boerman et al., 2015). Our study fed unsaturated fat and found the same relationship between insulin and milk protein yield. Additionally, the spared glucose from reduced de novo fatty acids synthesis could not only be used for lactose synthesis, but also used to induce protein synthesis by increasing mTOR activity via its metabolism in the TCA cycle to increase the NADH then leading to higher ATP in mammary epithelial cells (Bionaz et al., 2012).

Because UNSAT altered composition of milk, and because fat, protein, and lactose all contribute substantially to the energy value of milk (NE_l (Mcal/ kg)= 0.0929 x Fat % + 0.0547 x Crude Protein % + 0.0395 x Lactose %), ECM is a better way to evaluate diet effects on production. In addition, ECM:DMI is a better measurement for feed efficiency than is milk yield to DMI ratio. However, VandeHaar and St-Pierre (2006) argued that ECM: DMI is still not an ideal tool to evaluate feed efficiency because it does not account for the mobilization of body reserves that may be mobilized to support milk production. For example, cows that eat less but maintain high production may mobilize much of their body reserves, which can lead to some metabolic disorders; hurting the future production performance. Gross efficiency accounts for the

energy flow to both milk production and body tissue (VandeHaar and St-Pierre, 2006); however, milk is the major driving force in the dairy industry, so we need to distinguish energy flows more clearly. Therefore, ECM: DMI is also useful to evaluate feed efficiency in dairy industry. In this study, feed efficiency, defined as the ratio of ECM: DMI, was lower for UNSAT treated cows.

Body Tissue Gain

In our study, UNSAT did not affect BCS, change in BCS, change in rump fat thickness, and change in rib fat thickness, compared with SAT. However, UNSAT increased the final body weight and change in body weight. Currently, the relationship of diet-induced MFD to mobilization of body tissue is not well understood. Boerman and Lock (2014) found no change in BW or BCS of lactating dairy cows with soybean oil supplementation into a control diet (29 % starch and 28% NDF on the DM basis). In accordance with that, Boerman et al. (2014) reported no change in BW or BCS when corn oil was added into the control diet (28% starch and 29% NDF on the DM basis) of lactating cows at different concentrations. Possible explanations for the inconsistent results between those two studies and our current study could be that BCS and fat thickness are imprecise measures for which changes in short term studies are not sensitive and changes in BW may be confounded with gut fill effects (Harvatine et al., 2009). In our study, we used a 28-day treatment period, hence imprecise responding might not be an issue. Additionally, ultrasound measurements were included to detect subcutaneous fat thickness change between the hide and musculature over the rump and the 12th intercostal rib space in an effort to better assist measuring body fat deposition. No change in thickness at either measured location was observed during each period, which was consistent with the BCS change result in this study. In the study that did see difference in BCS and BW, CLA was increased though not significantly (Boerman et al., 2015). Considering the inducing effect of unsaturated fat on CLA,

the body fat mobilization effect of unsaturated fat could be mostly due to the effect of CLA (Palmquist, 2005). The effect of CLA on body fat reduction in non-ruminal animal studies has been commonly observed (Park et al., 1997; Delany et al., 1999; Wang and Jones, 2004) and two mechanisms were proposed to explain how CLA reduces body fat: 1) CLA can increase energy expenditure by increasing oxygen consumption; 2) CLA can reduce adipose cell mass and/or cell numbers by inhibiting lipoprotein lipase and enhancing apoptosis of adipocytes (Park and Pariza, 2006). However, the effect of CLA on body tissue mobilization in ruminants, specifically dairy cows, has not been given much attention. Observations of CLA effects in ruminant studies were not consistent and no systematic explanations have been given on the possible mechanism for body mobilization in ruminants during MFD. Gassman et al. (2002) found decreased subcutaneous fat accretion after feeding steers rumen-protected CLA while Sinclair et al. (2010) found no change in carcass composition in CLA fed lactating ewes. In mid-lactation goats, CLA tends to prevent excessive lipid mobilization from body tissue (Ghazal et al., 2014) while Kadegowda et al. (2013) reported that CLA had an effect on body fat mobilization in beef cattle. Harvatine et al. (2009) observed increased lipogenic enzyme expression in adipose tissue of lactating cows after an abomasal infusion of CLA. In accordance with that, Soosten et al. (2011) observed decreased body mass mobilization due to the effect of CLA in early lactating cows. Based on these previous studies, Lock (2015) proposed a possible explanation for the effect of CLA on ruminants body tissue mobilization: the effect of CLA on body tissue mobilization depends on the lactation status of ruminants; CLA diverts nutrients towards body tissue when ruminants are lactating but directs nutrients away from body tissue when ruminants are not lactating. Even though this hypothesis could also explain what happened to the cows during our study, I still propose the other possibility: CLA diverts nutrients away from body tissue to

prevent fat deposition while insulin diverts nutrients towards adipose tissue for fat deposition. Contrary results from previous studies might be due to the lack of attention to the interaction between CLA and insulin. If CLA production is positively related to plasma insulin concentration, as shown in this study, it is reasonable to assume that CLA and insulin could counteract each other for body fat deposition and that this interaction causes the inconclusive results observed for CLA to ruminants. In the future, direct quantitative relationship between CLA and insulin and their effects on body fat mobilization are needed to explain how CLA and insulin affect fat deposition in ruminants, independently or dependently. The other mechanism possibly explaining the lack of a significant change in body fat might be that the amount of insulin or/and CLA induced by UNSAT supplements in this study was not enough to alter subcutaneous fat. According to Allen (1976), the order of fat deposition in cattle is internal fat, subcutaneous, and then inter- and intra-muscular fat. I suspect that insulin and/or CLA in our study was only able to affect internal fat deposition but not subcutaneous fat deposition, which would also help explain why we did not observe any change in BCS nor fat thickness but a significant change in BW between the two treatment groups. Additionally, the seeming controversy results of BW and BCS could be due to the increased body protein mass, considering the effect of insulin on protein synthesis, deposition in body tissues (Tesseraud et al., 2007). However, we did not measure the body protein index of the cows during the experimental periods. Future studies should consider the possibility of including internal fat measurements and body protein index. Another possible reason for increased BW with no change in BCS is that UNSAT could have stimulated CCK (Bradford et al., 2008), thereby reducing rumen motility (Della and Baile, 1980), decreasing ruminal passage rate, and increasing the mass of ruminal contents. However, after looking at the BW change by date across treatment periods, I'm

assuming that the rumen gut fill effect could not explain the different BW increase between treatments (Figure 1).

Blood Metabolites and Hormones

UNSAT did not affect plasma glucose (composite at 3-h intervals during a 24-h period) concentrations in period 2, which is consistent with other studies (Litherland et al., 2005; Wang et al., 2010; Boerman et al., 2015). However, UNSAT did increase plasma glucose in period 1, which seems reasonable to me because it would have decreased the glucose needed to make milk fat. CLA, produced in the biohydrogenation pathway of unsaturated fat, has an inhibitory effect on de novo fatty acid synthesis in the mammary gland. When the mammary gland synthesizes milk fat, acetate and butyrate are the two main substrates used for de novo fatty acid synthesis, with the assistance of glucose to provide NADPH and glycerol (Palmquist and Jenkins, 1980; Bauman and Griinari, 2003; Voigt et al., 2005). In CLA-induced MFD, de novo fatty acid synthesis is mainly depressed; hence the requirement for NADPH and glycerol from glucose decreases, which in turn decreases the need for glucose from gluconeogenesis. Although both diets in this study had the same concentration of glucogenic precursors, cows fed UNSAT produced less de novo FA which means less glucose would have been needed to drive de novo FA synthesis; thus we might expect higher glucose in blood of UNSAT, which was observed in period 1. The unchanged glucose concentration in period 2 was likely the result of the tight regulatory function of insulin and the glucogenic role of the liver in dairy cows (Boerman et al., 2015).

UNSAT increased plasma insulin concentration in our study, likely because, less glucose was needed for de novo milk FA synthesis, so less gluconeogenesis was needed and thus more unused propionate would have circulated in blood. Propionate increases insulin secretion in

ruminants (Bines, 1984), and the increased insulin, in turn, would have regulated glucose concentration in blood so that overall treatment differences for glucose were small. Thus, it seems reasonable to me that the mean blood glucose levels over two periods were not different between the two treatment groups but the insulin was higher in the UNSAT group. Propionate concentration could be measured in the future study by high-performance liquid chromatography (HPLC). Additionally, *trans*-10*, cis-12* CLA can reduce insulin sensitivity by down-regulating PPARγto reduce the expression of key insulin-signaling genes (de Almeida et al., 2015). The cows might have adapted by increasing insulin concentration. The UNSAT might have also increased glucagon-like peptide 1 (GLP-1; Bradford et al., 2008), which could induce insulin secretion (Shigeto et al., 2015).

Plasma NEFA concentration (122 vs. 137μEq/L, respectively) in this study was within the range (0-460 μEq/L) given by Cozzi et al. (2011) and fits with other studies observing that mid-lactation cows mobilize less body fat than early lactation cows, but more than late lactation cows (Cozzi et al., 2011; van Kengsel et al., 2007; Grummer, 2008; Blum et al., 1983; Walters et al., 2002). UNSAT increased plasma NEFA in both periods but the increase would not be expected to affect milk fat synthesis directly because plasma NEFA was below 300 uEq/L (Kronfeld, 1965). The fact that UNSAT increased both plasma NEFA and insulin concentrations was surprising. Normally, an increase in insulin would be expected to decrease NEFA because insulin inhibits the hormone-sensitive lipase of adipose tissue (Vernon, 2005). However, FA digestion was greater in the UNSAT diet, and absorbed TG can enter the plasma NEFA pool (Evans et al., 2002; Karpe et al., 2011). Unfortunately, this explanation only seems reasonable for period 1, as UNSAT did not increase FA digestibility during period 2.

According to Cozzy et al. (2011), the reference range for plasma TAG is 6 - 22 mg/dL, regardless of lactation stage. We observed TAG concentration of ~8 mg/dL. UNSAT diet increased plasma TAG by 8%, which is relatively consistent with the 10% increase noted in NEFA concentration. Uptake of NEFA by hepatic cells for TAG synthesis is determined by supply, not need (Drackley et al, 2001). Additionally, as plasma TAG mainly come from digested and absorbed dietary fat, the higher TAG in blood from UNSAT is consistent with the higher FA digestion result in period 1 in this study. Hence, we observed higher TAG concentration in the UNSAT treated group. Still, this explanation does not apply to the TAG result in period 2.

Milk Fatty Acids

In our study, UNSAT increased CLA in milk, indicating increased CLA production in the rumen, and thus, decreased milk de novo FA yield, consistent with other studies (Ahnadi et al., 2002; Peterson et al., 2003; Harvatine and Bauman, 2006). UNSAT did not alter preformed milk FA yield. These effects on de novo and preformed FA are consistent with the biohydrogenation theory of MFD (Griinari and Bauman, 2006). UNSAT also decreased the yield of C16 FA, but this is likely because the SAT diet had a C16 FA supplement. When taking all components (de novo, mixed, preformed) into calculations on a g/100g milk fat basis, UNSAT reduced the de novo and mixed FA, but increased the preformed FA.

We also observed that UNSAT reduced the yield of saturated FA in milk by 27%, but did not alter the yield of unsaturated FA. On a concentration basis, UNSAT depressed saturated FA and increased unsaturated FA. The fact that UNSAT increased the concentration but not the yield of unsaturated FA is because UNSAT reduced milk fat yield. The decreased ratio of saturated FA to unsaturated FA with UNSAT treatment in this study was not consistent with previous studies

on the effects of CLA on milk saturated FA to unsaturated FA ratio. There are two reasons that CLA (and thus unsaturated fat supplements) alter saturated FA to unsaturated FA: 1) decreasedΔ-9 desaturase enzyme activity (Baumgard et al., 2000), and 2) decreased de novo FA synthesis. DecreasedΔ-9 desaturase activity would increase the saturated FA concentration, whereas decreased de novo FA synthesis would decrease saturated FA, because de novo FA mostly are saturated FA. In our study, we proposed that decreased de novo FA synthesis was more important than decreased desaturase activity. In the study done by Baumgard et al. (2001) and Peterson et al. (2002), increased saturated FA to unsaturated FA ratio along with higher CLA concentration only happened with a high CLA feeding or infusion dose. In our study, CLA was not fed but instead was only a byproduct of the rumen biohydrogenation pathway and rumen CLA outflow likely was less than the feeding or infusion dose discussed by Baumgard et al. and Peterson et al. As an indicator of the activity ofΔ-9 desaturase, the ratio of C14:1 to C14:0 + C14:1 was lower in UNSAT (0.07 for UNSAT vs. 0.08 for SAT; $P<0.01$). However, this C14:1 to C14:0 + C14:1 ratio was 0.05-0.06 in high dose CLA infused cows with control ratio 0.08 in the study done by Baumgard et al. (2001). The decrease of C14:1 to C14:0 + C14:1 ratio was 25% in the CLA infusion study while it was only 12% in our study, which confirmed that Δ-9 desaturase activity was not really inhibited by the low CLA concentration in our study.

In our study, UNSAT increased plasma insulin concentration by 8% but did not alter yield of preformed FA. This at first seems contradictory to other studies. Corl et al. (2006) observed reduced yield of preformed milk FA with increased plasma insulin concentration using a hyperinsulinemic-euglycemic clamp. Winkelman and Overton (2013) injected long-acting insulin and observed reduced performed milk FA. As they proposed, the reduced preformed milk FA yield was due to the inhibitory effect of insulin on lipolysis in adipose tissue, and the

partitioning of TAG into adipose tissue instead of mammary glands (Corl et al., 2006; Winkelmand and Overton, 2013). In our study, however, we observed not just higher insulin with UNSAT but also increased plasma TAG concentration. This increased plasma TAG was likely due to increased FA digestibility in the UNSAT diet, at least in period 1. Digested TAG, entering the bloodstream as chylomicrons, is taken up by the mammary glands, and is the largest contributor to the preformed FA in milk (Wattiaux and Grummer, 2003). Hence dietary FA source might have influenced preformed milk FA more significantly than insulin regulation.

Digestibility

Many studies reported that unsaturated fat had no effect on total tract DM digestibility (DePeters et al.,1987; Palmquist, 1991; Wu et al., 1994), which agrees with results of this study. Ruminal and total tract NDF digestibility also were not altered in most of studies (Pantoja et al., 1994; Pantoja et al. 1996; Drackley and Eliot, 1993;). Oldick and Firkins (2000) however, observed that unsaturated fat decreased ruminal NDF digestibility; they proposed that unsaturated fat increased acetate production and decreased butyrate as a proportion of total volatile fatty acids, which affected ruminal protozoa populations, and depressed NDF digestibility. Zinn (1989) and Pantoja et al. (1994) found that unsaturated fat decreased ruminal NDF digestibility, but digestion site in the hindgut compensated for this so that total tract NDF digestibility was not altered in their studies. Bateman and Jenkins (1998) found that soybean oil had no effect on total tract NDF digestibility, and they proposed that this lack of effect was because they fed a high fiber diet. Considering that we fed low NDF (~25%) in our basal diet, we expected that soybean oil would reduce NDF digestibility, as it tended to do. Total tract NDF digestibility was lower in our study than expected because of the high starch diet (Pirondini et al., 2015). We intentionally fed high starch diets in this study to cause MFD conditions. The low

NDF content along with high starch likely resulted in overall low NDF digestibility and, when combined with soybean oil, decreased NDF digestibility further.

Our data of total tract FA digestibility is not easy to interpret since FA digestibility was reduced by UNSAT in period 1 but not in period 2. However, the effect of FA saturation on FA digestibility in the literature is also not consistent. Oldick and Firkins (2000) and Doreau and Chilliard (1997) observed that total tract FA digestibility decreased as the degree of unsaturation of supplement fat increased. In contrast, Wu et al. (1991) found no effect of the degree of unsaturated of fat supplements on total tract FA digestibility. Interaction of fat source and period on total tract FA digestibility could be explained by the change in C16 and C18 FA digestibility in our study. In period 1, C16 digestibility was higher in UNSAT group, while no significant difference was detected for C18 digestibility; hence total FA digestibility was higher in UNSAT group. In period 2, C16 digestibility was still higher in UNSAT group but C18 digestibility was reduced by UNSAT supplement, hence total FA was not affected by the UNSAT treatment. Piantoni et al. (2013) reported palmitic acid reduced C16 FA digestibility, which is consistent with the result in our study that SAT containing palmitic fat reduced C16 digestibility. We propose that C18 digestiblity was reduced in period 2 for the same reason, as Piantoni et al. (2015) showed that C18 FA supplement decreased C18 FA digestibility, and our UNSAT fat supplement was soybean oil, which contains mostly C18 FA. However, we cannot explain why C16 and C18 digestibilities were different for the two periods.

Energy Partitioning

We measured the NE_L concentration of our diets based on energy output for milk production, body tissue gain and maintenance, as described by Boerman et al. (2015). However, we also calculated diet NE_L using nutrient digestibility and NRC (2001) equations. Since we did

not measure digestibility of non-fiber carbohydrate (NFC) and crude protein (CP) at the production level, NFC digestibility and CP digestibility were calculated based on the equations from NRC (2001). The equations used are as follows.

Equation 5-1: $TDN_{1x\ fat\ adjusted}$= TDN_{1x} -（EE%-3） x FA_d x 2.25

where TDN_{1x} was TDN value at maintenance intake level while diet ether extract level at 3% , EE% was ether extract percentage for each diet from Spartan Dairy 3.0 and FA_d was the FA digestibility for each individual cow during each treatment period.

Equation 5-2: MM = ($TDN_{1x\ fat\ adjusted}$ x DMI)/ (0.035 x $BW^{0.75}$)

where MM was multiple maintenance level, $TDN_{1x\ fat\ adjusted}$ was diet fat adjusted TND_{1x}, DMI was average DMI for each individual cow during each treatment period and BW was the average BW for each individual cow during each treatment period.

Equation 5-3: DD%=100- ((0.18 x $TDN_{1x\ fat\ adjusted}$-10.3) x (MM-1))/ $TDN_{1x\ fat\ adjusted}$

where DD% was digestibility discount%, $TDN_{1x\ fat\ adjusted}$ was diet fat adjusted TND_{1x} and MM was multiple maintenance level.

Based on the three equations above, we could get the digestibility discount coefficient at each intake (multiple maintenance) level for each cow. 93% was used to calculated apparent digestibility from true digestibility via Spartan Dairy 3.0 for each diet (A or B) (NRC2001). Using the digestibility discount coefficient and 93% value from NRC, we calculated the NFC and CP digestibility at production level for each cow.

Equation 5-4: DE_p (Mcal/kg)= NFCd/100 x 4.2 + NDFd x 4.2 + CPd x 5.6 +

FAd x 9.4 – 0.3

where DE was digestible energy of feed, NFCd was calculated apparent non-fiber carbohydrate digestibility as a percent of DM, NDFd was lab value for apparent neutral detergent fiber

digestibility as a percent of DM, CPd was calculated apparent crude protein digestibility as a percent of DM, FAd was lab value for apparent fatty acid digestibility as a percent of DM.

Equation 5-5: ME_p (Mcal/kg) = (1.01 x DE_P – 0.45) + 0.0046 (EE-3)

where ME_P was metaboliziable energy of feed, DE_P was digestible energy of feed and EE was ether extract percentage for each diet from Spartan Dairy 3.0.

Equation 5-6: NE_{LP} (Mcal/kg) = 0.703 x ME_p –0.19+ ((0.097 x ME + 0.19)/97) x (EE -3)

where NE_{LP} was net energy of feed, ME_P was metabolizable energy of feed and EE was ether extract percentage for each diet from Spartan Dairy 3.0.

The NE_L values for diets based on equations above was much lower than the NE_L values using the method described by Boerman et al. (2015). The reason was that calculated apparent digestibility of NFC (adNFC) was only ~78%, much lower than lab value from other studies (Boerman et al., 2015; Potts et al., 2015), which could cause the low calculated DE, hence ME and NE values. Correlation coefficient between the two sets of NE_L values using these two methods was 0.77, which means half of the production performance could be account for by the in vivo digestibility value.

The available energy from feed must first fulfill the energy requirements of maintenance and pregnancy before it can be partitioned to milk production and body tissue gain (NRC 2001). In our study, energy intake was not different between groups and energy used for maintenance was equal. However, cows fed UNSAT partitioned more energy towards body tissue gain, while cows fed SAT partitioned more nutrients towards milk production.

Treatment did not alter gross energy efficiency, defined as NE_L for production (milk and body tissue gain) as a fraction of NE_L intake. However, UNSAT partitioned more energy towards BW gains instead of milk, and therefore decreased energy efficiency for milk production.

Cows fed UNSAT gained 0.44 kg/d, whereas those fed SAT gained 0.19 kg/d. Thus UNSAT gained 0.27 kg/d more than SAT. Interestingly, no change in BCS or back/rib fat was seen during the study; therefore I suggest that the increased BW gain of UNSAT was due to gain in muscle, internal fat or rumen content. New procedures are needed to measure internal fat.

The 2 Mcal/d not used for body tissue gain in the SAT group was used for milk production. Insulin plays a key role in mediating energy partitioning because it inhibits mobilization of body fat and thus indirect decreases the availability of substrate for milk fatty acids (Hart et al., 1977). Unsaturated fat could alter partitioning by altering insulin secretion and also by altering DMI and nutrient digestibility (Harvatine and Allen, 2006). In our study, UNSAT decreased the total DM digestibility and NDF digestibility, but increased insulin, which would be consistent with increased partitioning to body fat. This is also consistent with results of Tyrrell and Moe (1972), who observed decreased efficiency during MFD, when efficiency is expressed as milk energy per unit of dietary ME.

To understand the effects of insulin and CLA on milk fat depression and nutrient partitioning independently, a comparison of our results with those of Boerman et al. (2015) is helpful. Treatments in Boerman et al differed in starch, whereas ours differed in fat saturation. The high starch diet in Boerman et al. (2015) and the UNSAT diet in our current study increased plasma insulin concentration (33% and 8%, respectively) and *trans*-10 C18:1 yield in milk (44% and 56%, respectively). However, Boerman et al. (2015) observed only a small increase of *trans*-10, *cis*-12 CLA in the high starch treatment group. In contrast, we observed a 69% increase of *trans*-10, *cis*-12 CLA in the UNSAT group. The increase of plasma insulin concentration was small in our study and thus did not help explain the increased BW gain and energy partitioning results. Even though the insulin, *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA responses were much

different, the depression in milk fat and the gain in body tissue caused by the MFD diets were similar when comparing these two studies (table 5-1). Boerman et al. (2015) observed no difference in DMI and energy intake, with an increase of milk yield but a decrease in milk fat yield, along with an increased BW and BCS gain in the high-starch feeding group. In our study, treatment did not alter DMI, energy intake, milk yield and BCS gain, but we saw a larger reduction in milk fat yield with the UNSAT diet; the effect on partitioning was consistent with Boerman et al. (2015). Our current study further explored the relationship between insulin and *trans*-10, *cis*-12 CLA in terms of nutrients partitioning effect, however, their effects are still confounded with each other due to the increase of both; therefore, differentiating the percentage of energy partitioning mediated by either insulin or CLA isomers is still difficult in our current study. Further studies are required to further examine the direct relationship between CLA and insulin and differentiate the effect on nutrient partitioning of each of them.

APPENDIX

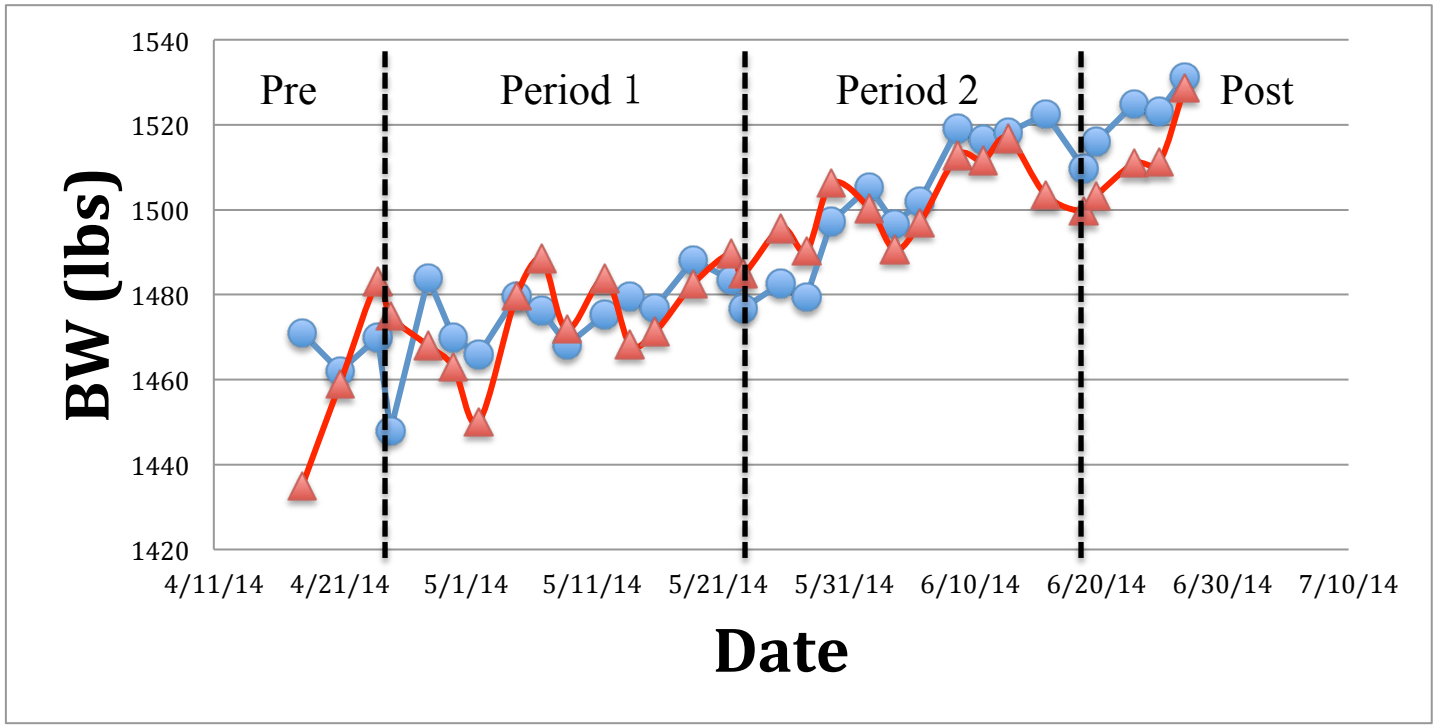

Figure 1: Mean cohort BW vs. date of experiment for cows to determine gut fill effects on BW change. Closed triangle represents mean BW for cows in cohort 1 fed the control diet in preliminary period, UNSAT in period1, SAT in period 2 and the control diet in post period. Closed circle represents mean BW for cows in cohort fed the control diet in preliminary period, SAT in period 1, UNSAT in period 2 and the control diet in post period.

Table 4-1: Major results comparison between Boerman et al. (2015) and current study[1]

	Boerman et al. (2015)[2]		Current Study[3]	
	HFF	HS[4]	SAT	UNSAT[5]
Insulin, µg/L		33% ↑		8% ↑
trans-10 C18:1		44% ↑		56% ↑
trans-10, *cis*-12 CLA, g/d		-		69% ↑
DMI, kg/d		-		-
NE_L, Mcal/kg		-		-
Milk Yield, kg/d		3% ↑		-
Milk Fat, kg/d		7% ↓		18% ↓
De novo FA, kg/d		16% ↑		22% ↓
Preformed FA, kg/d		-		-
BW Gain, kg/d		136% ↑		142% ↑
BCS Gain, pt/28d		250% ↑		-
NE for Milk, Mcal/d		1% ↓		7% ↓
NE for Body Tissue Gain, Mcal/d		151% ↑		138% ↑

[1] All numbers denote statistical significance at $P<0.05$.
[2]Treatments were either a high fiber and fat diet (HFF) diet containing a palmitic acid enriched FA supplement at 2.5% DM basis or a high starch diet (HS) diet containing a mixture of dry ground and high moisture corn.
[3]Treatments contained 2.5% added palmitic acid enriched triglyceride (SAT) or a diet containing 2.5% soybean oil (UNSAT) on a DM basis.
[4] Following comparisons are the results caused by HS, compared to HFF.
[5] Following comparisons are the results caused by UNSAT, compared to SAT.

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CHAPTER 5

CONCLUSIONS

As we found in this study, feeding diets differing in fat saturation with same level of starch, forage, crude protein and energy intake resulted in differences in production performance and energy partitioning in mid-late lactation dairy cows. The diet containing unsaturated fat supplements (soybean oil) decreased ECM and 3.5% FCM due to the depressed milk fat yield and concentration while the diet containing saturated fat supplements (palmitic-acids enriched triglyceride) increased milk protein yield and concentration. Most of the depressed milk fat was due to the de novo FA synthesis depression caused by higher biohydrogenation in the cows fed unsaturated fat, as indicated by the higher concentration of CLA isomers and *trans*-10, C18:1 in milk. With a 69% increase of milk *tran*-10*, cis*-12 CLA yield and a 8% increase of plasma insulin concentration, we observed a similar energy partitioning result with the study of Boerman et al. (2015), even though they observe little increase in CLA and a huge increase of plasma insulin and *trans*-10, C18:1 in the high starch diet. The higher portion of energy partitioning to body tissue gain instead of milk production indicated that plasma insulin and the rumen biohydrogenation intermediates are both important energy partitioning mediators, or more specifically, favoring more nutrients/energy storing in body tissue but not towards mammary glands. CLA isomers were more important mediators of energy flow in this study than was plasma insulin; however, the relationship between CLA and insulin is still not clear and requires further investigation.

CHAPTER 6

IMPLICATIONS

Based on our work, I suggest that if cows in early lactation are fed fat, the fat should be mostly unsaturated fatty acids. Unsaturated fat would help to minimize negative energy balance. However, unsaturated fat also reduced milk yield in this study, which is not consistent with the goal of maximizing milk production in lactation. Therefore I suggest that early lactation cows be fed higher starch diets, which would promote milk production as well as insulin secretion, which minimize the body condition loss, as shown in Boerman et al. (2015). For cows in mid to late lactation, if fats are fed, I suggest that the fat be mostly saturated, to promote partitioning towards milk instead of body tissues. These diets would prevent overfattening and improve health in the next parturition, maximize feed efficiency and maximize profitability.

Based on our work, I also suggest that future studies on the effect of feeding fats should measure the concentrations of CCK, GLP-1and propionate in blood. New methods to measure the mass of internal fat must be explored and applied. Finally, I think it is important to determine if changes in BW are the results of body tissue gain or rumen fill.