EFFECTS OF FEEDING SATURATED FAT SUPPLEMENTS ON PRODUCTION AND METABOLIC RESPONSES IN LACTATING DAIRY COWS

By

Paola Piantoni

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ABSTRACT

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Long-chain saturated fat supplements are used to increase yield of milk fat or improve energy balance in dairy cows. However, production responses to saturated fat supplements vary greatly, and therefore, it is currently not clear when these supplements should be fed. Our central hypothesis is that saturated fat supplements will have different effects on production responses depending on milk yield of dairy cows and composition of diets. In the first two experiments we evaluated the effects of palmitic (99% purity) and stearic (98% purity) acids on production and metabolic responses of past-peak dairy cows, compared with a diet with no supplemental fat. In both experiments, cows with a wide range of milk production were used to determine if cows at different levels of production responded differently to treatment diets. The first experiment showed that palmitic acid increased yields of milk (46.0 vs. 44.9 kg/d, $P = 0.04$), milk fat (1.53 vs. 1.45 g/d, $P < 0.01$), and 3.5% fat-corrected milk (3.5% FCM; 44.6 vs. 42.9 kg/d, $P < 0.01$) compared with a control diet with no supplemental fat, and that all cows responded similarly to treatment. The second experiment showed that stearic acid increased DMI (26.1 vs. 25.2 kg/d, $P = 0.01$) and yields of milk (40.2 vs. 38.5 kg/d, $P = 0.02$), milk fat (1.42 vs. 1.35 g/d, $P < 0.01$), and 3.5% FCM (40.5 vs. 38.6 kg/d, $P < 0.01$), with a greater response for high yielding cows (linear interaction $P < 0.10$). However, recovery of additional fatty acids consumed as additional yield of milk fatty acids was only 16.6% for palmitic acid and 8.2% for stearic acid supplementation. The third and last experiment evaluated the potential for a highly saturated

fatty acid supplement (FAT; >85% saturated FA; 46% stearic acid and 37% palmitic acid) fed at two levels of dietary forage NDF (fNDF; 20 and 26%) to improve metabolic status and energy balance in fresh cows. Regardless of dietary fNDF content, supplementation of FAT increased DMI (23.6 vs. 22.2 kg/d, $P = 0.04$) and tended to decrease milk yield (46.6 vs. 49.7 kg/d, $P = 0.10$), improving energy balance (-12.0 vs. -17.3 Mcal/d, $P < 0.01$) and decreasing BCS loss (0.7 vs. 0.9, $P = 0.02$) when fed during the first 4 wk postpartum. However, postpartum supplementation of FAT interacted ($P = 0.10$) with fNDF concentration for 3.5% FCM yield when cows were fed a common diet from 5 to 10 wk postpartum: FAT decreased 3.5% FCM yield in the 20% fNDF diet (51.1 vs. 58.7 kg/d), but not in the 26% fNDF diet (58.5 vs. 58.0 kg/d). Supplementation of saturated fats might benefit lactating dairy cows in some cases but results are dependent upon fat supplements fed, diet, stage of lactation, and milk yield of cows. Further work is needed to clarify these situations as well as the marginal economic return, if any, of specific fat supplements for different situations.

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LIST OF ABBREVIATIONS

ADF	Acid-detergent fiber
AUC	Area under the curve
BCS	Body condition score
BHBA	ß-hydroxybutyrate
BW	Body weight
CCK	Cholecystokinin
CONT	Control treatment
CP	Crude protein
DE	Digestible energy
DEI	Digestible energy intake
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
EBal	Energy balance
ECM	Energy-corrected milk
EDTA	Ethylenediaminetetraacetic Acid
F:C	Forage to concentrate ratio
FA	Fatty acids
FAME	Fatty acid methyl esters
FAT	Highly saturated free FA suplement
FAYR	Fatty acid yield response
FCM	Fat-corrected milk

FE	Feed efficiency
FFA	Free fatty acids
fNDF	Forage neutral-detergent fiber
GL	Galactolipids
GTT	Glucose tolerance test
HOT	Hepatic oxidation theory
iNDF	Indigestible neutral-detergent fiber
ITT	Insulin tolerance test
LDL	Low-density lipoproteins
LPL	Lipoprotein lipase
ME	Metabolizable energy
MEq	Mature equivalent milk production
MFD	Milk fat depression
MUN	Milk urea nitrogen
NDF	Neutral-detergent fiber
NEFA	Non-esterified fatty acids
NEL	Net energy of lactation
OM	Organic matter
PA	Palmitic acid treatment
pMY	Preliminary milk yield
PP	Post-partum
SA	Stearic acid treatment
SCC	Somatic cell count

SH	Soyhulls treatment
TG	Triglycerides
TMR	Total-mixed ration
VLDL	Very low-density lipoproteins

INTRODUCTION

Long-chain saturated fats have been used to increase the energy density of diets (Wang et al., 2010) and yield of milk fat (Steele and Moore, 1968; Steele, 1969; Wang et al., 2010) in dairy cows and have been reported to increase feed efficiency (FE, Wang et al., 2010; Lock et al., 2013) and milk yield (Steele, 1969; Enjalbert et al., 2000). Moreover, they are generally considered to be inert in the rumen (Grummer, 1988; Schauff and Clark, 1989), and have little effect on DMI (Allen, 2000) and nutrient digestibility (Grummer, 1988; Schauff and Clark, 1989; Elliott et al., 1996). However, production responses to highly saturated fats (≥ 85 % saturated) have varied greatly. This variability across experiments could be due to different types of fat supplements, rates of feeding, diets, and physiological state of cows. Fat supplements vary in chain length, degree of esterification, and saturation. Feeding rates have also varied widely across experiments from less than 2 % (Wang et al., 2010) to greater than 5 % of diet DM (Mosley et al., 2007). In addition, production responses may vary across experiments from interactions between the fat supplemented and diet composition. For instance, for cows in early lactation, fat increased energy partitioned to milk when supplemented in a diet with a low forage to concentrate ratio (F:C), but increased energy partitioned to body reserves when supplemented in a diet with a high F:C (Weiss and Pinos-Rodriguez, 2009). Variability across experiments might also be related to the level of milk production of the cows used. Harvatine and Allen (2005) showed that supplementation of saturated FA compared with a more unsaturated FA increased milk protein yield to a greater extent for high producing cows than lower producing cows. Furthermore, a palmitic acid supplement tended to increase milk yield overall in a field study, but lower producing cows responded better than higher producing cows (Warntjes et al., 2008). Because of the

inconsistent responses to feeding saturated fats, it is currently not clear when these supplements should be fed and to what extent their use can increase profitability of dairy farms.

Few studies have reported the use of highly pure saturated FA, and responses measured were related to milk yield, composition, and FA analysis. To our knowledge, there are no studies that have evaluated effects of supplementation of pure palmitic or stearic acid supplements on digestion, metabolic, and production responses in lactating dairy cows or how responses vary with level of milk production. Moreover, data regarding the interaction between a mixture of these FA and dietary fNDF during the immediate post-partum period are lacking. We expect that this research will allow nutritionists to strategically use currently available saturated fat supplements on farms to increase profitability.

Our objectives are to determine the effects of key, highly pure saturated FA in past-peak cows varying in milk yield and of a commercially available mixture of these FA (≥85 % saturated) in diets differing in fNDF fed to dairy cows in the immediate post-partum period. Our central hypothesis is that saturated FA supplements will have different effects on production responses depending on physiological state of dairy cows and composition of diets.

CHAPTER 1

LITERATURE REVIEW

Why feed fats to dairy cows?

Fats are fed to dairy cows to increase yields of milk (Steele, 1969; Enjalbert et al., 2000) and milk fat (Steele and Moore, 1968; Steele, 1969; Wang et al., 2010), increase energy density of diets (Wang et al., 2010), maintain milk yield under heat stress conditions (Wang et al., 2010), and improve energy balance (Weiss and Pinos-Rodriguez, 2009), reproductive performance (Staples et al., 1998), and feed efficiency (Wang et al., 2010; Lock et al., 2013). Even though the gross energy content of lipids is almost twice that of proteins and carbohydrates, lipids should not be used to increase energy density of diets without considering their various physiological effects. Lipids are primarily composed of fatty acids (FA) and different FA might have different effects on energy partitioning, intake, and production responses at different stages of lactation.

Digestion and metabolism of fats

In diets with no supplemental fat, sources of FA are forages and cereal grains. Forages contain mainly glycolipids (GL), which are composed of a molecule of glycerol, two FA, and a carbohydrate, while cereal grains contain mainly triglycerides (TG), which are composed of one molecule of glycerol and three molecules of FA (Jenkins and Harvatine, 2014). Fatty acid molecules contain a carboxylic acid group (-COOH) and an aliphatic chain ($-CH_n$), and can be classified by their chain-length and level of saturation. Most of the FA consumed by the dairy cow are long-chain (≥16C in length) and unsaturated (at least one

double bond between carbons of the aliphatic chain). Diets with no supplemental fats will normally contain large proportions of 18-carbon FA (linoleic acid (C18:2) would predominate in grains and linolenic (C18:3) acid in forages) and a smaller proportion of C16:0 (palmitic acid).

Once in the rumen, TG and GL undergo hydrolysis (cleavage of the FA or carbohydrate groups from glycerol) and FA undergo different degrees of biohydrogenation (saturation of the double bonds in FA; Harfoot, 1981; Jenkins, 1993). Ruminal bacterial enzymes perform both processes in a very efficient manner when conditions are appropriate. Conditions that might negatively affect the degree of biohydrogenation and increase exit of unsaturated FA to the duodenum are increased ruminal concentration of unsaturated FA, decreased ruminal pH, and the presence of ionophores (Jenkins and Harvatine, 2014). Triglycerides and GL that escape the rumen can be hydrolyzed by intestinal and pancreatic lipases in the small intestine, while unsaturated FA that escape biohydrogenation will be absorbed in the small intestine with other FA (Noble, 1981). As a consequence of biohydrogenation, most of the FA that reach the small intestine and are available for absorption are saturated FA. Due to FA profiles of dairy feeds, most FA reaching the small intestine for absorption will be stearic acid (C18:0) and palmitic acid when no supplemental fat is being fed (Bauman, 2003; Loften et al., 2014).

Once the chyme has reached the duodenum, it is mixed with bile and pancreatic juice. The bile will supply the bile acids and the lecithin and the pancreatic juice the phospholipases and bicarbonate required to increase pH. Phospholipases will transform the lecithin into lysolecithin and this, together with the bile salts, will desorb the FA from the feed particles and bacteria and emulsify them into micelles, structures with a hydrophobic

core and a hydrophilic coat (Davis, 1990; Bauman et al., 2003). The micelle is of extreme importance for FA absorption, since it will allow the FA to be absorbed through the mucosa of the small intestine, by making their hydrophobic aliphatic chain "soluble" in an aqueous layer of the intestinal cells (Loften et al., 2014). Fatty acids will then enter the enterocyte by simple diffusion. Absorption of these FA will occur predominantly in jejunum; there is no significant absorption of long-chain FA in the rumen-reticulum, omasum, or abomasum (Noble, 1981). In a summary of studies where intestinal digestibility of FA was measured, Lock et al. (2006) reported that total FA digestibility was ~74% and suggested that differences in digestibility among different individual FA did not contribute significantly to the variation among studies published.

Once in the enterocyte, FA are esterified into TG and associated with phospholipids, fat-soluble vitamins, cholesterol (originally from bile), and apoproteins into chylomicrons and very low-density lipoproteins (VLDL) that leave the enterocyte through pinocytosis into the lacteal and lymphatic circulation, which will ultimately end in the cranial vena cava (Noble, 1981). Therefore, chylomicrons and VLDL originated in the small intestine will access general circulation before they pass through the liver.

In extra-hepatic tissues, the enzyme lipoprotein lipase (LPL), that is located in the cell surface of endothelial cells, hydrolyzes TG contained in chylomicrons and VLDL and make FA available to go through the endothelial cell into interstitial space and then into the tissue cells (Moore and Christie, 1981). In muscle, FA from TG hydrolyzed by LPL will be oxidized for energy (Bell, 1981). In the mammary gland, long-chain FA will go through processes of desaturation and esterification into TG, to finally be translocated into milk fat droplets and secreted into the milk (Moore and Christie, 1981). Non-esterified FA (NEFA)

might also be a source of long-chain FA in milk, especially during periods of negative energy balance such as the post-partum period, when NEFA concentrations in blood are higher than normal (Kronfeld, 1965). In the liver, FA in chylomicrons and LDL, but mainly free FA, will be oxidized or repackaged into VLDL for subsequent transport in blood. In the liver, FA can be oxidized completely to carbon dioxide and water, incompletely to ketone bodies, or esterified to glycerol to form TG. If production of TG overcomes their export as VLDL, TG will accumulate in vacuoles in the hepatocyte, potentially affecting liver function (Bell, 1981).

Fats supplemented to dairy cows

Fat supplements available to use in dairy rations include specialty fats, sometimes referred to as rumen-inert fats (e.g. supplements high in palmitic acid (≥85% C16:0), highly saturated FA supplements with a mixture of FA (≥85% saturated; mainly composed of palmitic and stearic acids), calcium salts of unsaturated FA (either from palm or soybean oil) and commodity fats with variable degrees of hydrogenation (e.g. tallow, yellow grease, vegetable oils). Feed ingredients that can be used to increase FA content in dairy diets include extruded or whole oilseeds (e.g. cottonseeds, soybeans, canola) and by-products of corn milling (e.g. corn distillers). Specialty fats, non-hydrogenated commodity fats, and oilseeds have a more constant FA content and profile, while partially hydrogenated commodity fats will have a more variable FA profile (NRC, 2001) and corn distillers a more variable FA content (Hollman et al., 2011b).

Fat supplementation in dairy cow diets

In general, total fat in dairy diets will range from 3-7% of diet DM (NRC, 2001). The fat content of a diet with no supplemental fat will range from 2-3% of diet DM, and will be provided by cereal grains and forages. Therefore, a total of 3-4% of diet DM could be added as supplemental fat (NRC, 2001); half of the supplemental fat could be from feeds with elevated fat content (e.g. whole oilseeds) while half could be from specialty fats (commercial products with various degrees of rumen-protection). Even though positive responses to supplemental fats have been reported in the literature, production responses to fats in general, but also to highly saturated fats (≥ 85% saturated) in particular, have varied greatly (Rabiee et al., 2012). This variability across experiments could be due to different types of fat supplements (i.e. FA profile, esterification, degree of rumen-protection), rates of feeding, dietary ingredients, and physiological states of cows. In an extensive review of literature, Loften et al. (2014) concluded that palmitic and stearic acids might have specific and complementary roles in dairy cow metabolism, which might explain some of the differences observed when different FA are fed. For example, they suggested that since palmitic acid is the primary FA found in milk fat and its transfer to milk is usually higher than stearic acid, its supplementation could increase milk fat yield in lactating cows. In addition, stearic acid does not appear to be taken up by the ruminant liver (Mashek and Grummer, 2003) which would increase its availability for oxidation in extra-hepatic tissues and/or for secretion into milk, and therefore, stearic acid enriched fats could be fed in periods of negative energy balance without overloading the liver with FA. Sato et al. (2004) showed that the proportion of palmitic and oleic acids in liver increases in cows with liver lipidosis, compared with

healthy cows, but the opposite occurs for stearic acid, which is in agreement with results from perfused liver goats presented by Mashek and Grummer (2003).

Why focus on saturated long-chain free FA?

In an extensive summary of the literature, Allen (2000) showed that DMI decreased as FA content of the diet increased with the addition of unprocessed animal fat and Ca-salts of palm FA. The Ca-salts of palm FA decreased DMI 2.5% for each percentage unit increase in dietary FA over the control while the depression in DMI from addition of unprocessed animal fat was approximately half that of Ca-salts of palm FA. In addition, the effect of oilseeds on DMI was quadratic, with a maximum decrease in DMI with the addition of 2% of FA from this source. Hydrogenated fats did not have an effect on DMI when all studies were considered. When studies that included diets with >6% total FA content were excluded, hydrogenated fats affected DMI response quadratically, with the greatest decrease in DMI at inclusion of 2.3% of FA from this fat source.

To evaluate production responses to increasing proportion of unsaturated FA in the diet, Harvatine and Allen (2006) fed past-peak cows the following treatments in a 4x4 Latin square design experiment: control with no supplemental fat, a highly saturated long-chain FA supplement, a partially unsaturated long-chain FA supplement, and a supplement of intermediate level of saturation (combination of the highly saturated and partially unsaturated long-chain FA supplements). They showed that as level of unsaturation of the FA supplemented increased, DMI and 3.5% fat-corrected milk (FCM) also decreased, and that supplementation of 2.5% of Ca-salts of palm FA decreased DMI by 12% and 3.5% FCM by 16%. Not only the level of saturation of the FA, but also its chain-length might affect

production differently. Hollman et al. (2011a) evaluated two FA supplements that varied in FA chain-length and showed that coconut oil (>90% saturated FA; ~60% medium chain FA) decreased DMI by 18% compared with a highly saturated free FA supplement (≥85% long-chain FA) regardless of production level.

While unsaturated FA might affect ruminal fermentation (Jenkins and Jenny, 1992), saturated FA are usually considered to be inert in the rumen (Grummer et al., 1988; Schauff and Clark, 1989; Jerred et al., 1990; Jenkins and Jenny, 1992). Even though corn oil supplemented at 400 g/d depressed fiber and OM digestibility in 272 kg steers (Ward et al., 1997), total-tract OM and ADF digestibility was not affected by the addition of a prilled saturated fat supplement or canola oil included at 5% of diet DM in dairy cow rations (Jenkins and Jenny, 1992). In an extensive review of literature, Palmquist and Jenkins (1980) favored the idea that vegetable oils might have potential negative effects on fiber digestibility due to an inhibitory effect of the unsaturated FA on ruminal microbes. However, Palmquist and Jenkins (1980) mentioned that most of the research that demonstrated a negative effect of fats on fiber digestion was performed in lambs at maintenance intakes, and therefore, needed to be taken with caution when extrapolating to dairy cows with higher intakes. Palmquist (1991) compared four different commercial fat supplements ranging from approximately 27 to 80% saturated FA (Ca-soaps of palm FA, animal-vegetable blend, hydrogenated tallow, saturated free FA, and tallow) at two different inclusion rates (2.9 or 5.7% of diet DM) in high forage diets (59% of diet DM) against a control diet with no supplemental fat and showed that fat supplements did not affect DM, OM, and NDF digestibility compared with the control diet. In addition, digestibility of FA was not different among fat sources, but FA digestibility decreased as FA intake increased (Palmquist, 1991). More recently, Harvatine

and Allen (2006) tested three different FA treatments varying in degree of saturation and concluded that FA supplementation did not affect total-tract digestibility of DM, OM, or NDF compared with the control diet. In the same experiment, FA digestibility was higher for unsaturated compared with saturated FA treatment. Unfortunately, even after numerous studies conducted over the past decades that evaluated the effects of different fat sources on nutrient digestibility, the notion that fat supplements in general and at the inclusion rates that are normally recommended (<3% of diet DM) can depress digestibility of nutrients persists.

Supplementation of highly-saturated long-chain FA by stage of lactation

Ample research has examined the use of different fats in dairy rations at different stages of lactation, but few studies evaluated the use of pure sources of FA in an attempt to explain variability across experiments. Since unsaturated FA have the potential to decrease DMI and 3.5% FCM and medium-chain FA might also decrease DMI, we decided to focus our research on long-chain saturated FA. We also chose to work with free FA and not triglycerides because previous research suggests that digestibility of FA might be negatively affected by the proportion of triglycerides in the FA supplement (Weiss et al., 2011).

Past-peak lactation cows

Saturated fat supplements are fed to lactating cows past peak lactation usually to increase milk fat output and feed efficiency. Many experiments have been conducted to evaluate responses to fat supplements, but results are inconsistent. When positive results are observed, they are small, questioning the profitability of saturated fat supplements in dairy operations with current supplement costs.

Production responses to long-chain saturated FA in past-peak lactation cows

Long-chain FA mixtures in dairy cow rations Palmitic and stearic acids are common FA in saturated fat supplements and other feeds consumed by dairy cows. Weiss et al. (2011) reported that a supplement high in palmitic and stearic acid (~80% saturated; 38.2% C16:0 and 41.2% C18:0) fed at 3% of diet DM did not affect DMI, milk yield, or milk protein yield but increased milk fat percent (3.94 vs. 3.59; $P < 0.05$) and yield (1.63 vs. 1.45; $P < 0.05$), and therefore, 4% FCM (41.4 vs. 38.2 kg/d; $P < 0.05$) compared with a diet with no supplemental fat (~32% NDF; ~31% starch). Wu et al. (1993) compared the same FA supplement fed at 2.5% of diet DM with a control diet with no supplemental fat (~32% NDF; ~23.5% starch) and reported that fat inclusion did not affect DMI, or milk protein yield, but increased 3.5% FCM (33.4 vs. 30.4 kg/d; $P = 0.05$) and milk fat yield (1.15 vs. 1.02; $P = 0.03$). Simas et al. (1998) also fed the same prilled saturated FA supplement at 2.5% of diet DM in diets that contained either dry-rolled sorghum or steam-flaked sorghum (~31% NDF; ~26.5% starch) and showed that fat supplementation did not affect DMI or BW change, but tended to increase milk protein yield and increased yields of milk (45.8 vs. 43.4; $P < 0.05$), milk fat (1.39 vs. 1.29; $P < 0.05$), and 3.5% FCM (42.4 vs. 39.7; $P < 0.05$) regardless of sorghum grain processing. Importantly, Simas et al. (1998) also evaluated the inclusion of 5% of the same FA supplement in a steam-flaked corn diet and did not detect any benefit of increasing the inclusion rate of the FA from 2.5 to 5% of diet DM. Fat supplementation increased feed efficiency by 9.9% ($P < 0.05$) in one of these studies (Weiss et al., 2011), but did not affect it in two (Wu et al., 1993; Simas et al., 1998). Even though fat supplementation did not affect digestibility of nutrients in two of the studies mentioned, it decreased 18-

carbon FA digestibility by ~8.5% compared with a control diet with no supplemental fat (Simas et al., 1998; Weiss et al., 2011).

Grum et al. (1996) evaluated the supplementation of prilled saturated FA (3% diet DM; mixture of palmitic and stearic acids) at two different levels of concentrate in the diets (~28 and 33% NDF). Overall, fat supplementation did not affect DMI, milk yield, 4% FCM, or milk fat and protein yields compared with a diet with no fat added. In addition, fat supplementation did not affect total-tract digestibility of OM, NDF, or total 16-carbon FA, but tended to decrease that of total FA and total 18-carbon FA. However, fat supplementation interacted with concentrate level of the diet for DMI and OM digestibility, which will be discussed in a later section of this review.

Palmitic acid in dairy cow rations Steele and Moore (1968) evaluated a highly pure (96%) palmitic acid supplement on production response for cows in mid-lactation, but milk yield of cows was low (~12 kg/d) and responses measured were limited. In that study, palmitic acid (fed at ~4% of diet DM; ~40:60 F:C) increased milk fat concentration (4.17% vs. 3.31%; $P < 0.001$) and milk fat yield (492 g/d vs. 404 g/d; $P < 0.01$), but did not affect milk yield (11.8 kg/d vs. 12.2 kg/d), compared with a control with no added fat (~50:50 F:C). In a later study, Steele (1969) showed that palmitic acid (85% pure, fed at ~4.25% of diet DM; ~55:45 F:C) increased not only milk fat concentration (4.53% vs. 4.01%; $P < 0.001$) and milk fat yield (661 g/d vs. 546 g/d; $P < 0.001$), but also milk yield (14.6 kg/d vs. 13.6 kg/d; $P < 0.001$), compared with a control diet with no added fat.

More recently, a FA supplement high in palmitic acid (~85 % pure) fed at 2 % of diet DM (~50:50 F:C) increased milk fat yield (1.32 vs. 1.23 kg/d), 3.5 % FCM (35.1 vs. 33.6

kg/d), and FE (1.51 vs. 1.38 3.5% FCM/DMI; all $P < 0.05$; Lock et al., 2013). The increase in FE was a result of increased 3.5 % FCM and a decrease in DMI (23.3 vs. 24.7 kg/d; $P < 0.01$) observed with fat supplementation. In a dose response experiment, Mosley et al. (2007) fed a palmitic acid supplement (>85% pure) at 0, 500, 1,000, and 1,500 g/d and showed that DMI was affected quadratically by fat supplementation ($P < 0.05$) with the 500 g/d inclusion rate increasing DMI compared with the non-supplemented diet (26.4 vs. 23.3 kg/d; $P < 0.05$); inclusion rates of 1,000 and 1,500 g/d did not affect DMI compared with the non-supplemented diet. In addition, it was reported that an inclusion rate of 500 g/d of palmitic acid in the diet increased milk yield (34.0 vs. 30.9 kg/d), milk fat yield (1.30 vs. 1.02 kg/d), and milk protein yield (0.97 vs. 0.88 kg/d; all $P < 0.05$) and higher inclusion rates did not provide greater responses compared with 500 g/d. In a more recent experiment, Rico et al. (2014) reported that a highly enriched source of palmitic acid (84% pure; fed at 1.9% of diet DM) did not affect milk yield or milk fat and protein yields in high- or low-producing cows (~48 and ~34 kg/d, respectively), but decreased DMI in both groups by ~2.1 kg/d, increasing FE in high-producing cows only (1.53 vs. 1.41 ECM/DMI; both $P < 0.05$) all compared with a diet with no fat added.

In general, supplementation of saturated fats has not affected OM digestibility. While a tendency for an increase in the digestibility of nutrients has been reported when a highly enriched palmitic acid supplement was fed in a field study (Warntjes et al. 2008), most experiments have found no effects of saturated fat supplementation on nutrient digestibility (Schauff and Clark, 1989; Grum et al., 1996; Harvatine and Allen, 2006c). However, no previous experiment has measured digestibility responses to a pure palmitic acid supplement.

In summary, when a highly enriched source of palmitic acid (~85 % pure) was compared with a control diet with no supplemental fat, there was usually a positive response in milk fat output (4 out of 5 experiments reported), but inconsistent effects on DMI, which was reduced in two studies (Lock et al., 2013; Rico et al., 2014) and increased in another study (at 500 g/d in Mosley et al., 2007) at similar inclusion rates (~2% of diet DM), but not affected when included at higher feeding rates of 1,000 or 1,500 g/d (Mosley et al., 2007).

Stearic acid in dairy cow rations Stearic acid is also commonly found in saturated fat supplements and, due to ruminal biohydrogenation, it is the FA that reaches the duodenum in greatest proportions when diets have no supplemental fats added. However, fewer studies have been reported with highly enriched stearic acid supplements, compared with palmitic acid supplements. Steele and Moore (1968) also evaluated a pure (94%) stearic acid supplement on production responses for cows in mid-lactation. They found that stearic acid (fed at ~4% of diet DM; ~40:60 F:C) increased milk fat yield (459 kg/d vs. 404 g/d; $P < 0.05$), but had no effect on milk fat concentration or milk yield compared with a control with no supplemental fat added (~50:50 F:C). In a later study, Steele (1969) showed that stearic acid (85% pure; fed at ~4.25% of diet DM; ~55:45 F:C) increased milk yield (14.2 kg/d vs. 13.6 kg/d; $P < 0.001$) and did not affect milk fat concentration or yield compared with a control diet with no supplemental fat added. Interestingly, and in the same experiment, stearic acid (85% pure) fed at half the inclusion rate previously mentioned (~2.1% of diet DM) increased not only milk yield (14.8 kg/d vs. 13.6 kg/d; $P < 0.001$), but also milk fat yield (585 g/d vs. 546 g/d; $P < 0.05$) compared with the control (Steele, 1969).

Palmitic acid vs. stearic acid in dairy cow rations Studies that compared palmitic acid with stearic acid generally report that palmitic acid has higher potential to increase milk fat yield than stearic acid. Duodenal infusions (500 g/d) of palmitic acid (98.6% pure) increased total FA in milk compared with a control (4.55 % vs. 3.57%; $P < 0.05$), while duodenal infusions of stearic acid (92.3% pure) did not (Enjalbert et al., 2000). However, total FA in milk were not different between stearic and palmitic acid duodenal infusions (4.09% vs. 4.55%). Unfortunately, Steele (1969) and Steele and Moore (1968) did not compare both FA treatments with each other, but only with a control diet with no supplemental fat added. In these studies, even though palmitic and stearic acids increased milk fat yield compared with the control diet, a greater increase was observed for palmitic acid. Recently, Rico et al. (2014) reported that a palmitic acid supplement (99% pure) increased milk fat percent (3.66% vs. 3.55%; $P < 0.05$), milk fat yield (1.68 vs. 1.59 kg/d; $P < 0.01$) and 3.5% FCM (47.46 vs. 45.56 kg/d; $P < 0.01$), but did not affect DMI, BW, BCS, milk yield, or milk protein yield compared with a stearic acid supplement (98% pure). These data indicate that individual FA may affect production responses differently.

Fatty acid yield response to additional FA intake Transfer efficiency of FA into milk is important to consider when feeding fat supplements. In a field study, Warntjes et al. (2008) reported that the palmitic acid supplement fed had a transfer efficiency of 16.5%. That means that for every 100 g of the palmitic acid supplement fed, they detected an increase of 16.5 g of palmitic acid in milk. However, higher transfer efficiencies have been reported for long-chain saturated FA supplements in the literature. Enjalbert et al. (2000), reported a transfer efficiency of 46.7% for palmitic acid and 12.0% for stearic acid after duodenal infusions,

while Lock et al. (2013) reported a transfer efficiency of 29.7% for a palmitic acid supplement fed in a TMR.

Metabolic responses to long-chain saturated FA in past-peak lactation cows Fat feeding has affected plasma metabolites and insulin concentrations differently across studies with cows past peak lactation. Enjalbert et al. (1998) continuously infused 500 g/d of palmitic or stearic acids in the duodenum of lactating cows, and even though they showed that individual FA infusions increased the corresponding FA in arterial blood, they did not affect their concentrations in venous blood or total FA in arterial or venous blood. In that experiment, palmitic acid increased its arterial concentration more than stearic acid did, compared with control. Choi et al. (2000) fed a FA supplement with a mixture of palmitic and stearic acids (at 8.1 % of diet DM) and also observed an increase in plasma NEFA and TG concentrations, but a decrease in plasma insulin concentration (all pre-feeding). These effects on plasma metabolites could be related to the fact that corn was removed from the basal diet to add the fat (decrease in insulin) and due to dietary fat addition itself (increase in NEFA). In contrast, Harvatine and Allen (2006) observed an increase in plasma insulin concentration, while Grum et al. (1996) reported no effect on plasma insulin but a tendency for the FA supplement to increase plasma NEFA concentrations. Consistent with Harvatine and Allen (2006), saturated FA were also reported to have an insulinotropic effect in perfused pancreas of fasted rats (Stein et al., 1997). Even though stearic acid was more insulinotropic than palmitic acid in rats (Stein et al., 1997), Rico et al. (2014) reported no difference in plasma insulin concentration in past-peak cows fed a 99% pure palmitic acid supplement or a 98% pure stearic acid supplement (both fed at 2% of diet DM). In that experiment, palmitic acid

increased plasma glucose and NEFA concentrations ($P < 0.05$), but did not affect plasma BHBA concentrations compared with the 98% pure stearic acid supplement.

Post-partum and early lactation cows

Adaptation to the rapid increase in energy demand at parturition is challenging for dairy cows. Many experiments have been conducted to evaluate the effects of fats in fresh and early lactation cows in an attempt to improve energy density of diets and energy balance during these periods of high metabolic demands, but few fed prilled saturated FA during the post-partum period alone. Several studies that evaluated supplementation of saturated long-chain FA during the post-partum period also fed these FA during the prepartum period, making it impossible to differentiate between pre- and post-partum effects (Moallem et al., 2007a and b; Petit et al., 2007) or started the experiments after day 1 post-partum (Carrol et al., 1990; Jerred et al, 1990; Weiss and P. Rodriguez, 2009).

Production responses to long-chain saturated FA mixtures in post-partum and early lactation cows Experiments that evaluated the inclusion of different saturated fat supplementation during the transition period and continuing in early lactation have shown negative effects on DMI pre- and post-partum. For example, Moallem et al. (2007a) showed that feeding a prilled saturated FA supplement (230 g/d; mixture of palmitic and stearic acids) during the close-up dry period until past-peak lactation (256 d pregnant to 100 DIM) decreased DMI pre-partum (11.4 vs. 12.1 kg/d), and increased DMI post-partum (25.1 vs. 24.3 kg/d) and milk yield (44.4 vs. 42.3 kg/d; both $P < 0.05$), but did not affect milk protein or fat yields compared with a control diet with no supplemental fat (~19% dietary fNDF).

However, when only the first 21 DIM where considered, feeding 230 g/d of prilled saturated fat during the transition period decreased DMI (18.4 vs. 19.8 kg/d) and calculated EBal (-4.16 vs. -1.71 Mcal/d; both $P < 0.05$), but did not affect yields of milk and milk components, BW, or BCS (Moallem et al. 2007b). Because of the decrease in DMI and no effect on milk yield, prilled saturated FA increased FE (2.13 vs. 1.86 3.5% FCM/DMI). Petit et al. (2007) fed the same FA supplement during the whole dry period (at 1.7% of diet DM) until 28 DIM (at 3.5% of diet DM) and showed that fat supplementation decreased pre- and postpartum DMI (12.1 vs. 13.4 kg/d and 14.6 vs. 18.1 kg/d, respectively) and milk yield (29.1 vs. 33.3 kg/d; all both $P < 0.05$), but did not affect milk components yield compared with a control with no added fat. However, with the objective of having isoenergetic diets, NDF of the control diet was 33.5% while NDF of the supplemented diet was 38.4%, and therefore, the decrease in DMI post-partum observed of 3.5 kg/d could have been due to an increase in the filling effect of the diet and not due to the fat supplementation alone. Other researchers evaluated metabolic adaptation to lactation when feeding a prilled saturated FA supplement during the dry period only and reported no effect on DMI, BW, or BCS pre-partum compared with a control diet with no supplemental fat (Andersen et al., 2008; Castañeda-Gutiérrez et al., 2009). In addition, fat supplementation did not have an effect on DMI, BW, BCS, or milk yield post-partum in those experiments.

Experiments in which prilled saturated FA supplements were fed immediately after parturition and for 3 to 4 wk postpartum only are rare. Fat could be supplemented during this period to increase energy density of the diet and allow the cow to reach a more positive energy balance sooner during a period when DMI is not sufficient to support lactation. Beam and Butler (1998) supplemented a prilled saturated FA (2.6% of diet DM) to fresh cows in a

33% NDF diet and reported no effect on DMI, yields of milk and 4% FCM, or net energy intake during the first 6 wk post-partum compared with a control diet with no supplemental fat. However, fat supplementation decreased DMI (15.5 vs. 17.3 kg/d; $P < 0.05$) and increased BW and BCS loss (-42.6 vs. -26.5 kg and -0.56 vs. -0.34, respectively; both $P < 0.05$) when only the first 4 wk post-partum were considered. In addition, an interaction between diet and week was detected for yields of 4% FCM and milk (both $P < 0.10$): fat supplementation decreased yields of 4% FCM and milk during the first 3 wk PP, but increased them during the last 3 wk on experiment compared with a control diet with no fat added (Beam and Butler, 1998).

Other experiments have evaluated saturated fat supplementation at different dietary forage levels early in lactation but not immediately post-partum. For example, Jerred et al. (1990) evaluated the supplementation of prilled saturated FA (5% of diet DM; mixture of palmitic and stearic acids) in diets with three different dietary NDF contents (25, 30, and 35%) from day 5 post-partum until 100 DIM. Overall, fat supplementation decreased DMI (22.1 vs. 23.6 kg/d, $P < 0.05$), but did not affect calculated energy intake or balance, milk yield, milk protein and fat yield, or BW change compared with a diet with no supplemental fat. An interaction between fat supplementation and week was detected for DMI ($P < 0.05$), and indicated that fat supplementation did not affect DMI during the first 2 wk post-partum, but depressed it during the rest of the experimental period (Jerred et al., 1990). Weiss and Pinos-Rodriguez (2009) fed the same FA supplement (at 2.25% of diet DM) but at two dietary fNDF levels (~17 and 25%) from 21 to 126 d post-partum and reported an interaction between dietary NDF and fat supplementation for energy partitioning: fat supplementation increased BCS with no effect on milk yield when supplemented in the high fNDF diet, but

increased milk yield with no change in BCS when supplemented in the low fNDF diet. Overall, fat supplementation did not affect production responses significantly (Weiss and Pinos-Rodriguez, 2009). Data from this experiment indicate that there is a potential for manipulating energy partitioning by altering dietary contents of fNDF and saturated fat in early lactation.

Metabolic responses to long-chain FA mixtures in post-partum and early lactation cows

When fat was fed only during the dry period, it did not affect glucose, but decreased insulin and increased NEFA pre-partum (Andersen et al., 2008). Post-partum, a prilled saturated fat supplement fed during the pre-partum period decreased NEFA and liver fat content in early lactation compared with a control diet with no supplemental fat; therefore, Andersen et al. (2008) concluded that fat supplementation could be a useful strategy to prime the cows for fat mobilization early in lactation. In a similar experiment, Castañeda-Gutiérrez et al. (2009) showed that prilled saturated fats did not affect glucose but decreased insulin pre-partum, consistent with findings reported by Andersen et al. (2008). However, supplementing prilled saturated fats during the close-up period did not affect plasma NEFA concentration or liver fat content during the transition period and they failed to detect any positive metabolic response to fat supplementation during the close-up period.

Moallem et al. (2007b) showed that prilled saturated FA fed during the transition period decreased calculated EBal (-4.16 vs. -1.71 Mcal/d) and plasma insulin (126 vs. 275 pg/mL; both $P < 0.05$), and increased plasma NEFA (608 vs. 423 μEq/L) and BHBA concentrations (6.2 vs. 4.5 mg/dL; both $P < 0.05$) post-partum. Fat supplementation also decreased plasma insulin (239 vs. 396 pg/mL) and increased plasma NEFA (333 vs. 232

μEq/L), and BHBA (4.0 vs. 3.1 mg/dL; all $P < 0.05$) concentrations during the dry period (Moallem et al., 2007a). In that experiment, plasma glucose concentration was not affected by fat supplementation either during the pre- or the post-partum period. Petit et al. (2007) showed similar results when feeding a prilled saturated FA during the dry period and for several weeks into lactation: fat supplementation decreased calculated EBal, plasma glucose and liver glycogen content, and increased plasma NEFA, BHBA, and liver total TG in multiparous cows after parturition compared with a diet with no fat added.

When fat was supplemented during the post-partum period only, it did not affect plasma concentrations of insulin, glucose, or NEFA during the first 4 wk post-partum, EBal over the first 6 wk post-partum, or days to EBal nadir or first ovulation (Beam and Butler, 1998). Moreover, Carrol et al. (1990) presented reproductive data from the experiment by Jerred et al. (1990), and reported that supplementation of prilled saturated FA during the early post-partum period did not affect days to positive EBal or first ovulation compared with the control diet with no supplemental fat.

Interaction between saturated fat supplementation and dietary forage content

Results for saturated fat supplementation at different forage concentrations have been inconsistent. Grum et al. (1996) compared a diet with no fat added to a diet supplemented with prilled saturated FA (3% of diet DM) at two different dietary NDF contents (~33% NDF vs. ~28% NDF) obtained by altering F:C in past-peak cows producing ~28 kg milk/d. In that experiment, fat supplementation had opposite effects in terms of DMI when supplemented in the low or high NDF diets (interaction $P = 0.02$): fat increased DMI when fed in the high NDF diet (20.7 vs. 19.2 kg/d), but decreased it when fed in the low NDF diet (19.4 vs. 20.2

kg/d). Grum et al. (1996) also showed that fat added to the high NDF diet increased OM (67.8 vs. 66.4%) and CP digestibility (64.3 vs. 62.2%), but decreased digestibility of both for the low NDF diet (65.5 vs. 70.9% and 61.8 vs. 67.6%, respectively; interactions $P \leq 0.10$). However, no interactions between dietary NDF and fat supplementation were detected for any other production response measured, plasma glucose, insulin, NEFA, or BHBA concentrations, or liver glycogen and total lipids (Grum et al., 1996). Weiss and Pinos-Rodriguez (2009) also evaluated the addition of a prilled saturated fat (2.25% of diet DM) to diets varying in fNDF content (~17 or 25% fNDF) from 21 to 126 DIM in cows producing ~46 kg milk/d, and detected an interaction for milk yield (interaction $P < 0.10$): the low fNDF diet with fat added increased milk yield during the entire treatment period compared with the other three diets. In addition, an interaction between fNDF and fat was detected for BCS (interaction $P < 0.05$), indicating that the fat added to the high fNDF diet decreased the loss in BCS observed in the high fNDF diet with no fat added.

Effects of fat supplements on energy partitioning between mammary gland and peripheral tissues

Fatty acids can have an impact on energy partitioning of dairy cows, and this will depend on the FA itself as well as on characteristics of the diets (Weiss and Pinos-Rodriguez, 2009; Bauman et al., 2011). An example would be the milk-fat-depression (MFD) syndrome, characterized by a depression in milk fat concentration and yield, with no change in other milk components, milk yield, or DMI (Baumgard et al., 2001). During MFD, a shift in the normal ruminal biohydrogenation process determines an increased production of several trans FA intermediates that will reach the duodenum for absorption and will then be secreted

into milk. Fatty acids that have been associated with milk fat depression are: *trans*-10, *cis*-12 C18:2, *cis*-10, *trans*-12 C18:2, and *trans*-9, *cis*-11 C18:2 (Bauman et al., 2011). Small amounts of these FA intermediates can have great impact on milk fat production. In an abomasal infusion study, Baumgard et al. (2001) showed that 0.016% of dietary DM of *trans*-10, *cis*-12 C18:2 could decrease milk fat synthesis by 25% in less than 2 days of infusion. The effect of this FA on milk fat was further studied by Baumgard et al. (2002), who showed that *trans*-10, *cis*-12 C18:2 decreased FA in milk through the down-regulation of key mammary lipogenic enzymes. Later, Harvatine et al. (2009) evaluated adipose tissue gene expression in cows abomasally infused with *trans*-10, *cis*-12 C18:2 and observed an up-regulation in key lipogenic enzymes in adipose tissue. This finding lead Harvatine et al. (2009) to conclude that the increase in body weight usually observed in cows with MFD was due to an increase in adipose tissue lipogenesis either from a direct effect of *trans*-10, *cis*-12 C18:2 on adipose tissue or from an indirect effect of increased fuel availability from decreased milk fat synthesis. Nutritional factors that have been related to MFD are: increased load of dietary unsaturated FA, increased diet fermentability (sometimes associated with lower ruminal pH), and inclusion of ionophores in the diet (Bauman et al., 2011; Jenkins and Harvatine, 2014). Ruminal pH has been positively related to milk fat percent in dairy cows (Allen, 1997), and even though low ruminal pH benefits microorganisms with alternative biohydrogenation pathways, increasing the synthesis of trans 10 intermediates and the risk of MFD, a low ruminal pH is not a prerequisite for the shift in biohydrogenation to occur (Jenkins and Harvatine, 2014).

Fatty acids could also affect energy partitioning through an increase in plasma insulin concentration or modulation of insulin resistance. Insulin responses to FA supplementation

have not been consistent across experiments (Grummer and Carroll, 1991). Saturated fat supplements have increased insulin concentration in dairy cows (Harvatine and Allen, 2006) and in rats (Stein et al., 1997). In addition, unsaturated FA have also increased insulin secretion in perfused pancreas in rats (Stein et al., 1997). However, increasing amounts of unsaturated FA decreased plasma insulin linearly in dairy cows ($P = 0.0001$; Choi and Palmquist, 1996). Chilliard (1993) suggested that the reason for inconsistent insulin responses to fat supplementation might be related to their effect on DMI, to which dietary ingredient was removed to add the fat supplement, and/or to the glucose sparing effect that fats might have if they decrease milk fat synthesis. If increased, insulin would stimulate insulin responsive tissues, such as muscle and adipose tissues to increase glucose uptake, protein synthesis in skeletal muscle, and lipogenesis in adipose tissue. An increase in glucose uptake by insulin responsive tissues might decrease glucose available for lactose synthesis in mammary gland, potentially decreasing milk yield.

Research evaluating the effects of fat supplementation on insulin and glucose responses has been more extensive in laboratory animals than in dairy cows. In a recent review of literature Kennedy et al. (2009), indicated that saturated FA increase adipose tissue expansion, inducing insulin resistance, and impairing insulin signaling in laboratory animals. In dairy cows, Blum et al. (1999) used euglycemic-hyperinsulinemic clamps and hyperglycemic clamps fed a free FA supplement (200 g/d, ~84% long-chain saturated FA) or a high starch diet from calving through 20 wk into lactation to evaluate if free FA supplementation induces insulin resistance. Contrary to their expectations, supplementation of free FA did not affect BW or energy-corrected milk yield, plasma insulin concentration before clamps, insulin-dependent glucose utilization, insulin metabolic clearance rate, or

insulin secretion compared with the high starch diet. Later, Pires et al. (2007) infused intravenously tallow or saline as control treatment in nonlactating, nonpregnant cows and conducted both insulin (ITT) and glucose tolerance tests (GTT). Hyperlipidemia from tallow infusions decreased glucose clearance during the GTT, despite greater plasma insulin concentrations, and also during the ITT, indicating a reduction in responsiveness to insulin during the ITT; results from both tests indicated that tallow infusions increased insulin resistance. In a later experiment, Salin et al. (2012) abomasally infused tallow, camelina oil, or water in dry, late-pregnant cows to evaluate responses to ITT and GTT. Consistent with results of Pires et al. (2007), both lipid infusions increased basal plasma NEFA concentration and impaired glucose clearance during both GTT and ITT. However, lipid infusions tended to decrease basal insulin concentration. In this experiment, researchers also concluded that tallow infusion increased insulin resistance compared with control (Salin et al., 2012). In contrast, researchers suggested that camelina oil infusion might have had an insulin-sensitizing effect.

Effects of fat supplements on control of feed intake

Fat supplements might decrease DMI through gut peptide secretion (Choi et al., 2000; Bradford et al., 2008) and/or through an increase in plasma NEFA concentration, which increases availability of fuels for oxidation in the liver (Allen et al., 2009). Choi et al. (2000) related the depression in DMI observed with long-chain FA supplemented diets to an increase in plasma cholecystokinin concentration. Cholecystokinin is a gut peptide that can inhibit gastric emptying (Reidelberger, 1994), and likely increase ruminal retention time. An increase in ruminal retention time will likely increase the filling effect of the diet, inducing

satiety in high yielding cows, whose control of intake is predominantly related to gut-distension.

Fat supplementation and the Hepatic Oxidation Theory Oxidation of fuels in the liver can modulate satiety or hunger according to the Hepatic Oxidation Theory (HOT) of the control of feed intake. It is likely the energy status of the liver and not the hepatic oxidation of fuels per se what has an effect of control of intake (Friedman, 1997). While most of the research done in the area has been done in laboratory animals, its application to ruminants has been described (Allen et al., 2009). Fuels that can be oxidized in the ruminant liver and could induce satiety include metabolites mobilized from body reserves and provided by the diet such as NEFA, glycerol, and amino acids.

In dairy cows, NEFA are the preferred source of energy in the liver in both negative and positive energy balance. Liver uptake and oxidation of different FA might differ with their chain-length and degree of saturation, which could explain the fact that different FA affect feed intake differently. Mashek and Grummer (2003) showed that the net uptake of stearic acid was lower than that of palmitic, oleic, linoleic, linolenic, eicosapentaenoic, and docosahexaenoic acids in perfused liver of goats. Authors concluded that liver uptake of all FA tested, except for stearic acid, was similar. This could suggest that stearic acid would not induce satiety in ruminants directly, since it is not taken up or oxidized in the liver. Nevertheless, stearic acid could be oxidized in extra-hepatic tissues, sparing nutrients for oxidation in the liver, inducing satiety indirectly.

Research in rats indicates that unsaturated FA are more readily oxidized in the liver compared with saturated FA (Leyton et al., 1987), which could explain the greater depression

in DMI usually observed with unsaturated FA compared with saturated FA in dairy cows. Harvatine and Allen (2006a) fed three different long-chain FA supplements varying in degree of saturation versus a control diet with no supplemental fat and reported a linear decrease in DMI as degree of unsaturation of the FA supplement increased. Fatty acids varying in chain-length might also have different effects on DMI in dairy cows. Hollman at al. (2011a) showed that coconut oil (~90% saturated FA, ~60% medium-chain FA) decreased DMI by 18% compared with a source of long-chain saturated FA. While long-chain FA transport into the liver mitochondria requires a transporter, medium-chain FA do not; therefore, medium-chain FA will likely induce satiety sooner by being more readily oxidized compared with long-chain FA (Friedman et al., 1990).

Non-esterified FA are taken up by the liver in proportion to their concentration in plasma (Emery et al. 1992). During periods of negative EBal such as the post-partum period, dairy cows mobilize fat to cover their energy demands, increasing plasma NEFA concentrations. An increase in plasma NEFA concentration will likely induce satiety because of increased hepatic FA oxidation and hepatic energy status. A strategy that has been evaluated in dairy cows to decrease fat mobilization during the post-partum period is the use of certain trans FA that would induce a "controlled" MFD and improve energy balance (Moore et al., 2004). The use of C18:2 isomers (i.e. *trans*-10, *cis*-12 C18:2) could improve energy balance not only through a decrease in milk energy output, but also through an increase in DMI, according to HOT. An increase in DMI would be related to a decrease in the flux of NEFA to the liver from reduced fat mobilization.

Justification for research in saturated fat supplementation, hypothesis, and objectives

Milk components and not milk volume continue to be the principal driver of producer milk prices. The concentration and yield of milk fat is affected by the nutrition of the dairy cow; therefore, diets that increase milk fat yield would potentially be economically advantageous. However, data available do not consistently support the use of saturated fats. Inconsistent results in terms of production responses previously mentioned in dairy cows make it difficult or impossible to be certain when to recommend the use of available saturated fats on farms. Nevertheless, and regardless of the lack of conclusive data, farmers in the US are usually feeding saturated fats in their lactating herds even though the cost-benefit ratio of currently available supplements might is questionable (cost of commercial fat sources is higher than $1.20/kg). Moreover, many farmers and nutritionists might not recognize the difference between saturated and unsaturated FA, and less even between different saturated FA, and therefore, they do not grasp the idea that the composition of the fat supplement can have variable effects on cow performance. Currently, in the US, fats (usually indistinctively) are being commercialized and fed in farms to increase milk and milk fat yields, feed efficiency, and energy intake in all stages of lactation and to improve energy balance and reproductive performance early in lactation. There is a poor understanding of what effects fats have on cow performance and if these effects justify their use on farms. Almost 20 years ago, Jordan and Fourdraine (1993) published the results of a survey designed to characterize management practices of the top milking herds in the US. They reported that, of the 61 producers interviewed (11,096 kg yearly rolling-herd milk average), 41% were feeding by-pass fat, 45.9% were feeding tallow, and 21.3% were feeding other sources of fat to their cows. With the increased number of fat products available, it is likely

that the feeding of specialty fats, represented in the survey by "by-pass fat", has increased on farms. A conservative assumption that 40% of the dairy cows in the US (~9 million) consume an average of 0.25 kg/d, at $1.2/kg of specialty fat, this amounts to more than $350 million spent by dairy producers per year on these products. Therefore, considering the price of fat supplements and their extensive use, effects of fat supplements on cows at different stages of lactation and their interaction with different diets need to be clearly established to help producers make knowledgeable decisions on whether to use fat supplements based on scientific data.

Our long-term goal is to improve our understanding of fat feeding on farms through the study and integration of nutrient digestibility and metabolic and production responses to saturated fat supplementation. Our objectives are to determine the effects of key, highly pure saturated FA in post-peak cows varying in milk yield and of a commercially available mixture of these FA (≥85 % saturated) in post-partum cows when included in diets differing in fNDF content. Our central hypothesis is that saturated FA supplements will have different effects on production responses depending on stage of lactation of dairy cows and composition of diets. Our rationale is that results of this research will increase our current knowledge allowing us to strategically supplement and formulate lactating cow rations using saturated fats to increase profitability of dairy farms.

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CHAPTER 2

PALMITIC ACID INCREASED YIELDS OF MILK AND MILK FAT AND NUTRIENT DIGESTIBILIY ACROSS PRODUCTION LEVEL OF LACTATING COWS

Piantoni, P., A.L. Lock, and M.S. Allen. 2013. J. Dairy Sci. 96:7143–7154.

ABSTRACT

The effects of palmitic acid supplementation on feed intake, digestibility, and metabolic and production responses were evaluated in dairy cows with a wide range of milk production (34.5 to 66.2 kg/d) in a crossover design experiment with a covariate period. Thirty-two multiparous Holstein cows (151 ± 66 DIM) were randomly assigned to treatment sequence within level of milk production. Treatments were diets supplemented (2% of diet DM) with palmitic acid (PA, 99% C16:0) or control (SH, soyhulls). Treatment periods were 21 d with the final 4 d used for data and sample collection. Immediately prior to the first treatment period, cows were fed the control diet for 21 d and baseline values were obtained for all variables (covariate period). Milk production measured during the covariate period (preliminary milk yield) was used as covariate. In general, no interactions were detected between treatment and preliminary milk yield for the response variables measured. PA increased milk fat percent (3.40 vs. 3.29%) and yields of milk (46.0 vs. 44.9 kg/d), milk fat (1.53 vs. 1.45 kg/d), and 3.5% FCM (44.6 vs. 42. 9 kg/d), compared with SH. Concentrations and yields of protein and lactose were not affected by treatment. PA did not affect DMI or BW, tended to decrease BCS (2.93 vs. 2.99), and increased feed efficiency (3.5% FCM/DMI, 1.60 vs. 1.54), compared with SH. PA increased total tract digestibility of NDF (39.0 vs. 35.7%) and OM (67.9 vs. 66.2%), but decreased fatty acid (FA) digestibility (61.2 vs. 71.3).

As total FA intake increased, total FA digestibility decreased ($R^2 = 0.51$) and total FA absorbed increased (quadratic $R^2 = 0.82$). Fatty acid yield response, calculated as the additional FA yield secreted in milk per unit of additional FA intake, was 11.7% for total FA and 16.5% for C16:0 plus C16:1 *cis*-9 FA. PA increased plasma concentration of NEFA (101 vs. 90.0 μEq/L) and cholecystokinin (19.7 vs. 17.6 pmol/L), and tended to increase plasma concentration of insulin (10.7 vs. 9.57 μIU/mL). Results show that palmitic acid fed at 2% of diet DM has the potential to increase yields of milk and milk fat, independent of production level without increasing body condition score or body weight. However, a small percentage of the supplemented FA was partitioned to milk.

INTRODUCTION

Long-chain saturated fat supplements have been used to increase the energy density of diets (Wang et al., 2010) and milk fat yield (Steele and Moore, 1968; Steele, 1969; Wang et al., 2010) in dairy cows and have been reported to increase feed efficiency (Wang et al., 2010; Lock et al., submitted) and milk yield (Steele, 1969; Enjalbert et al., 2000). Moreover, they are considered to be inert in the rumen (Grummer, 1988; Schauff and Clark, 1989), and have little effect on DMI (Allen, 2000) and nutrient digestibility (Grummer, 1988; Schauff and Clark, 1989; Elliott et al., 1996). However, production responses to highly saturated fats (> 85% saturated) have varied greatly. For instance, supplementation of a highly saturated fat fed at 1.5 to 2% of diet DM had various effects on productive performance when compared with a control diet with no fat added: increasing milk yield by 3.1 kg/d (Mosley et al., 2007) and 2.2 kg/d (Wang et al., 2010), or not affecting milk yield (Lock et al., submitted); increasing fat yield by 286 g/d (Mosley et al., 2007) and 90 g/d (Lock et al.,

submitted), or not affecting fat yield (Warntjes et al., 2008); and increasing DMI by 3.1 kg/d (Mosley et al., 2007), not affecting DMI (Wang et al., 2010), or decreasing DMI by 1.4 kg/d (Lock et al., submitted).

Variability across experiments could be due to the level of milk production of the cows used. Harvatine and Allen (2005) showed that milk protein yield was increased to a greater extent for high producing cows than lower producing cows for saturated compared with unsaturated FA supplements. Furthermore, early lactation cows with lower milk yield responded more favorably to the dietary inclusion of a highly saturated fat supplement than cows with higher milk production in a field study (Warntjes et al., 2008). Variability across experiments could also be related to the use of different types of fat supplements and rates of feeding. Fat supplements vary in FA chain lengths and degree of esterification and in their feeding rates, which vary widely from less than 2% (Wang et al., 2010) to greater than 5% of diet DM (Mosley et al., 2007) across experiments. In addition, substitution method might also affect production responses if the supplement is added in place of a source of glucose precursors such as corn (Wang et al., 2010), a fermentable fiber source such as soyhulls (Lock et al., submitted), or the base diet (Mosley et al., 2007). Because of inconsistent responses to feeding saturated fats, it is currently not clear when these supplements should be fed and whether their use can increase production and feed efficiency of cows and profitability of dairy farms.

To identify the effects of specific FA on dairy cow performance, studies involving the use of pure FA are required. Palmitic acid is a saturated FA that is commonly found in many different saturated fat supplements and dairy cow feedstuffs. Although several studies have been reported with fat sources containing ~ 85% palmitic acid, the remaining FA might have

influenced responses to treatment, so studies with pure FA are required. Steele and Moore (1968) evaluated a pure (96%) palmitic acid supplement on production responses for cows in mid-lactation but the milk yield of the cows was low (~12 kg/d) and responses measured were limited. To our knowledge, there are no studies that have evaluated the effects of supplementation of a pure palmitic acid supplement on digestion and metabolic and production responses in lactating dairy cows or how responses vary with level of milk production. The objectives of this experiment were to evaluate the effects of palmitic acid supplementation and its interaction with level of milk production on digestion, metabolism, and production of lactating dairy cows. We hypothesized that a palmitic acid enriched supplement compared with soyhulls would increase milk yield, milk fat yield, and feed efficiency of dairy cows and that responses would differ across production levels.

MATERIALS AND METHODS

Animal housing and care

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University. All cows were housed in the same tie-stall throughout the entire experiment. Cows were fed once daily (0800 h) at 110% of expected intake and milked twice daily (0400 and 1500 h). Amounts of feed offered and orts were weighed for each cow daily.

Design and treatment diets

Thirty-two multiparous Holstein cows (151 ± 66 DIM; mean ± SD) at the Michigan State University Dairy Field Laboratory were used in a crossover design experiment with a

covariate period. Cows were selected from the herd to provide a uniform distribution and a wide range of milk yield (34.5 to 66.2 kg/d). Cows were randomly assigned to treatment sequence within levels of milk production varying by 5 kg/d. The experiment was 63 d in duration and consisted of a 21-d preliminary (covariate) period and two 21-d treatment periods. During the preliminary period, cows were fed the control diet and baseline values were obtained for all variables (Table 2.1). During the first treatment period, half of the cows were fed the control diet (SH) with no supplemental fat added while the remaining cows were fed the palmitic acid supplemented diet (PA; prilled free FA supplement: 99% C16:0; Emery Oleochemicals, Selangor, Malaysia). The palmitic acid supplement was added at 2% of diet DM, replacing soyhulls, compared with the control diet. Diets were switched for the second treatment period. The ingredient and nutrient composition of the diets fed as TMR are described in Table 2.2**.** Diets were formulated to meet requirements of the average cow in the group according to NRC (2001).

Data and sample collection

Samples and data were collected during the last 4 d of the second week of the covariate period (d 11 to 15) and 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. Milk yield was recorded and two milk samples were collected at each milking. One milk sample was stored without preservative at -20°C for determination of FA profile and the other was stored with preservative at 4°C for component analysis (Universal Labs; East Lansing, MI). Fecal (500 g) and blood samples (~ 15 mL) were collected every 15 h resulting in eight samples per cow per period, representing every 3 h of

Table 2.1. Baseline data for cows used in this study, obtained during the preliminary period when cows were fed a common diet (n = 32)

Parameter	Mean	SD	Minimum	Maximum
Yields, kg/d				
Milk	48.3	9.39	31.3	64.6
Fat	1.49	0.31	0.72	2.14
Protein	1.46	0.22	1.01	1.84
Lactose	2.34	0.49	1.46	3.17
3.5% FCM	45.1	8.04	30.3	60.0
ECM	45.5	7.69	31.7	59.1
Milk composition, %				
Fat	3.14	0.58	1.68	4.05
Protein	3.05	0.20	2.73	3.71
Lactose	4.83	0.16	4.36	5.08
SCC, 1000/mL	44.5	77.4	1.93	363
DMI, kg	28.0	3.34	20.9	34.3
3.5% FCM/DMI	1.61	0.18	1.30	2.01
BW, kg	702	74.6	556	902
BCS	2.90	0.81	1.92	4.67
Total tract digestibility, %				
DM	62.5	3.04	56.1	68.3
OM	64.8	2.86	59.1	70.1
NDF	36.2	6.24	22.6	49.1
CP	61.9	3.82	53.3	66.5
Starch	96.2	0.95	93.6	98.2
Plasma metabolites and hormones				
Insulin, μIU/mL	11.1	4.40	4.08	24.8
Glucagon, pg/mL	147	16.9	114	191
Ratio Insulin:Glucagon	0.076	0.030	0.027	0.165
Glucose, mg/dL	55.9	2.55	52.0	63.0
NEFA, μEq/L	107	21.4	82.2	155.9
Trygliceride, mg/dL	9.77	1.22	7.31	12.7
Glucose tolerance test				
Glucose baseline, mg/dL	59.9	2.65	55.4	67.5
Maximum glucose, mg/dL[1]	232	64.5	170	430
Glucose area under the curve[1]	421	83.8	274	601
Insulin baseline, μIU/mL	4.04	1.44	3.05	9.53
Maximum insulin, μIU/mL[1]	44.8	16.3	19.8	89.8
Insulin area under the curve[1]	118	51.8	26.8	248
Insulin tolerance test				
Glucose baseline, mg/dL	59.2	3.05	54.1	64.8
Minimum glucose, mg/dL	40.1	5.82	21.5	48.6
Glucose area under the curve	-115	50.2	-249	-26.8
Insulin baseline, μIU/mL	3.72	1.24	3.01	8.86
Maximum insulin, μIU/mL[1]	325	306	76.0	1,446
Insulin area under the curve[1]	528	342	217	1,650

[1]n = 31

Table 2.2. Ingredient and nutrient composition of SH and PA diets fed during the treatment periods

	Diet	
	SH	PA
Ingredients, % DM		
Corn silage	25.8	25.8
Alfalfa silage	7.33	7.34
Chopped alfalfa hay	6.23	6.23
Dry ground corn	31.2	31.2
Soybean meal	12.5	12.5
Soyhulls	9.20	7.19
Cottonseed with lint	3.64	3.64
Vitamin-mineral mix[1]	4.18	4.18
Palmitic acid supplement (99% C16:0)	0.00	1.98
Nutrient composition		
DM, %	62.0	62.1
OM, % DM	93.4	93.5
NDF, % DM	30.4	29.1
% Forage NDF	19.1	19.1
% NDF from forage	62.7	65.5
iNDF[2], % DM	9.51	9.40
CP, % DM	15.9	15.7
Starch, % DM	29.3	29.2
Total FA, % DM	2.52	4.47
C16:0, % DM	0.464	2.41

[1]Vitamin-mineral mix contained (DM basis): 30.1% limestone, 25.3% sodium bicarbonate, 10.1% salt-white, 7.07% urea, 6.00% potassium chloride, 5.98% dicalcium phosphate, 5.68% magnesium sulfate, 5.68% animal fat, 3.94% trace mineral pre-mix and vitamins, 0.21% selenium yeast 600.
[2]iNDF = indigestible NDF

a 24-h period to account for diurnal variation. Feces were stored in a sealed plastic cup at -20°C until dried. 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 x g for 15 min at 4°C (within 30 min of sample collection). Two aliquots (1 mL) of plasma from the potassium EDTA tube were stored in 0.05 M of benzamidine (final concentration) to prevent enzymatic degradation of glucagon or

cholecystokinin (CCK). The remaining plasma was transferred into micro-centrifuge tubes and stored at -20°C until composited by cow by period. BW and BCS were recorded at the end of each period. Body condition was scored by three trained investigators on a 5-point scale, where 1 = thin and 5 = fat (in 0.25 point increments), as described by Wildman et al. (1982).

Glucose and insulin tolerance tests

Glucose and insulin tolerance tests were performed during the last week of the covariate period to further characterize the physiological state of individual cows. Cows were divided into two groups of 16 cows each for catheterization and tolerance tests to provide a 2-d resting period between procedures. The glucose tolerance test (GTT) was conducted according to Bradford and Allen (2007) on d 18 and 19 and the insulin tolerance test (ITT) was conducted according to Smith et al. (2007) on d 20 and 21. All cows were fitted with a single jugular catheter 2 d prior to the GTT. Indwelling 14 gauge x 13 cm radiopaque polyurethane extended-use catheters were used for infusions and blood collection. Catheter patency was checked daily with 10 mL of heparinized saline (20 IU heparin/mL saline) until removed after the ITT. On the day of the GTT and ITT, cows were blocked from feed at 0730 h and not allowed access until the tests were completed. For the GTT, a sterile solution of 50% dextrose (wt/vol) was administered by intra-jugular bolus at a dose of 1.67 mmol glucose/kg of BW within 8 min. For the ITT, insulin (Sigma-Aldrich, product # I5500) was infused at a dose of 1.2 μg insulin/kg of BW within 1 min. Catheters were flushed with 5 to 10 mL of heparinized saline (4 IU heparin/mL saline) after infusions and after blood collections. Samples were processed as described above, within 1 h of collection.

Sample analysis

Feed, orts, and fecal samples were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration. All samples were ground in a Wiley mill (1-mm screen; Arthur H Thomas, Philadelphia, PA) and analyzed for ash, NDF, indigestible NDF, CP, starch, and FA. Feces were composited on an equal DM basis by cow by period before analysis. All nutrients are expressed as percentages of DM determined by drying at 105°C in a forced air oven for more than 8 h. Ash concentration was determined after 5 h of oxidation at 500°C. Concentration of NDF was determined according to Mertens (2002). Indigestible NDF, which was used as an internal marker to estimate fecal output and nutrient digestibility (Cochran et al., 1986), was estimated as NDF residue after 240 h *in vitro* fermentation (Goering and Van Soest, 1970); flasks were re-inoculated at 120 h to ensure a viable microbial population. Ruminal fluid for the *in vitro* incubations was collected from a non-pregnant dry cow fed dry hay only. Crude protein was determined according to Hach et al. (1987). Starch was gelatinized with sodium hydroxide and hydrolyzed using an enzymatic method (Karkalas, 1985); glucose was then measured using a glucose oxidase method (PGO Enzyme product No. P7119, Sigma Chemical Co., St. Louis) and by determination of absorbance with a micro-plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Fatty acids from feed ingredients and orts were determined using a one-step trans-esterification method, according to Sukhija and Palmquist (1988). Briefly, 1 mg of C17:1 10*c*, diluted in acetone, was added to the oven-dried ground samples (sample weight chosen to provide 10 to 50 mg of FA) to calculate total FA yield. Fatty acid methyl esters (FAME) were then prepared by adding 2 mL of 5% methanolic sulfuric acid to the samples. After samples were incubated overnight at 50°C, they were allowed to cool down and neutralized

with a 5% sodium chloride solution. Fatty acids from feces were extracted with a two-step methylation procedure, as described by Jenkins et al. (2010). An internal standard, C17:1 *cis*-10, was added to the oven-dried ground fecal samples as described above for feed ingredients and orts. FAME from feed ingredients, orts, and feces were extracted with hexane and filtered through silica gel and charcoal. Hexane was evaporated under N_2 at 30°C, FAME were weighed, and samples reconstituted in hexane to obtain a 1% solution. FAME were quantified by gas-liquid chromatography (GC-2010 Plus; Shimadzu, Kyoto, Japan), using a CP-8827 WCOT fused silica column (30 m × 0.32 mm i.d. x 0.025-µm film thickness; Varian Inc., Lake Forest, CA). The chromatograph was equipped with a split injector (1:100 split ratio) and a flame-ionization detector (FID). Hydrogen was used as the carrier gas at a flow rate of 1 mL/min and for the FID at 40 mL/min. Purified air was used at a flow rate of 400 mL/min and nitrogen makeup gas at 30 mL/min. Injector and detector temperatures were kept at 270°C. Initially, oven temperature was 140°C for 1 min, then increased by 5°C/min to 225°C and then by 50 °C/min to 250°C, and held for 5.5 min. The injection volume was 1 µL of FAME-hexane mixture. Integration was performed with GCSolution software (version 2.32.00). FAME were identified by comparison of retention times with known FAME standards (GLC 63A and GLC 455 from Nu-Check Prep, Elysian, MN).

All plasma samples were analyzed in duplicate, unless otherwise specified. Commercial kits were used to determine plasma concentrations of NEFA (NEFA-HR (2) kit, Wako Chemicals USA, Inc., Richmond, VA; intra-assay CV: 2.5%, inter-assay CV: 3.1%), triglyceride (L-Type Triglyceride M kit, Wako Chemicals USA, Inc., Richmond, VA; intra-assay CV: 14.6 %, inter-assay CV: 8.8 %), insulin (Coat-A-Count RIA kit, Siemens

Healthcare Diagnostics, Deerfield, IL; intra-assay CV: 7.8 %), glucagon (Glucagon RIA kit #GL-32K, Millipore, St. Charles, MA; intra-assay CV: 4.4%), and CCK (Euria-CCK kit # RB 302 US, Euro Diagnostica, Malmö, Sweden; singlicate analysis; intra-assay CV: 3%). Plasma glucose concentration was analyzed using a glucose oxidase method (PGO Enzyme product No. P7119, Sigma Chemical Co., St. Louis, MO; intra-assay CV: 1.1%, inter-assay CV: 1.0%).

Milk samples stored with preservative were analyzed for fat, true protein, lactose, MUN, and somatic cell count by infrared spectroscopy (AOAC, 1997), by the Michigan Herd Improvement Association (Universal Labs; East Lansing, MI). Milk samples stored without preservative were composited by milk fat yield and centrifuged at 17,800 x g for 30 min at 4°C to collect the fat cake. Lipids were extracted according to Hara and Radin (1978) and FAME prepared according to Christie (1989). Quantification of FAMEs using gas-liquid chromatography was performed as described by Caldari-Torres et al. (2011). A total of ~80 individual FA were quantified per sample. Even though all quantified FA were used for summation by source and concentration calculations, only select FA were included in the tables. Yield of individual FA in milk fat were calculated by correcting for glycerol content, according to Schauff et al. (1992), and other milk lipid classes, according to Glasser et al. (2007). The FA yield response to additional FA intake (FAYR), was calculated for total FA and for C16:0 plus C16:1 *cis*-9 with the following equation:

FAYR, % = (FA yield for PA – FA yield for SH)/(FA intake for PA – FA intake for SH)

Statistical analysis

All data were analyzed using the fit model procedure of JMP (Version 9.0.2, SAS

Institute, Cary, NC) according to the following model:

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

where Y_{ijk} = dependent variable, μ = overall mean, C_i = random effect of cow (i = 1 to 32), P_j = fixed effect of period (j = 1 to 2), T_k = fixed effect of treatment (k = 1 to 2), pMY = preliminary milk yield used as covariate, pMY x T_k = interaction between treatment and preliminary milk yield, pMY^2 = preliminary milk yield squared, pMY^2 x T_k = interaction between treatment and preliminary milk yield squared, and e_{ijk} = residual error. Linear and quadratic effects for the interaction between pMY and treatment were added to evaluate responses to treatment by level of milk yield. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. When necessary, data was transformed and this was noted in the tables. 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 $P \leq 0.15$. Interactions were evaluated, but removed from the statistical model when not significant ($P > 0.15$). In general, period by treatment interaction was not significant, but variables with significant interactions are noted in the tables. All data was expressed as least square means and standard error of the means, unless otherwise specified.

RESULTS

Production responses

Treatment did not interact with preliminary milk yield for any production response measured (Table 2.3). PA increased milk fat percent (3.40 vs. 3.27%; $P = 0.01$) and yields of milk (46.0 vs. 44.9 kg/d; $P < 0.05$) and milk fat (1.53 vs. 1.45 kg/d; $P < 0.01$).

Concentrations and yields of protein, lactose, solids, solids non-fat, and MUN were not affected by treatment. PA increased 3.5% FCM yield by 1.74 kg/d and ECM yield by 1.57 kg/d (both $P < 0.01$) because of the increases in both milk yield and milk fat percent. PA did not affect DMI or BW, tended to decrease BCS slightly ($P = 0.06$), and increased feed efficiency (3.5% FCM/DMI) by 0.06 units ($P < 0.01$).

Total tract digestibility

Treatment did not interact with preliminary milk production for total tract digestibility of any feed fraction measured, except for 16-carbon FA (Table 2.4). PA increased DM and OM total tract digestibility by 2.8 and 2.6%, respectively (both $P < 0.01$), mainly from higher NDF digestibility, which increased by 9.2% ($P < 0.001$), and CP digestibility, which increased by 3.3% ($P < 0.05$). A treatment by period interaction was detected for CP digestibility ($P = 0.07$); PA increased CP digestibility substantially during period 1 (65.1 vs. 61.2 ± 0.87%), but not during period 2 (67.9 vs. 67.6 ± 0.87%). In contrast, PA did not affect digestibility of starch and decreased total FA digestibility by 14.2% ($P < 0.001$). Moreover, PA decreased 16-carbon FA digestibility by 25.7% ($P < 0.001$) and increased 18-carbon FA digestibility slightly ($P = 0.02$) compared with SH. The interaction between preliminary milk yield and treatment for 16-carbon FA digestibility indicated that cows with higher milk yield had lower 16-carbon FA digestibility than cows with lower milk yield during the preliminary period (interaction $P = 0.07$). Further, total FA digestibility decreased as total FA intake increased ($R^2 = 0.51$; $P < 0.0001$; Figure 2.1).

Table 2.3. Dry matter intake, milk production and composition, BW, and BCS for cows fed treatment diets (n = 32)

	Trt[1]			Significance, *P*-values	
Item	SH	PA	SEM	Trt	Trt x Period
DMI, kg/d	27.8	27.8	0.54	0.98	0.84
Yield, kg/d					
Milk	44.9	46.0	1.74	0.04	0.87
Fat	1.45	1.53	0.05	0.001	0.45
Protein	1.38	1.41	0.04	0.13	0.84
Lactose	2.19	2.23	0.09	0.12	0.98
3.5% FCM	42.9	44.6	1.35	<0.01	0.75
ECM	43.2	44.8	1.31	<0.01	0.82
3.5% FCM/DMI	1.54	1.60	0.03	<0.0001	0.73
Milk composition, %					
Fat	3.29	3.40	0.11	0.01	0.50
Protein	3.11	3.09	0.05	0.57	0.98
Lactose	4.85	4.82	0.03	0.28	0.17
SCC (1000/ml)	36.5	43.6	11.6	0.48	0.25
Average MUN, mg/dl	15.0	14.8	0.27	0.49	0.11
BW, kg	722	723	14.7	0.58	0.82
BCS	2.99	2.93	0.15	0.06	0.81

[1]Trt = dietary treatments. Treatments were either SH (with 2% of diet DM as added soyhulls) or PA (with 2% of diet DM as added palmitic acid supplement; 99% C16:0).

Table 2.4. Total-tract digestibility for cows fed treatment diets (n = 32)

	Trt[1]			Significance, *P*-values			
Item, %	SH	PA	SEM	Trt	Trt x Period	pMY	pMY x Trt
DM	64.4	66.2	0.40	0.001	0.67	0.20	0.63
OM	66.2	67.9	0.38	0.001	0.96	0.14	0.95
NDF	35.7	39.0	0.74	<0.001	0.24	0.25	0.87
CP	64.4	66.5	0.62	0.01	0.07	0.54	0.17
Starch	96.7	96.8	0.15	0.57	0.62	0.48	0.44
Total FA	71.3	61.2	1.13	<0.0001	0.17	<0.01	0.56
16-carbon FA	67.6	50.2	1.07	<0.0001	0.28	0.09	0.07
18-carbon FA	73.3	75.5	1.28	0.02	0.47	<0.01	0.73

[1]Trt = dietary treatments. Treatments were either SH (with 2% of diet DM as added soyhulls) or PA (with 2% of diet DM as added palmitic acid supplement; 99% C16:0).

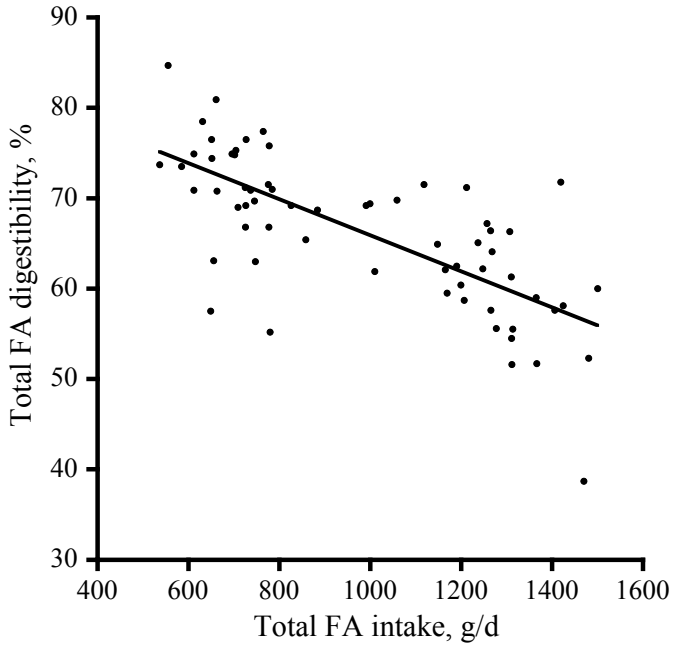

Figure 2.1. Relationship between total FA digestibility and total FA intake of cows fed treatment diets (Total FA digestibility, % = 85.9 – 0.020 x Total FA intake, g/d; R^2 = 0.51; *P* < 0.0001). Cows with FA intakes higher than 950 g/d were on the PA diet (n = 32), while cows with FA intakes lower than 950 g/d were on the SH diet (n = 32).

As expected, PA increased intakes of total FA and 16-carbon FA by 551 g/d (both *P* < 0.0001; Table 2.5). The interaction between preliminary milk yield and treatment for the intakes of total FA and 16-carbon FA (both *P* < 0.01) indicated that the difference in intakes for cows with lower milk production was slightly less than for cows with higher milk production. Intake of 18-carbon FA was not affected by diet (*P* = 0.75). PA increased absorption of total FA and 16-carbon FA by 262 and 250 g/d, respectively (both *P* < 0.0001; Table 5), and tended to increase absorption of 18-carbon FA by 10 g/d (*P* = 0.10). Total FA absorption increased at a decreasing rate as total FA intake increased (quadratic R^2 = 0.82; *P* = 0.01; Figure 2.2). An interaction between treatment and period was detected for total FA absorption (*P* = 0.05); PA increased total FA absorbed by 216 g/d during period 1, but by

308 g/d during period 2. A similar interaction between treatment and period was observed for 16-carbon FA absorption ($P = 0.001$).

Table 2.5. Total FA intake and absorbed for cows fed treatment diets (n = 32)

	Trt[1]			Significance, *P*-values			
Item, %	SH	PA	SEM	Trt	Trt x Period	pMY	pMY x Trt
DMI, kg/d	27.8	27.8	0.54	0.98	0.84	<0.0001	0.84
Total FA intake, g/d	707	1258	14.2	<0.0001	0.85	<0.0001	<0.01
16-carbon FA intake, g/d	136	687	7.05	<0.0001	0.95	<0.0001	<0.0001
18-carbon FA intake, g/d	533	531	10.6	0.75	0.78	<0.0001	0.57
Total FA absorbed, g/d	502	764	12.4	<0.0001	0.05	0.04	0.80
16-carbon FA absorbed, g/d	91.9	342	5.65	<0.0001	0.001	0.12	0.96
18-carbon FA absorbed, g/d	389	399	7.80	0.10	0.36	0.04	0.59

[1]Trt = dietary treatments. Treatments were either SH (with 2% of diet DM as added soyhulls) or PA (with 2% of diet DM as added palmitic acid supplement; 99% C16:0).

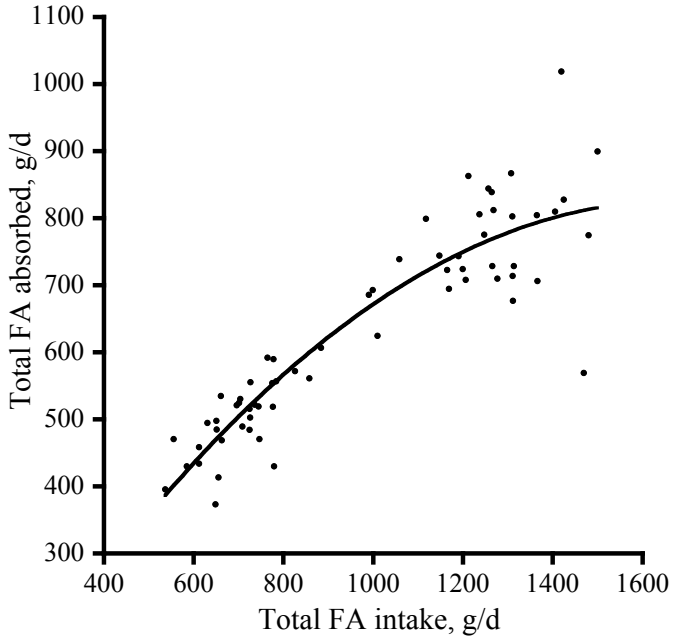

Figure 2.2. Relationship between total FA absorbed and total FA intake of cows fed treatment diets (Total FA absorbed, g/d = 202 + 0.470 x Total FA intake, g/d – 0.0003 x (FA intake, g/d – 983)^2; $R^2 = 0.82$; $P = 0.01$). Cows with FA intakes higher than 950 g/d were on the PA diet (n = 32), while cows with FA intakes lower than 950 g/d were on the SH diet (n = 32).

Plasma metabolites and hormones

Treatment did not interact with preliminary milk yield for plasma concentration of any hormone or metabolite, except glucagon (Table 2.6). PA decreased plasma glucagon concentration, compared with SH (interaction $P = 0.07$), for cows with preliminary milk yield greater than 55 kg/d only (data not shown). Although PA did not affect plasma glucose concentration, it tended to increase plasma insulin concentration by 11.7% ($P = 0.06$) and insulin to glucagon ratio by 9.6% ($P = 0.07$). PA also increased plasma CCK concentration by 11.9% ($P < 0.001$). Plasma triglyceride concentration was not affected by treatment, but PA increased plasma NEFA concentration by 12.7% ($P < 0.001$).

Milk fatty acid profile and yields

PA increased concentrations of mixed source FA in milk, but decreased concentrations of *de novo* and preformed FA ($P < 0.001$; Table 2.7). Mixed source FA (C16:0 plus C16:1 *cis*-9) can originate from both preformed (dietary or mobilized) and *de novo* synthesis in the mammary gland (Bauman and Griinari, 2003). Interactions between treatment and preliminary milk yield with respect to the concentrations of preformed and mixed source FA indicate that treatment affected cows differently depending on their level of milk production; for cows with higher milk production the difference in concentrations of preformed and mixed source FA between diets was less than for cows with lower milk production (both $P < 0.15$).

There were no interactions between treatment and preliminary milk yield for yields of individual FA in milk (Table 2.8). PA increased yields of mixed source FA in milk (564 vs.

473 g/d; $P < 0.0001$) and PA decreased slightly the yield of *de novo* FA (395 vs. 408 g/d; $P = 0.05$) and did not affect yields of preformed FA.

FAYR to additional FA intake

No interactions were detected between treatment and preliminary milk production for FAYR to the additional FA intake when calculated for either total FA or for C16:0 plus C16:1 *cis*-9. For each additional 100 g intake of total FAs, milk FA increased by 11.7 g compared with SH. When only C16:0 plus C16:1 *cis*-9 were considered, for each additional 100 g intake of these FA, C16:0 plus C16:1 *cis*-9 increased by 16.5 g compared with SH.

Table 2.6. Plasma metabolites and hormones of cows fed treatment diets (n = 32)

	Trt[1]			Significance, *P*-values				
Item	SH	PA	SEM	Trt	Trt x Period	pMY	pMY x Trt	pMY x pMY	pMY x pMY x Trt
Insulin, μIU/mL[2]	9.57	10.69	1.07	0.06	0.96	<0.01	0.37	0.48	0.49
Glucagon, pg/mL	149	155	6.35	0.17	0.21	0.82	0.18	0.90	0.07
Insulin:glucagon[2]	0.065	0.071	1.08	0.07	0.57	<0.01	0.22	0.48	0.21
Glucose, mg/dL	55.1	55.0	0.56	0.80	0.68	0.14	0.90	0.66	0.90
NEFA, μEq/L	90.0	101	2.64	<0.0001	0.14	0.16	0.19	0.82	0.44
Triglycerides, mg/dL	9.39	9.64	0.32	0.37	0.85	0.05	0.68	0.93	0.63
Cholecystokinin, pmol/L	17.6	19.7	0.86	<0.001	0.26	0.36	0.74	0.41	1.00

[1]Trt = dietary treatments. Treatments were either SH (with 2% of diet DM as added soyhulls) or PA (with 2% of diet DM as added palmitic acid supplement; 99% C16:0).
[2]Data was transformed (base-10 log) before analysis to meet the assumption of homogeneity of variance. For interpretation purposes, means and SEM were back-transformed and included in the table.

Table 2.7. Milk fatty acid concentrations[1] of cows fed treatment diets (n = 32)

	Trt[2]			Significance, *P*-values			
Item, g/100 g	SH	PA	SEM	Trt	Trt x Period	pMY	pMY x Trt
Summation by Source[3]							
De novo	29.9	27.5	0.33	<0.0001	0.21	0.19	0.61
Mixed	34.7	39.4	0.33	<0.0001	0.11	0.03	0.03
Preformed	35.4	33.1	0.43	<0.0001	0.81	0.01	0.10
Selected Individual Fatty Acids							
4:0	2.95	2.94	0.06	0.75	0.06	0.19	0.58
6:0	2.11	2.02	0.04	<0.0001	0.04	0.30	0.83
8:0	1.31	1.21	0.03	<0.0001	0.02	0.48	0.91
10:0	3.43	3.10	0.07	<0.0001	0.06	0.67	0.72
12:0	4.11	3.68	0.08	<0.0001	0.20	0.94	0.54
14:0	12.4	11.3	0.13	<0.0001	0.83	0.18	0.64
14:1 *cis*-9	0.962	0.920	0.04	<0.0001	0.27	0.45	0.93
16:0	33.0	37.6	0.34	<0.0001	0.23	0.04	0.03
16:1 *cis*-9	1.66	1.80	0.06	<0.0001	0.05	0.90	0.23
18:0	8.77	7.77	0.18	<0.0001	0.02	<0.01	0.05
18:1 *trans*[4]	1.97	1.81	0.20	0.13	0.17	0.89	0.40
18:1 *cis*-9	17.1	16.4	0.25	<0.0001	0.76	<0.01	0.31

[1]A total of ~80 individual FA were quantified and used for calculations (summation by source and concentrations). Only select FA are reported in the table.
[2]Trt = dietary treatments. Treatments were either SH (with 2% of diet DM as added soyhulls) or PA (with 2% of diet DM as added palmitic acid supplement; 99% C16:0).
[3]*De novo* fatty acids originate from mammary de novo synthesis (< 16 carbons), preformed fatty acids originate from extraction from plasma (> 16 carbons), and mixed fatty acids originate from both sources (C16:0 plus C16:1 *cis*-9).
[4]Total 18:1 *trans* fatty acids.

Table 2.8. Milk fatty acid yields[1] of cows fed treatment diets (n = 32)

	Trt[2]			Significance, *P*-values			
Item, g/d	SH	PA	SEM	Trt	Trt x Period	pMY	pMY x Trt
Summation by Source[3]							
De novo	408	395	14.2	0.05	0.30	<0.0001	0.94
Mixed	473	564	18.4	<0.0001	0.81	<0.0001	0.64
Preformed	475	470	11.6	0.45	0.26	0.0001	0.45
Selected Individual Fatty Acids							
4:0	40.4	42.4	1.72	<0.01	0.16	<0.001	0.43
6:0	29.0	29.2	1.23	0.74	0.14	<0.001	0.88
8:0	18.0	17.4	0.73	0.08	0.10	0.001	0.96
10:0	46.9	44.7	1.86	<0.01	0.14	0.002	0.98
12:0	56.0	53.0	2.09	<0.01	0.22	0.002	0.94
14:0	169	162	5.72	<0.01	0.46	<0.0001	1.00
14:1 *cis*-9	12.9	13.1	0.53	0.42	0.84	<0.001	0.88
16:0	450	539	17.8	<0.0001	0.76	<0.0001	0.63
16:1 *cis*-9	22.2	25.5	0.83	<0.0001	0.28	0.001	0.88
18:0	118	111	4.17	<0.001	0.05	0.13	0.48
18:1 *trans*[4]	25.2	25.0	1.65	0.85	0.16	0.02	0.44
18:1 *cis*-9	230	233	6.06	0.33	0.29	0.001	0.56

[1]A total of ~80 individual FA were quantified and used for calculations (summation by source). Only select FA are reported in the table.
[2]Trt = dietary treatments. Treatments were either SH (with 2% of diet DM as added soyhulls) or PA (with 2% of diet DM as added palmitic acid supplement; 99% C16:0).
[3]*De novo* fatty acids originate from mammary de novo synthesis (< 16 carbons), preformed fatty acids originate from extraction from plasma (> 16 carbons), and mixed fatty acids originate from both sources (C16:0 plus C16:1 *cis*-9).
[4]Total 18:1 *trans* fatty acids.

DISCUSSION

Previous research on saturated fat supplementation suggests that cows at different levels of milk production can respond differently to a treatment diet (Harvatine and Allen, 2005; Warntjes et al., 2008). However, all cows in this study responded similarly to palmitic acid supplementation, as evidenced by a lack of interaction between treatment and preliminary milk production for nearly every response variable measured. Exceptions were plasma glucagon concentration, 16-carbon FA digestibility, total FA intake, 16-carbon FA intake, and profiles of several individual milk FA. Interactions between treatment and preliminary milk yield for these variables were small and likely not biologically important. Preliminary milk yield was used as a covariate since this information is readily available to the dairy producer, and therefore can easily be used for grouping and feeding cows. GTT and ITT results were added to the statistical model and evaluated as possible covariates, but interactions with treatment were not significant (data not shown).

Palmitic acid supplementation did not affect DMI, but increased milk yield, which, together with an increase in milk fat concentration, resulted in an increase in 3.5% FCM. The lack of an effect on DMI and the increase in 3.5% FCM resulted in a slight increase in FE. Steele (1969) reported that a supplement high in palmitic acid (~ 85% C16:0) fed at ~ 4.25% of diet DM increased milk yield by 1 kg/d, milk fat yield by 115 g/d, and milk fat percent by 13%, when compared with a non-supplemented control diet. Moreover, palmitic acid supplementation decreased milk protein concentration by 6.4% without affecting milk protein yield and increased milk lactose yield by 8.6% with no effect on lactose concentration (Steele, 1969). In the present experiment, PA did not affect milk protein concentration or lactose yield despite increased yields of milk and milk fat, consistent with

results reported by Steele (1969). More recently, and consistent with our results, a FA supplement high in palmitic acid (~ 85%) fed at 2% of diet DM increased milk fat yield by 90 g/d and 3.5% FCM by 1.5 kg/d as well as increased FE (Lock et al., submitted). The increase in FE was a result of increased 3.5% FCM and a decrease in DMI of 1.4 kg/d, which was not observed in the current study. Since DMI was not affected by PA, the milk yield response observed could be because of an increase in energy consumed per day from the FA supplementation, to higher OM digestibility, or both. In addition, the increase in milk fat percent and yield observed for PA is likely directly related to the higher level of palmitic acid in the experimental diet, which raised plasma NEFA, but not TG concentration. Saturated fat addition to diets has previously been reported to increase plasma concentrations of NEFA and TG (Choi et al., 2000). Kronfeld (1965) showed that a plasma NEFA concentration higher than 300 μEq/L was associated with increased milk fat output in fresh cows. However, in our study, plasma NEFA was only a third of that threshold and PA increased NEFA by only 12.7%. The NEFA concentration we observed may have been related to the higher FA intake as well as to an increased mobilization of body fat reserves related the slight decrease in BCS observed for PA ($P = 0.06$). Palmitic acid tended to increase insulin concentration, consistent with the effects of dietary saturated fat supplementation for cows (Harvatine and Allen, 2006) and rats (Stein et al., 1997). Increased insulin concentration is expected to decrease lipolysis, which is inconsistent with the tendency for the decrease in BCS observed. The increase in concentration and yield of milk fat could also be explained by a longer retention time of digesta in the rumen, consistent with the increase in NDF digestibility and CCK concentration observed, which could favor a more complete biohydrogenation of CLA isomers associated with milk fat depression in dairy cows (e.g.

C18:2 t10, c12; Bauman et al., 2011). These biohydrogenation intermediates decrease milk fat synthesis in the mammary gland (Baumgard et al., 2002), but have opposite effects in adipose tissue (Harvatine et al., 2009). Nevertheless, we did not find evidence of lower concentrations in milk of previously described FA isomers related to milk fat depression (data not shown) and, therefore, this explanation for the increased milk fat yield observed is speculative. A decrease in the concentration of currently unknown bioactive FA isomers could explain both the increase in milk fat production and the tendency for the change in BCS observed when PA was fed in our study.

The effect of palmitic acid supplementation on milk FA yields was consistent with that observed by Steele and Moore (1968) in regard to a slight decrease in *de novo* FA (< 16-carbon FA) synthesis and an increase in C16:0 in milk, but not with the decrease in preformed FA (> 16-carbon FA) in milk. In agreement with Lock et al. (submitted), we did not see an effect of palmitic acid on preformed FA yields. FAYR was only 11.7% for total FA and 16.5% for C16:0 plus C16:1 *cis*-9 FA. Since the average intake of 16-carbon FA for PA was ~ 5 times higher than SH, the low FAYR to palmitic acid supplementation could not be explained by decreased FA digestibility alone. Similarly, a transfer efficiency of 16.5%, calculated as the fraction of digested C16:0 partitioned to milk, was reported previously in a field study where a ~85% palmitic acid supplement was fed at 450 g/d (Warntjes et al., 2008). The authors suggested that this low transfer efficiency was related to oxidation of the FA as metabolic fuel rather than export as milk triglycerides. In contrast, a higher FAYR (46.7%), calculated as the additional C16:0 consumed and partitioned to milk relative to control, has been reported when 490 g of palmitic acid was infused in the duodenum (Enjalbert et al.,

2000). In our study, increased milk fat yield was accounted for by the increase in total 16-carbon FA in milk.

Saturated FA generally do not affect DMI when added to diets at normal inclusion rates (up to 3% of total DM; Palmquist and Jenkins, 1980). Allen (2000) showed that DMI was not affected by hydrogenated FA in a meta-analysis of 29 treatment means reported in the literature. Consistent with this, we did not detect a difference in DMI between treatments in the present study. Results are inconsistent however, for studies in which highly enriched (≥ 85%) sources of palmitic acid were supplemented; supplementation of an enriched palmitic acid supplement decreased DMI (Lock et al., submitted) but also increased DMI (Mosley et al., 2007) when all treatments were compared to a control diet with no supplemental fat.

In general, supplementation of saturated fats has not affected OM digestibility. While saturated fat supplementation did not affect nutrient digestibility in several studies (Schauff and Clark, 1989; Grum et al., 1996; Harvatine and Allen, 2006), a highly enriched palmitic acid supplement tended to increase digestibility for several nutrient fractions in a field study (Warntjes et al. 2008). However, no previous experiment has measured digestibility responses to a pure palmitic acid supplement. The increase in nutrient digestibility observed in the present study might have been caused by an increase in ruminal retention time, possibly from a reduction in rumen motility from increased plasma CCK concentration, which has previously been reported when saturated fats were fed (Choi et al., 2000). An increased ruminal retention time might decrease passage of FA biohydrogenation intermediates (e.g. CLA) to the duodenum, previously mentioned as one of the possibilities for increased milk fat yield.

Studies from our laboratory (Harvatine and Allen, 2006) and others (Wu et al., 1993; Elliot et al., 1996; Grum et al., 1996) have reported decreased FA digestibility when saturated FA were fed. Palmquist (1991) showed a reduction in FA digestibility as FA intake increased, consistent with our results. Furthermore, total FA absorption increased at a decreasing rate with greater total FA intake. Decreased FA digestibility and absorption at high FA intakes might be related to excessive amounts of FA reaching the small intestine (Palmquist, 1991). Alternative explanations are an alteration of the micelle formation in the duodenum or biliary salts production in response to increased proportions of saturated vs. unsaturated FA (Doreau and Chilliard, 1997). Nevertheless, neither of these hypotheses has been tested. We only report total FA and 16- and 18-carbon FA in feces because of the biohydrogenation of unsaturated FA that occurs in the rumen and large intestine that can lead to overestimation of unsaturated FA digestibility and underestimation of saturated FA digestibility.

Since milk income is primarily dependent on protein and fat yields in most markets, dietary FA supplements have the potential to increase profitability of dairy farms. Increased profitability would depend on the cost of the supplement relative to other diet ingredients, the value of the production and feed efficiency responses in relation to milk price, and other intangibles related to reproduction and health. All of these factors need to be considered to determine the feasibility of the utilization of any dietary supplement in dairy herds. In the present study, palmitic acid substituted for soyhulls increased milk fat yield by 80 g/d, and did not affect milk protein yield or DMI. In view of these results, producers would have to consider only the slight increase in milk fat yield to evaluate whether it is profitable to feed a similar supplement to their herds. Research utilizing highly enriched palmitic acid

supplements has often reported increases in milk fat yield and various responses in terms of DMI, and therefore feed efficiency. For these reasons, their use on dairy farms might be justifiable in some cases, but the marginal return on any such supplement must be carefully considered.

CONCLUSION AND IMPLICATIONS

Our results confirmed our hypothesis that palmitic acid supplementation compared with soyhulls can increase milk yield, milk fat yield, and feed efficiency of dairy cows. However, production responses to palmitic acid did not differ across production level of cows. Further studies are required to evaluate the effects on performance of other long-chain saturated fatty acids, such as stearic acid, interactions of FA supplements with other dietary components, and to understand the reasons for differences in DMI and FAYR across studies with palmitic acid supplements.

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CHAPTER 3

MILK PRODUCTION RESPONSES TO DIETARY STEARIC ACID VARY BY PRODUCTION LEVEL IN DAIRY CATTLE

Piantoni, P., A.L. Lock, and M.S. Allen. 2005. J. Dairy Sci. doi:10.3168/jds.2014-8634.

ABSTRACT

Effects of stearic acid supplementation on feed intake and metabolic and production responses of dairy cows with a wide range of milk production (32.2 to 64.4 kg/d) were evaluated in a crossover design experiment with a covariate period. Thirty-two multiparous Holstein cows (142 ± 55 DIM) were assigned randomly within level of milk yield to treatment sequence. Treatments were diets supplemented (2% of diet dry matter) with stearic acid (SA; 98% C18:0) or control (CONT; soyhulls). The corn silage and alfalfa based diets contained 24.5% forage NDF, 25.1% starch and 17.3% CP. Treatment periods were 21 d with the final 4 d used for data and sample collection. Compared with CONT, SA increased dry matter intake (DMI, 26.1 vs. 25.2 kg/d) and milk yield (40.2 vs. 38.5 kg/d). Stearic acid had no effect on the concentration of milk components, but increased yields of fat (1.42 vs. 1.35 kg/d), protein (1.19 vs. 1.14 kg/d), and lactose (1.96 vs. 1.87 kg/d). The SA treatment increased 3.5% fat-corrected milk (3.5% FCM, 40.5 vs. 38.6 kg/d), but did not affect feed efficiency (3.5% FCM/DMI, 1.55 vs. 1.53), body weight, or body condition score compared with CONT. Linear interactions between treatment and level of milk yield during the covariate period were detected for DMI and yields of milk, fat, protein, lactose, and 3.5% FCM; responses to SA were positively related to milk yield of cows. The SA treatment increased CP digestibility (67.4 vs. 65.5%), tended to increase NDF digestibility (43.6 vs. 42.3%), decreased fatty acid (FA) digestibility (56.6 vs. 76.1%), and did not affect organic

matter digestibility. Fatty acid yield response (FAYR), calculated as the additional FA yield secreted in milk per unit of additional FA intake, was only 13.3% for total FA and 8.2% for C18:0 plus cis-9 C18:1 FA. Low estimated digestibility of the FA supplement was at least partly responsible for the low FAYR. Treatment did not affect plasma insulin, glucagon, glucose, and NEFA concentrations. Results show that stearic acid has the potential to increase DMI and yields of milk and milk components, without affecting conversion of feed to milk, body condition score, or body weight. Moreover, effects on DMI and yields of milk and milk components were more pronounced for higher yielding cows than for lower yielding cows.

INTRODUCTION

Production responses to highly saturated fats (≥ 85% saturated) have varied greatly in past experiments. Reasons for variability across experiments could be from different types of fat supplements, diets, and physiological states of cows. Variation in response among cows was demonstrated by Harvatine and Allen (2005) by comparing saturated and unsaturated fatty acid (FA) supplements fed to mid-lactation cows with a wide range of milk production. In that experiment, response to treatment for yield of milk protein varied across milk yield of cows; high producing cows responded better to the saturated FA supplement, while low producing cows responded better to the unsaturated FA supplement. Moreover, Palmquist and Jenkins (1980) reported that cows with low production potential did not respond to fat supplementation compared with cows with high production potential in their feeding trials. Saturated long-chain FA often increase milk fat yield in dairy cows (Steele and Moore, 1968; Steele, 1969; Wang et al., 2010). In addition, saturated long-chain FA supplements have been

shown to increase milk yield (Steele, 1969; Piantoni et al., 2013) and feed efficiency (FE; Wang et al., 2010; Lock et al., 2013; Piantoni et al. 2013) in some experiments. Interestingly, Piantoni et al. (2013) showed that a palmitic acid supplement increased milk yield, milk fat yield, and feed efficiency regardless of level of milk production.

Several studies evaluated the use of palmitic acid supplements (Mosley et al. 2007; Lock et al., 2013; Piantoni et al., 2013), but few reported the use of highly enriched stearic acid supplements. Steele and Moore (1968) evaluated a stearic acid supplement (94% pure), fed at ~4% of diet DM, on production responses for cows in mid-lactation: the supplement increased milk fat yield but did not affect milk fat concentration or milk yield compared with a control with no supplemental fat added. In a later study, stearic acid (85% pure; fed at ~4.25% of diet DM) increased milk yield, but did not affect milk fat concentration or yield compared with a control diet with no supplemental fat added (Steele, 1969). Interestingly, and in the same experiment, the same stearic acid supplement fed at half that inclusion rate (~2.1% of diet DM) increased not only milk yield but also milk fat yield compared with the control (Steele, 1969). Even though Steele and colleagues evaluated effects of highly enriched stearic acid supplements on production of lactating cows (Steele and Moore, 1968; Steele, 1969), the cows used had low milk yield (~12 kg/d), and responses measured were related to milk yield, composition, and FA analysis only and not to DMI, digestibility, metabolic responses, or feed conversion efficiency.

Inconsistent responses to feeding saturated fats requires research with pure FA sources to identify the effects of specific FA on production response of cows varying in milk yield to clarify when these supplements should be fed and their potential for increasing profitability of dairy farms. To our knowledge, there are no studies that have evaluated the

effects of a pure stearic acid supplement on digestion and metabolic and production responses in lactating dairy cows with a wide range of milk production. The objectives of this experiment were to evaluate the effects of stearic acid supplementation on digestion, metabolism, and production of lactating dairy cows and its interaction with level of milk production. Our hypothesis was that a highly pure (98%) stearic acid supplement will increase milk yield, milk fat yield, and feed efficiency of dairy cows and that responses to treatment will differ across levels of milk production.

MATERIALS AND METHODS

Animal housing and care

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University (East Lansing). All cows were housed in the same tie-stall throughout the entire experiment. Cows were fed once daily (1000 h) at 110% of expected intake and milked twice daily (0500 and 1600 h). The amounts of feed offered and orts were weighed for each cow daily.

Design and treatment diets

Thirty-two multiparous Holstein cows (142 ± 55 DIM; mean ± SD) at the Michigan State University Dairy Field Laboratory were used in a crossover design experiment with a covariate period. Cows were selected from the herd to provide a uniform distribution and a wide range of milk yield (32.2 to 64.4 kg/d). Cows were randomly assigned to treatment sequence within levels of milk production that varied by approximately 5 kg/d. The experiment was 56 d in duration and consisted of a 14-d preliminary (covariate) period and

two 21-d treatment periods. During the preliminary period, cows were fed the control diet and baseline values were obtained for all variables (Table 3.1). During the first treatment period, half of the cows (n = 16) were fed the control diet (CONT) with no supplemental fat added, whereas the other half (n = 16) was fed the stearic acid-supplemented diet (SA; prilled free FA supplement: 98% C18:0; Emery Oleochemicals, Selangor, Malaysia). The stearic acid supplement was added at 2% of diet DM, replacing 2% of soyhulls in the control diet. Diets were switched for the second treatment period. The ingredient and nutrient composition of the diets fed as TMR are described in Table 3.2. Diets were formulated to meet requirements of the average cow in the group according to NRC (2001).

Table 3.1. Baseline data for cows used in this study, obtained during the preliminary period when cows were fed a common diet (n = 32)

Parameter	Mean	SD	Minimum	Maximum
DMI, kg/d	28.9	3.11	22.7	35.4
Yields, kg/d				
Milk	46.1	9.20	31.9	62.5
Fat	1.54	0.29	1.03	2.15
Protein	1.41	0.24	1.03	1.94
Lactose	2.27	0.47	1.48	3.05
3.5% FCM	44.9	8.07	31.4	57.3
ECM	45.0	7.92	31.8	57.1
Milk composition, %				
Fat	3.37	0.47	2.34	4.38
Protein	3.08	0.33	2.72	4.41
Lactose	4.91	0.30	4.63	6.42
BW, kg	727	71.7	615	907
BCS	2.59	0.60	1.67	3.75
Plasma metabolites and hormones				
Insulin, μIU/mL	7.67	1.97	4.17	11.8
Glucagon, pg/mL	139	25.5	98.4	206
Ratio Insulin:Glucagon	0.056	0.012	0.033	0.081
Glucose, mg/dL	55.9	3.38	50.6	63.8
NEFA, μEq/L	95.1	36.5	61.2	228

Table 3.2. Ingredient and nutrient composition of the treatment diets[1]

	Diets	
	CONT	SA
Ingredients, % DM		
Corn silage	35.5	35.5
Alfalfa silage	12.6	12.6
Chopped alfalfa hay	7.17	7.16
Dry ground corn	16.6	16.5
Soybean meal	15.5	15.5
Soyhulls	6.55	4.67
Cottonseed with lint	3.44	3.44
Vitamin-mineral mix[2]	2.70	2.70
Stearic acid supplement (98% C18:0)	0.00	1.92
Nutrient composition		
DM, %	55.0	55.1
OM, % of DM	92.6	92.7
NDF, % of DM	33.2	31.9
% Forage NDF	24.5	24.5
% NDF from forage	73.7	76.6
iNDF, [3] % of DM	11.5	11.4
CP, % of DM	17.4	17.2
Starch, % of DM	25.1	25.1
Total FA, % of DM	2.73	4.60
C18:0, % of DM	0.085	1.96

[1]Treatments were either control (CONT; with 2% of diet DM as added soyhulls) or a stearic acid-supplemented diet (SA; with 2% of diet DM as stearic acid; 98% C18:0).
[2]Vitamin-mineral mix contained (DM basis) 34.5% sodium chloride, 29.4% calcium carbonate, 13.4% magnesium oxide, 12.5% monocalcium phosphate, 5.40% soybean oil, 4.85% trace minerals and vitamins.
[3]Indigestible NDF.

Data and sample collection

Samples and data were collected during the last 4 d of the covariate period (d 11 to 15) and 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. Milk yield was recorded and 2 milk samples were collected at each milking. One milk sample was stored without preservative at −20°C for determination of FA profile and the other was stored with a Bronopol tablet added as preservative at 4°C for component

analysis. Fecal (500 g) and blood samples (~15 mL) were collected every 15 h, 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. Blood was collected by coccygeal venipuncture into 3 evacuated tubes; 2 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 centrifuged at 2,000 × g for 15 min at 4°C (within 30 min of sample collection). Two aliquots (1 mL) of plasma from the potassium EDTA tube were stored in 0.05 M benzamidine (final concentration) to prevent enzymatic degradation of glucagon. The remaining plasma was transferred into microcentrifuge tubes and stored at −20°C until composited by cow by period. Body weight and BCS were recorded at the end of each period. Body condition was scored by 3 trained investigators on a 5-point scale, where 1 = thin and 5 = fat, as described by Wildman et al. (1982).

Sample analysis

Feed, orts, and fecal samples were processed and analyzed for ash, NDF, indigestible NDF, CP, starch, and FA as described by Piantoni et al. (2013). Indigestible NDF was used as internal marker to calculate digestibility. Particle size of the FA supplement was determined in duplicate as described by ASAE (1997). All plasma samples were analyzed in duplicate. Commercial kits were used to determine plasma concentrations of NEFA [NEFA-HR (2) kit; Wako Chemicals USA Inc., Richmond, VA; intraassay CV: 3.2%, interassay CV: 5.9%], insulin (Coat-A-Count RIA kit; Siemens Healthcare Diagnostics, Deerfield, IL; intraassay CV: 6.0%, interassay CV: 14%), and glucagon (Glucagon RIA kit no. GL-32K;

Millipore Corp., St. Charles, MA; intraassay CV: 4.4%). Plasma glucose concentration was analyzed using a glucose oxidase method (PGO Enzyme Product No. P7119; Sigma Chemical Co.; intraassay CV: 2.3%, interassay CV: 2.0%).

Milk samples stored with preservative were analyzed for fat, true protein, lactose, MUN, and SCC by infrared spectroscopy (AOAC International, 2000), by the Michigan Herd Improvement Association (Universal Lab Services, East Lansing, MI). Milk samples stored without preservative were composited by milk fat yield and centrifuged at 17,800 × g for 30 min at 4°C to collect the fat and FA composition was determined as described by Lock et al. (2013). A total of approximately 80 individual FA were quantified per sample. Even though all quantified FA were used for summation by source and concentration calculations, only select FA were included in the tables. Yield of individual FA in milk fat were calculated by correcting for glycerol content according to Schauff et al. (1992), and other milk lipid classes according to Glasser et al. (2007). The FA yield response (FAYR) to additional FA intake was calculated for total FA and for C18:0 plus *cis*-9 C18:1 with the following equation: FAYR (%) = (FA yield for SA − FA yield for CONT)/(FA intake for SA − FA intake for CONT).

Statistical analysis

All data were analyzed using the fit model procedure of JMP (version 9.0.2; SAS Institute, Cary, NC) according to the following model:

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

where Y_{ijk} = dependent variable, μ = overall mean, C_i = random effect of cow (i = 1 to 32),

P_j = fixed effect of period (j = 1 to 2), T_k = fixed effect of treatment (k = 1 to 2), $P_j \times T_k$ = interaction between period and treatment, pMY = preliminary milk yield used as covariate, pMY × T_k = interaction between pMY and treatment, pMY × pMY = pMY squared, pMY × pMY × T_k = interaction between pMY × pMY and treatment, and e_{ijk} = residual error. Linear and quadratic effects for the interaction between pMY and treatment were added to evaluate responses to treatment by level of milk yield. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. 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 $P \leq 0.15$. Interactions were evaluated, but removed from the statistical model when not significant ($P > 0.15$). All data were expressed as least squares means and standard error of the means, unless otherwise specified.

RESULTS

Production responses

Treatment interacted with preliminary milk yield for DMI and yields of milk, milk fat, milk protein, milk lactose, 3.5% FCM, and ECM (Table 3.3). Interactions indicated that responses were positively related to preliminary milk yield, and therefore, higher producing cows responded more favorably to SA than lower producing cows (Table 3.4). All interactions mentioned followed the same pattern, and the relationship between 3.5% FCM and preliminary milk yield is shown as an example in Figure 3.1. Overall, SA increased DMI 0.9 kg/d ($P = 0.01$), milk yield 1.7 kg/d ($P = 0.02$), 3.5% FCM 1.9 kg/d ($P < 0.01$), and

Table 3.3. Dry matter intake, milk production and composition, BW, and BCS for cows fed treatment diets (n = 32)

	Trt[1]				Significance, *P*-values[2]				
Item	CONT	SA	SEM	Trt	Trt x Period	pMY	pMY x Trt	pMY x pMY	pMY x pMY x Trt
DMI, kg/d	25.2	26.1	0.42	0.01	0.29	0.01	0.08	0.97	0.55
Yield, kg/d									
Milk	38.5	40.2	0.70	0.02	0.13	<0.0001	0.03	0.78	0.97
Fat	1.35	1.42	0.03	<0.01	0.08	<0.0001	0.04	0.75	0.60
Protein	1.14	1.19	0.02	0.02	0.15	<0.0001	0.04	0.47	0.94
Lactose	1.87	1.96	0.04	0.02	0.12	<0.0001	0.05	0.75	0.90
3.5% FCM	38.6	40.5	0.76	<0.01	0.06	<0.0001	0.03	0.89	0.75
ECM	38.2	40.1	0.75	<0.01	0.07	<0.0001	0.03	0.78	0.79
3.5% FCM/DMI	1.53	1.55	0.04	0.25	0.40	<0.0001	0.67	0.79	0.70
Milk composition, %									
Fat	3.60	3.59	0.12	0.82	0.44	0.11	0.22	0.72	0.09
Protein	3.00	2.99	0.05	0.93	0.98	<0.001	0.64	0.36	0.16
Lactose	4.83	4.86	0.02	0.16	0.75	0.11	0.94	0.93	0.94
SCC, x1,000/ml	78.1	62.0	21.8	0.47	0.26	0.16	0.51	0.21	0.67
MUN, mg/dl	19.3	18.8	0.32	0.08	0.49	0.49	0.62	0.94	0.79
BW, kg	727	730	12.80	0.28	0.60	0.10	0.07	0.89	0.63
BCS	2.67	2.67	0.11	0.81	0.82	0.02	0.41	0.56	0.44

[1]Trt = dietary treatment. Treatments were either control (CONT; with 2% of diet DM as added soyhulls) or a stearic acid-supplemented diet (SA; with 2% of diet DM as stearic acid; 98% C18:0).
[2]pMY = preliminary milk yield.

Table 3.4. Treatment response for DMI and yields of milk and milk components for cows varying in preliminary milk yield calculated using equations of the fitted lines between response variable and preliminary milk yield by diet (n = 32)

	pMY,[1] kg/d		
Item[2]	31.9	46.1	62.5
DMI[3], kg/d			
CONT	24.0	25.2	26.6
SA	24.0	26.1	28.4
Yield, kg/d			
Milk[4]			
CONT	28.9	38.5	49.5
SA	28.4	40.2	53.8
Fat[5]			
CONT	1.10	1.36	1.65
SA	1.10	1.43	1.81
Protein[6]			
CONT	0.942	1.126	1.340
SA	0.937	1.193	1.488
3.5% FCM[7]			
CONT	30.3	38.5	48.1
SA	30.1	40.5	52.4

[1]pMY = preliminary milk yield.
[2]Responses to treatment diets (control (CONT, with 2% of diet DM as added soyhulls) or a stearic acid-supplemented diet (SA; with 2% of diet DM as stearic acid; 98% C18:0) were calculated using the equations of the fitted lines between response variable and preliminary milk yield by diet (n = 32).
[3]DMI, kg/d (CONT) = 21.3 + 0.084 × pMY; R^2 = 0.06; *P*-value = 0.19); DMI, kg/d (SA) = 19.4 + 0.144 × pMY; R^2 = 0.25; *P*-value < 0.01).
[4]Milk yield, kg/d (CONT) = 7.29 + 0.676 × pMY; R^2 = 0.54; *P*-value < 0.0001); Milk yield, kg/d (SA) = 1.86 + 0.831 × pMY; R^2 = 0.80; *P*-value < 0.0001).
[5]Milk fat yield, kg/d (CONT) = 0.527 + 0.018 × pMY; R^2 = 0.32; *P*-value < 0.0001); Milk fat yield, kg/d (SA) = 0.370 + 0.230 × pMY; R^2 = 0.56; *P*-value < 0.0001).
[6]Milk protein yield, kg/d (CONT) = 0.527 + 0.013 × pMY; R^2 = 0.35; *P*-value < 0.001); Milk protein yield, kg/d (SA) = 0.363 + 0.018 × pMY; R^2 = 0.62; *P*-value < 0.0001).
[7]3.5% FCM yield, kg/d (CONT) = 11.7 + 0.582 × pMY; R^2 = 0.45; *P*-value < 0.0001); 3.5% FCM yield, kg/d (SA) = 6.81 + 0.730 × pMY; R^2 = 0.73; *P*-< 0.0001).

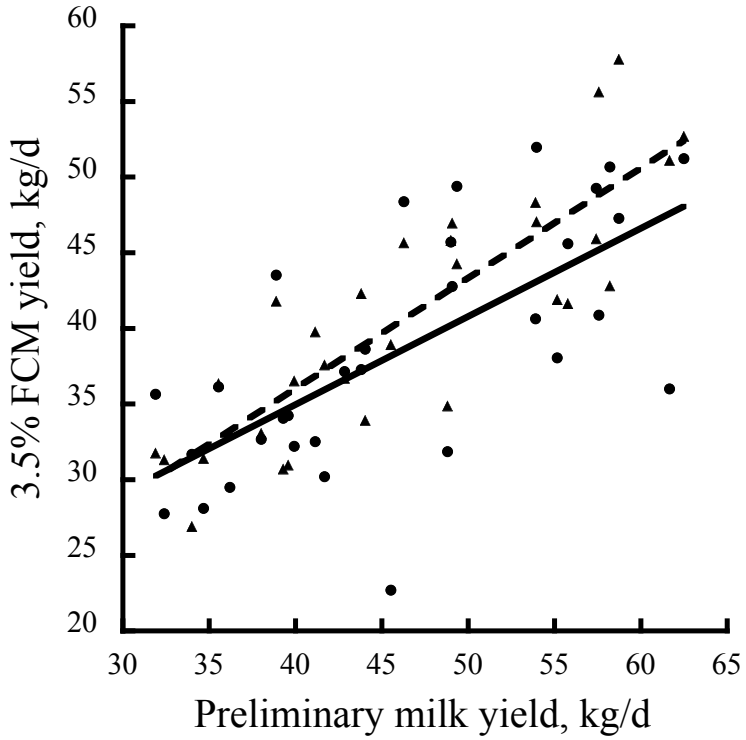

Figure 3.1. Relationship between 3.5% fat-corrected milk (3.5% FCM) and preliminary milk yield of cows fed treatment diets. Cows were either fed a control (CONT; with 2% of diet DM as added soyhulls; n = 32; 3.5% FCM (kg/d) = 11.7 + 0.582 × pMY (kg/d); R^2 = 0.45; $P < 0.0001$; solid line) or a stearic acid-supplemented diet (SA; with 2% of diet DM as stearic acid; 98% C18:0; n = 32; 3.5% FCM (kg/d) = 6.81 + 0.730 × pMY (kg/d); $R^2 = 0.73$; $P < 0.0001$; dashed line).

ECM 1.9kg/d ($P < 0.01$) compared with CONT. The SA treatment did not affect the concentration of milk components, but increased yields of fat 70 g/d ($P < 0.01$), protein 50 g/d ($P = 0.02$), and lactose 90 g/d ($P = 0.02$) and tended to decrease MUN. The SA treatment did not affect feed efficiency (FE; 3.5% FCM/DMI), BW, or BCS compared with CONT. Interactions were detected between preliminary milk yield and treatment for milk fat concentration (quadratic, $P < 0.09$) and BW (linear, $P < 0.07$) but effects were small. Period by treatment interactions ($P \leq 0.15$) were detected for yields of milk and milk components indicating that period 2 was entirely responsible for the overall treatment effects (Table 3.5).

We evaluated linear and quadratic interactions between DIM and treatment for DMI and yields of milk, 3.5% FCM, and components to test whether there was an interaction between DIM and treatment; however, we did not detect a relationship between these responses and DIM.

Table 3.5. Treatment by period interactions for DMI and yields of milk and milk components for cows fed treatment diets (n = 32)

	Trt,[1] Period 1		Trt, Period 2		
Item	CONT	SA	CONT	SA	SEM
DMI, kg/d	27.1	27.1	23.3	25.0	0.59
Yield, kg/d					
Milk	42.2	42.0	34.8	38.5	1.00
Fat	1.49	1.45	1.21	1.40	0.05
Protein	1.24	1.23	1.04	1.15	0.03
Lactose	2.05	2.04	1.68	1.87	0.05
3.5% FCM	42.4	41.6	34.7	39.4	1.08
ECM	41.9	41.2	34.5	38.9	1.06

[1]Trt = dietary treatment. Treatments were either control (CONT; with 2% of diet DM as added soyhulls) or a stearic acid-supplemented diet (SA; with 2% of diet DM as stearic acid; 98% C18:0).

Total-tract digestibility and plasma metabolites and hormones

Treatment did not interact with preliminary milk yield to affect DM, OM, NDF, CP, or 16-carbon FA total-tract digestibility (Table 3.6). A tendency for a quadratic interaction between preliminary milk yield and treatment was detected for starch total-tract digestibility ($P < 0.15$); SA increased starch digestibility in high and low producing cows but decreased it in cows producing between 40 and 60 kg of milk a day compared with CONT, although differences were small.

The SA treatment did not affect DM, OM, or starch digestibility, but increased CP digestibility (67.4 vs. 65.5%, $P < 0.01$), and tended to increase NDF digestibility (43.6 vs. 42.3%, $P = 0.10$) compared with CONT. Tendencies for linear interactions between

Table 3.6. Total-tract digestibility for cows fed treatment diets (n = 32)

	Trt[1]			Significance, *P*-values[2]					
Item, %	CONT	SA	SEM	Trt	Trt x Period	pMY	pMY x Trt	pMY x pMY	pMY x pMY x Trt
DM	64.0	64.8	0.44	0.13	0.31	0.34	0.49	0.40	0.93
OM	65.3	65.9	0.43	0.22	0.38	0.34	0.56	0.38	0.86
NDF	42.3	43.6	0.66	0.10	0.97	0.85	0.86	0.23	0.78
CP	65.5	67.4	0.62	<0.01	0.35	0.12	0.30	0.88	0.72
Starch	95.5	95.2	0.26	0.42	0.56	0.85	0.19	0.46	0.13
Total FA	76.1	56.6	1.47	<0.0001	0.22	0.22	0.14	0.44	0.83
16-carbon FA	76.2	75.9	1.10	0.79	0.03	0.07	0.28	0.63	0.86
18-carbon FA	79.1	55.3	1.54	<0.0001	0.32	0.26	0.15	0.45	0.83

[1]Trt = dietary treatment. Treatments were either control (CONT; with 2% of diet DM as added soyhulls) or a stearic acid-supplemented diet (SA; with 2% of diet DM as stearic acid; 98% C18:0).
[2]pMY = preliminary milk yield.

treatment and preliminary milk yield were detected for digestibility of total FA and 18-carbon FA; while SA decreased digestibility of total FA (56.6 vs. 76.1%, $P < 0.0001$) and 18-carbon FA (55.3 vs. 79.1%, $P < 0.0001$) at all levels of milk production compared with CONT, the difference between treatments decreased as milk yield increased. The SA treatment did not affect 16-carbon FA digestibility compared with CONT. Total FA digestibility was related quadratically to total FA intake for both treatment diets (Figure 3.2A); digestibility of FA increased as FA intake increased up to ~700 g/d for cows fed CONT, but then it remained ~80 to 85% ($R^2 = 0.48$; $P = 0.003$). In contrast, FA digestibility was less than 70% for all cows when fed SA and initially decreased as FA intake increased from 800 to 1,000 g/d fed SA and then increased over 1,200 g/d ($R^2 = 0.20$; $P = 0.05$). At similar total FA intakes (~900 g/d), total FA digestibility for cows in the CONT treatment was approximately 30% units higher than for cows in the SA treatment. A period by treatment interaction was detected for 16-carbon FA digestibility ($P = 0.03$); SA decreased 16-carbon FA digestibility during period 1 (77.4 vs. 81.9 ± 1.55%), but increased it during period 2 (74.3 vs. 70.5 ± 1.55%).

Preliminary milk yield interacted with treatment for intake and absorption of total FA and 18-carbon FA and tended to interact for intake and absorption of 16-carbon FA (both $P < 0.05$; Table 3.7). Interactions indicated that differences between treatments for intakes and absorption of FA in cows with lower milk production was less than for cows with higher milk production. As expected, SA increased total FA (1213 vs. 692 g/d, $P < 0.0001$) and 18-carbon FA (1052 vs. 542 g/d, $P < 0.0001$) intakes compared with CONT. The SA treatment also increased total FA and 18-carbon FA absorbed ($P < 0.0001$). Total FA absorbed was related quadratically to total FA intake for both treatment diets, and as FA intake increased

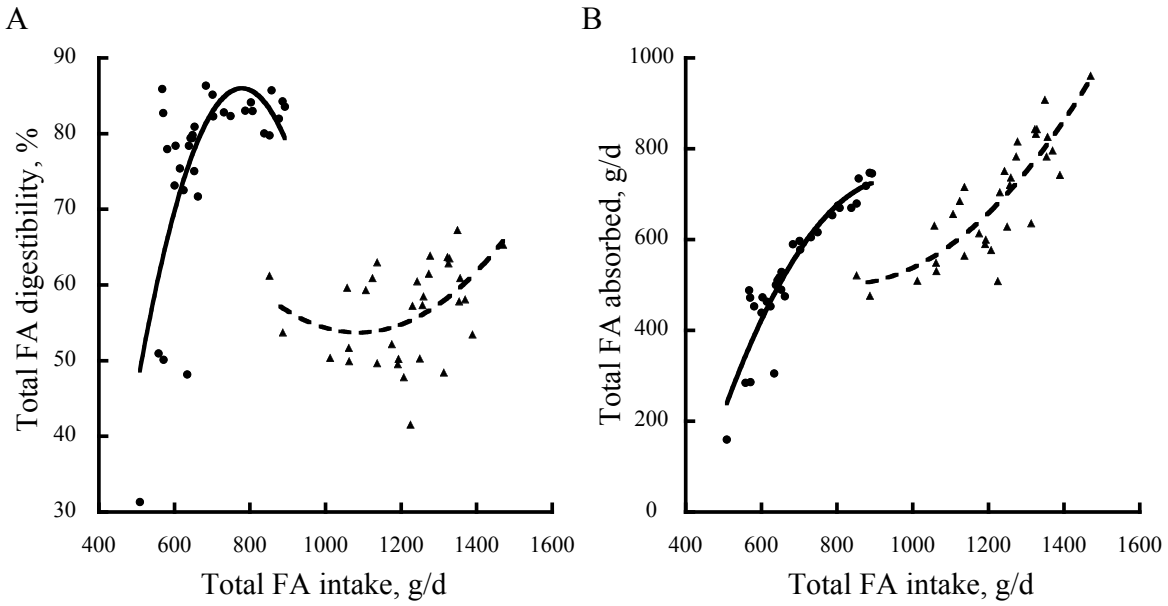

Figure 3.2. Relationship between total FA digestibility and absorbed and total FA intake of cows fed treatment diets. Panel A: Relationship between total FA digestibility and total FA intake of cows fed either control (CONT; with 2% of diet DM as added soyhulls; n = 32; Total FA digestibility (%) = 240 – 0.178 × total FA intake (g/d) – 0.0005 × (total FA intake (g/d) – 952)^2; R^2 = 0.48; *P* = 0.003; solid line) or a stearic acid-supplemented diet (SA; with 2% of diet DM as stearic acid; 98% C18:0; n = 32; Total FA digestibility (%) = 76.1 – 0.022 × total FA intake (g/d) – 0.00008 × (total FA intake (g/d) – 952)^2; R^2 = 0.20; *P* = 0.05; broken line). Panel B: Relationship between total FA absorbed and total FA intake of cows fed either CONT (n = 32; Total FA absorbed (g/d) = 738 – 0.005 × total FA intake (g/d) – 0.003 × (total FA intake (g/d) – 952)^2; R^2 = 0.86; *P* = 0.01; solid line) or SA (n = 32; Total FA absorbed (g/d) = 240 – 0.295 × total FA intake (g/d) – 0.001 × (total FA intake (g/d) – 952)^2; R^2 = 0.71; *P* = 0.04; broken line).

Table 3.7. Total FA intake and absorbed for cows fed treatment diets (n = 32)

	Trt[1]				Significance, *P*-values[2]		
Item, %	CONT	SA	SEM	Trt	Trt x Period	pMY	pMY x Trt
Total FA intake, g/d	692	1213	15.1	<0.0001	0.82	0.01	<0.01
16-carbon intake, g/d	114	118	1.88	<0.01	0.27	0.01	0.14
18-carbon intake, g/d	542	1052	12.8	<0.0001	0.65	0.01	<0.01
Total FA absorbed, g/d	534	689	15.4	<0.0001	0.72	0.29	0.02
16-carbon FA absorbed, g/d	87.4	89.5	1.98	0.21	0.05	0.30	0.12
18-carbon FA absorbed, g/d	435	584	13.0	<0.0001	1.00	0.26	0.02

[1]Trt = dietary treatment. Treatments were either control (CONT; with 2% of diet DM as added soyhulls) or a stearic acid-supplemented diet (SA; with 2% of diet DM as stearic acid; 98% C18:0).
[2]pMY = preliminary milk yield.

FA absorbed increased for both diets (CONT: $R^2 = 0.86$, $P = 0.01$; SA: $R^2 = 0.71$, $P = 0.04$; Figure 3.2B). At similar total FA intakes (~900 g/d), cows in the CONT treatment absorbed approximately 200 g/d more FA than cows in the SA treatment. An interaction between treatment and period was detected for absorption of 16-carbon FA ($P = 0.05$); SA decreased 16-carbon absorbed (97.4 vs. 103 g/d) during period 1, but increased it (81.6 vs. 72.1 g/d) during period 2.

No interactions were detected between treatment and preliminary milk production for plasma metabolites and hormones measured (Table 3.8). Moreover, treatment did not affect plasma insulin, glucagon, glucose, or NEFA concentrations ($P > 0.50$).

Table 3.8. Plasma metabolites and hormones of cows fed treatment diets (n = 32)

	Trt[1]			Significance, *P*-values	
Item	CONT	SA	SEM	Trt	Trt x Period
Insulin, μIU/mL	8.62	8.66	0.39	0.89	0.75
Glucagon, pg/mL	141	141	4.81	0.72	0.36
Insulin:glucagon	0.062	0.063	0.003	0.57	0.32
Glucose, mg/dL	56.1	55.8	0.52	0.51	0.65
NEFA, μEq/L	83.7	85.3	3.02	0.51	0.65

[1]Trt = dietary treatment. Treatments were either control (CONT; with 2% of diet DM as added soyhulls) or a stearic acid-supplemented diet (SA; with 2% of diet DM as stearic acid; 98% C18:0).

Milk FA profile and yields

In general, there were no interactions between treatment and preliminary milk yield for FA profile (Table 3.9). The SA treatment tended to decrease the concentration of FA from de novo synthesis ($P = 0.09$), decreased that of mixed-source ($P < 0.0001$), but increased preformed FA concentration in milk ($P < 0.001$) compared to CONT. Interactions between treatment and level of milk production indicated that high producing cows

responded more positively to SA than low producing cows in terms of FA yields (Table 3.10) compared with CONT. The SA treatment increased yields of preformed FA (515 vs. 471 g/d, $P < 0.0001$) and FA from de novo synthesis (359 vs. 344 g/d, $P = 0.03$) in milk, but did not affect yield of mixed-source FA compared with CONT. Period by treatment interactions were detected for the yield of several FA in milk and indicate that period 2 was responsible for the overall treatment effects.

FAYR to additional FA intake

Fatty acid yield response to additional FA intake was not related to preliminary milk yield when calculated for either total FA or for C18:0 plus *cis*-9 C18:1. For each additional 100 g intake of total FA, milk FA increased by 13.3 g compared with CONT. When only C18:0 plus *cis*-9 C18:1 were considered, for each additional 100 g intake of these FA, C18:0 plus *cis*-9 C18:1 increased by 8.2 g compared with CONT.

Table 3.9. Milk FA concentrations[1] of cows fed treatment diets (n = 32)

	Trt[2]				Significance, *P*-values[3]				
Item, g/100 g	CONT	SA	SEM	Trt	Trt x Period	pMY	pMY x Trt	pMY x pMY	pMY x pMY x Trt
Summation by Source[4]									
De novo	27.1	26.8	0.25	0.09	0.85	0.77	0.38	0.41	0.68
Mixed	35.6	34.4	0.44	<0.0001	0.42	0.16	0.37	0.92	0.53
Preformed	37.3	38.8	0.52	<0.001	0.54	0.18	0.9	0.62	0.84
Select Individual Fatty Acids									
4:0	3.02	3.06	0.04	0.08	0.03	0.07	0.38	0.10	0.57
6:0	2.11	2.13	0.02	0.10	0.03	0.05	0.11	0.69	0.54
8:0	1.29	1.30	0.02	0.67	0.17	0.25	0.22	0.47	0.32
10:0	3.37	3.31	0.06	0.21	0.83	0.92	0.42	0.19	0.50
12:0	3.99	3.87	0.08	0.06	0.67	0.72	0.67	0.13	0.68
14:0	12.5	12.3	0.11	0.01	0.57	0.75	0.45	0.75	0.93
14:1 *cis*-9	0.874	0.849	0.03	0.03	0.23	0.73	0.94	0.35	0.63
16:0	34.1	33.0	0.44	0.0001	0.47	0.15	0.36	0.94	0.59
16:1 *cis*-9	1.56	1.45	0.05	<0.001	0.25	0.88	0.61	0.01	0.11
18:0	9.38	10.4	0.23	<0.0001	0.72	0.73	0.68	0.26	0.93
18:1 *trans*[5]	1.66	1.61	0.05	0.12	0.93	0.59	0.17	0.86	0.27
18:1 *cis*-9	16.7	17.6	0.31	<0.001	0.37	0.22	0.65	0.62	0.87

[1]A total of approximately 80 individual FA were quantified and used for calculations (summation by source and concentrations). Only select FA are reported in the table.
[2]Trt = dietary treatment. Treatments were either control (CONT; with 2% of diet DM as added soyhulls) or a stearic acid-supplemented diet (SA; with 2% of diet DM as stearic acid; 98% C18:0).
[3]pMY = preliminary milk yield.
[4]De novo FA originate from mammary de novo synthesis (<16 carbons), preformed FA originate from dietary or mobilized FA extracted from plasma (>16 carbons), and mixed FA originate from both sources (C16:0 plus *cis*-9 C16:1).
[5]Total 18:1 *trans* FA.

Table 3.10. Milk FA yield[1] of cows fed treatment diets (n = 32)

	Trt[2]			Significance, *P*-values[3]					
Item, g/d	CONT	SA	SEM	Trt	Trt x Period	pMY	pMY x Trt	pMY x pMY	pMY x pMY x Trt
Summation by Source[4]									
De novo	344	359	9.55	0.03	0.07	<0.0001	0.04	0.58	0.87
Mixed	451	461	13.8	0.14	0.22	<0.0001	0.12	0.77	0.43
Preformed	471	515	12.1	<0.0001	0.07	<0.0001	0.05	0.93	0.63
Select Individual Fatty Acids									
4:0	38.5	41.0	1.10	<0.01	0.01	<0.0001	0.04	0.58	0.51
6:0	26.9	28.7	0.83	<0.01	0.03	<0.0001	0.02	0.94	0.59
8:0	16.5	17.5	0.53	<0.01	0.05	<0.0001	0.03	0.59	0.91
10:0	42.7	44.5	1.51	0.07	0.13	<0.001	0.07	0.34	0.87
12:0	50.4	51.9	1.75	0.20	0.20	0.001	0.15	0.27	0.79
14:0	158	164	4.10	0.03	0.10	<0.0001	0.04	0.69	0.79
14:1 *cis*-9	10.9	11.3	0.50	0.14	0.86	<0.01	0.27	0.30	0.67
16:0	433	443	13.3	0.13	0.22	<0.0001	0.12	0.83	0.44
16:1 *cis*-9	18.3	18.4	0.75	0.75	0.55	<0.01	0.23	0.06	0.45
18:0	119	139	4.30	<0.0001	0.25	<0.001	0.02	0.63	0.37
18:1 *trans*[5]	20.9	21.3	0.59	0.43	0.11	<0.0001	0.07	0.74	0.80
18:1 *cis*-9	212	233	6.01	<0.001	0.06	< 0.0001	0.15	0.92	0.72

[1]A total of approximately 80 individual FA were quantified and used for calculations (summation by source). Only select FA are reported in the table.
[2]Trt = dietary treatments. Treatments were either control (CONT; with 2% of diet DM as added soyhulls) or a stearic acid-supplemented diet (SA; with 2% of diet DM as stearic acid; 98% C18:0).
[3]pMY = preliminary milk yield.
[4]De novo FA originate from mammary de novo synthesis (<16 carbons), preformed FA originate from dietary or mobilized FA extracted from plasma (>16 carbons), and mixed FA originate from both sources (C16:0 plus *cis*-9 C16:1) (Bauman and Griinari, 2003).
[5]Total 18:1 *trans* FA.

DISCUSSION

We conducted three experiments to evaluate the effects of palmitic and stearic acids on cow performance, each of which used very pure supplements (≥ 98% pure palmitic and/or stearic acids) and cows with a wide range of milk production. We chose to use cows with a wide range of milk production to evaluate if cows of different production levels, and therefore, physiological states would respond differently to the same diet. Piantoni et al. (2013) compared a highly pure palmitic acid supplement (99% C16:0) with a control diet with no supplemental fat, while Rico et al. (2014) compared the same FA supplement with a highly pure stearic acid supplement (98% C18:0). The experiment described herein is the third of these experiments, and compared the highly pure stearic acid supplement used by Rico et al. (2014) with a control diet with no supplemental fat. Milk yield measured during the covariate period was used as a covariate in all three experiments because this information is readily available to the producer and can be used for grouping and feeding cows.

Interaction between stearic acid supplementation and level of production

We previously reported that palmitic acid supplementation did not interact with level of milk production, and therefore, cows with a wide range of milk production responded similarly to treatment (Piantoni et al., 2013). However, production responses to stearic acid supplementation did vary by level of milk production in the current experiment, which signifies the importance of testing pure sources of FA at different stages of lactation. To our knowledge, responses to stearic acid have not been previously reported using cows at different levels of production or high producing cows. In this experiment, higher yielding cows responded more favorably to SA than to CONT for DMI and yields of milk and milk

components compared with lower yielding cows. We were not able to discern if the increase in DMI followed the increase in milk production or vice versa. In a recent literature review, Bionaz et al. (2013) indicated that FA can affect gene expression, and more specifically gluconeogenesis. White et al. (2011) examined the effect of different FA on gene expression of rat hepatoma cells transfected with specific bovine promoters and showed that stearic acid decreased expression of pyruvate carboxylase, a key gluconeogenic enzyme. Even though results from that experiment suggest stearic acid would decrease milk yield through a decrease in gluconeogenesis, which is opposite to what we observed in the current experiment, a potential effect of stearic acid on gene expression at the level of mammary gland or liver cannot be ruled out.

Since one of the objectives of this study was to evaluate linear or quadratic relationships between preliminary milk yield (measured during the covariate period) and treatment, cows with a wide range and uniform distribution of milk yield were selected from the herd so there were no distinct groups (e.g. high and low milk yield groups) to compare statistically. However, we calculated the response to CONT and SA for cows using the minimum, mean, and maximum preliminary milk yield (Table 3.1; 31.9, 46.1, and 62.5 kg/d) using the equations of the fitted lines between response variable and preliminary milk yield by diet (see Figure 3.1). Compared with CONT, SA increased DMI and yields of milk, milk fat and protein, and 3.5% FCM for cows producing 62.5 kg/d and 46.1 kg/d. In contrast, SA did not affect DMI and yields of milk, milk fat and protein, and 3.5% FCM for cows producing 31.9 kg/d, compared with CONT (Table 3.4).

Interactions between treatment and preliminary milk yield that were not discussed were small and likely not biologically meaningful. In addition, we reported interactions that

were significant or tended to be significant between treatment and period for several responses measured, including yields of milk and milk components. Unfortunately, we could not determine the reasons for these interactions. However, interactions between period and treatment were detected only when preliminary milk yield was included in the model as a covariate; when preliminary milk yield was removed from the model, interactions between period and treatment were not significant ($P > 0.15$).

Production responses to stearic acid supplementation

Overall, SA increased milk yield and milk fat yield, which is in agreement with previous findings reported by Steele (1969), who fed a stearic acid supplement (85% pure) at approximately 2.1% of diet DM and showed that stearic acid not only increased milk yield by 1.2 kg/d, but also milk fat yield by 41 g/d compared with a control diet with no supplemental fat. However, Steele (1969) used low producing cows (~12 kg/d), and considering the interaction with level of production reported in the current experiment, we would not have expected a positive result in those cows. In contrast and consistent with our results, duodenal infusions of stearic acid (92.3% pure) did not affect milk production or total FA concentration in milk in cows producing less than 30 kg of milk daily (Enjalbert et al., 2000).

The SA treatment also increased DMI and 3.5% FCM but did not affect FE or BCS in the current experiment. Rabiee et al. (2012) conducted a meta-analysis of treatment means from the literature and concluded that fat feeding can improve FE through decreased DMI and increased milk yield and milk fat yield. However, in that meta-analysis, FE was not calculated separately for each study, which could have altered the conclusion reached. Nevertheless, and in agreement with Rabiee et al. (2012), several experiments have reported

an increase in FE when long-chain saturated FA sources were fed to dairy cows (Wang et al., 2010; Lock et al., 2013; Piantoni et al., 2013). Lack of treatment effect on BCS observed in this experiment is consistent with the lack of treatment effects on metabolic and hormonal profiles. Effects of supplemental saturated FA on metabolic response have been inconsistent and are likely dependent upon physiological state of cows (Harvatine and Allen, 2006).

Stearic acid increased DMI in the current experiment, and DMI response increased with milk yield of cows. Consistent with our findings, Mosley et al. (2007) showed that a palmitic acid supplement (> 85% pure) fed at 500 g/d increased DMI by 3.1 kg/d compared with a control diet with no supplemental fat. A statistical analysis of treatment means from the literature indicated that saturated FA supplements had little or no effect on DMI, but none of the supplements evaluated were pure stearic or palmitic acid supplements (Allen, 2000). Consistent with this, our companion studies reported that a highly pure source of palmitic acid did not affect DMI compared with a control diet with no supplemental fat (Piantoni et al., 2013) or with stearic acid (Rico et al., 2014). Moreover, FA supplements did not interact with preliminary milk yield to affect DMI in either experiment. Different DMI responses within the set of experiments might be related to the different FA (palmitic or stearic) and diets fed: experiments were done in different years and forage NDF of the diets reported by Piantoni et al. (2013) and Rico et al. (2014) was 19.1%, which is 5.4 percentage units lower than the one reported in this experiment. Diets for all three experiments were formulated to contain 30% NDF, but diets fed in this experiment resulted in slightly higher NDF concentrations.

Fatty acid yield response and digestibility of nutrients

We calculated FAYR to evaluate the efficiency of utilization of the stearic acid supplement for milk fat yield. In agreement with Enjalbert et al. (2000) we observed a lower FAYR to additional dietary stearic acid, compared with additional dietary palmitic acid (Piantoni et al., 2013). Enjalbert et al. (2000) reported an apparent FAYR of 46.7% for palmitic acid and 12.0% for stearic acid, both infused in the duodenum, while we reported a FAYR of 16.6% for palmitic acid (Piantoni et al., 2013) and 8.2% for stearic acid in this study. Other experiments that calculated FAYR to palmitic acid supplementation also reported lower FAYR than that of Enjalbert et al. (2000): Lock et al. (2013) reported a FAYR of 29.7%, while Warntjes et al. (2008) reported a FAYR of 16.5% in a field study. The 18-carbon FAYR of 8.2% in this experiment was approximately one half of that reported for 16-carbon FA in Piantoni et al. (2013) and the difference observed is likely because of the digestibility and tissue availability of the FA itself or the different prill size of the FA supplements fed; the prill size of the palmitic acid supplement was smaller and much more uniform than the stearic acid supplement [0.89 ± 0.14 vs. 0.98 ± 0.39 mm (mean ± SD)], which could have affected absorption of the FA. The calculated digestibility for the palmitic acid supplement used by Piantoni et al. (2013) was 48.1%, while the calculated digestibility for the stearic acid used in this experiment was only 28.4%. However, utilization of absorbed 18-carbon FA was also low so a large proportion of the stearic acid absorbed was likely oxidized by extra-hepatic tissues decreasing its export in milk but sparing other fuels for milk synthesis.

Organic matter digestibility was not altered by treatment diets, which is consistent with previous experiments and the notion that saturated FA supplements are inert in the

rumen (Grummer, 1988; Schauff and Clark, 1989). The SA treatment not only did not affect OM digestibility but also tended to increase NDF digestibility. Saturated fats can increase digestibility of nutrients (Piantoni et al., 2013) and this might be from release of cholecystokinin (CCK) from the duodenum (Choi et al., 2000), which can reduce gut motility and increase ruminal retention time. Piantoni et al. (2013) showed that a palmitic acid supplement increased total-tract NDF digestibility (39.0 vs. 35.7%; $P < 0.001$) compared with a control diet with no supplemental fat, and this increase was at least partially explained by the observed increase in plasma CCK concentration. In contrast, SA decreased total FA and 18-carbon FA digestibility in the current experiment. The overall decrease in FA digestibility was expected since it has been previously shown that increased FA intake is related to decreased FA digestibility (Palmquist, 1991; Piantoni et al., 2013). However, we did not expect SA would decrease total FA digestibility so markedly (56.6 vs. 76.1%). Interestingly, cows in the CONT diet with lower total FA intakes showed great variation in total FA digestibility (from 31 to 86%), and total FA intake and digestibility were positively related at lower intakes of total FA but negatively related at higher intakes of total FA for cows in CONT (Figure 3.2). In addition, variation in total FA digestibility from cows in the SA diet was not very well explained by total FA intake ($R^2 = 0.20$), but total FA digestibility and intake were positively correlated. Interactions between preliminary milk yield and treatment for intake and absorption of total and 18-carbon FA followed the DMI response pattern, and therefore, cows with higher milk production had higher intake and absorption of total and 18-carbon FA compared with cows with lower milk production when fed SA compared with CONT. Total FA absorbed increased as total FA intake increased for both CONT and SA cows. In agreement with others (Palmquist, 1991; Piantoni et al., 2013), total

FA absorbed increased at a slower rate as total FA intake increased for cows in the CONT treatment. In contrast, total FA absorbed increased at a faster rate as total FA intake increased for cows receiving the SA treatment.

Milk FA

As expected, the SA treatment increased the yield of preformed FA in milk, mainly composed of stearic acid and other 18-carbon FA varying in their degree of saturation, consistent with increased supply of preformed FA in the diet (Steele and Moore, 1968; Enjalbert et al., 2000). In addition, the interaction between preliminary milk yield and treatment detected for yield of milk FA followed the same pattern as DMI and milk yield response, so it was related to the effect of SA on intake and milk yield. While we expected SA to increase preformed FA in milk, increased de novo synthesis was not expected (Grummer et al., 1991; Enjalbert et al., 2000). Fatty acids of different carbon chain lengths appear to have preferences in positional distributions with C18:0 usually found in the first carbon of TG, while C18:1 is either found in the first or third carbon and de novo FA in carbons second and third (Parodi, 1983). The increase in short-chain FA might help maintain fluidity of milk when there is an increase in long-chain FA in milk (Barbano and Sherbon, 1980).

CONCLUSIONS

Stearic acid has the potential to increase DMI and yields of milk and milk components, without affecting FE, BCS, or BW. Moreover, stearic acid increased DMI and yields of milk and milk components more as milk yield of cows increased. Reasons why

higher yielding cows responded more positively to stearic acid supplementation than lower yielding cows could not be determined in this study. The low FAYR to stearic acid supplementation was a result of the low digestibility of the supplement and low apparent uptake of stearic acid by the mammary gland. To evaluate the potential use of a stearic acid supplement on farms, producers need to calculate the marginal economic return of the supplement, considering not only the increase in yields of milk and milk solids, but also the increase in DMI observed and the group of cows this supplement is to be fed.

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CHAPTER 4

SATURATED FAT SUPPLEMENTATION INTERACTS WITH DIETARY FORAGE NDF CONTENT DURING THE IMMEDIATE POSTPARTUM AND CARRYOVER PERIODS IN HOLSTEIN COWS: PRODUCTION RESPONSES AND DIGESTIBILITY OF NUTRIENTS

ABSTRACT

Forty-eight multiparous cows were used in a randomized complete block design experiment with a 2x2 factorial arrangement of treatments to determine the interaction between a highly saturated free FA supplement (FAT) and dietary forage NDF (fNDF) content on production responses and nutrient digestibility of dairy cows in the postpartum period. Treatment diets were offered from 1 to 29 d postpartum (postpartum period; PP) and contained 20% or 26% fNDF (50:50 corn silage:alfalfa silage and hay, dry matter basis) and 0% or 2% FAT (Energy Booster 100; 96.1% FA: 46.2% C18:0 and 37.0% C16:0). From 30 to 71 d postpartum (carryover period), a common diet (~23% fNDF, 0% FAT) was offered to all cows to evaluate carryover effects of the treatment diets early in lactation. During the PP, higher fNDF decreased dry matter intake (DMI) by 2.0 kg/d, while FAT supplementation increased it by 1.4 kg/d. In addition, high fNDF with 0% FAT decreased DMI compared with the other diets and this difference increased throughout PP. Treatments did not affect 3.5% fat-corrected milk yield during PP but did during the carryover period when FAT supplementation decreased 3.5% fat-corrected milk yield for the low fNDF diet (51.1 vs. 58.7 kg/d), but not for the high fNDF diet (58.5 vs. 58.0 kg/d). During the PP, lower fNDF and FAT supplementation decreased body condition score loss. A tendency for an interaction between fNDF and FAT indicated that low fNDF with 2% FAT decreased body condition score loss compared with the other diets (-0.49 vs. -0.89). During the PP, lower fNDF and

2% FAT supplementation decreased feed efficiency (3.5% fat-corrected milk/DMI) by 0.30 and 0.23 units, respectively. The low fNDF with 2% FAT diet decreased feed efficiency compared with other diets early in the PP, but this difference decreased over time. Supplementation of FAT in the PP favored energy partitioning to body reserves and limited DMI depression for the high fNDF diet, which might allow higher fNDF diets to be fed to cows in the PP. However, FAT supplemented in the low fNDF diet during the PP affected production negatively in the carryover period. Dietary fNDF and FAT interacted affecting performance in the PP period with carryover effects when cows were fed a common diet in early lactation.

INTRODUCTION

Following parturition cows enter a period of negative energy balance because they cannot consume enough DM to support lactation. Approaches to increase energy intake of postpartum cows include increasing starch content of the diet by decreasing dietary forage level and supplementing fat to increase the energy density of the diet. However, because of greater ruminal fermentation from high starch and less buffering from low forage, high starch diets might increase the risk of ruminal acidosis and displaced abomasum (Allen and Piantoni, 2013). Different FA, on the other hand, can affect metabolism and animal response differently. For example, unsaturated FA can depress feed intake (Allen, 2000), modulate insulin action (Pires and Grummer, 2008), and alter ruminal biohydrogenation, which can potentially induce milk fat depression (Baumgard, et al., 2002) and increase energy partitioning to body reserves (Harvatine et al., 2006a; Harvatine et al., 2009), whereas saturated FA are considered to be inert in the rumen (Grummer, 1988) and have little effect

on DMI (Allen, 2000), and can increase milk fat output (Wang et al., 2010; Lock et al., 2013; Piantoni et al., 2013). However, variation has been observed among responses to FA supplements, which is likely related to the FA profiles and physical form of the fat supplements, diet composition, and physiological states of cows.

There is scant research available on production responses to diets fed in the PP, especially regarding optimal forage level, fat supplementation, and their interaction. Rabelo et al. (2003) reported that a low forage to concentrate ratio (F:C) diet (40:60; 25% NDF) fed during the first 20 d postpartum tended to increase DMI (16.5 vs. 15.4 kg/d) and increased calculated energy intake (27.7 vs. 25.1 Mcal/d) compared with a high F:C diet (60:40; 30% NDF). Beam and Butler (1998) reported that a highly saturated (≥ 85% saturated) free FA supplement at 2.6% of diet DM in a 45% forage diet (~33% NDF) decreased yields of milk and 4% FCM during the first 4 wk postpartum and increased them during the following 2 wk on experiment. Importantly, Weiss and Pinos-Rodriguez (2009) reported that the same FA supplement used by Beam and Butler (1998), fed at 2.25% of diet DM from 21 to 126 d postpartum, affected energy partitioning differently depending on forage NDF (fNDF) content of the diets. In that experiment, supplemental fat increased BCS with no change in milk yield when supplemented in a 25% fNDF diet (60:40 F:C), but increased milk yield and DMI with no change in BCS when supplemented in a 17% fNDF diet (40:60 F:C).

Although benefits of supplementing a highly saturated free FA to cows in the immediate postpartum period were not identified in the experiment reported by Beam and Butler (1998), the interaction between the same FA supplement and dietary fNDF content reported by Weiss and Pinos Rodriguez (2009) on energy partitioning in early lactation cows deserves further investigation. Our objectives were to determine the interaction between a

highly saturated free FA supplement and dietary fNDF content on yields of milk and milk components, intake, and nutrient digestibility of dairy cows in the postpartum period and to evaluate carryover effects of the treatment diets early in lactation. We hypothesized that the saturated FA supplement would increase BCS when added to the high fNDF diet and milk yield when added to the low fNDF diet during the postpartum period, considering results reported by Weiss and Pinos-Rodriguez (2009) with cows in early lactation.

MATERIALS AND METHODS

Animal housing and care

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University (East Lansing). The experiment began on September 30th, 2011 and finished on May 1st, 2012. All cows were housed in the same tie-stall, assigned by parturition order, throughout the entire treatment period. Cows were fed once daily (1000 h) at 120% and 110% of expected intake during the treatment and carryover periods, respectively, and milked twice daily (0400 and 1430 h). The amounts of feed offered and orts were weighed for each cow daily. Standard reproduction and health herd checks and breeding practices were maintained during this study.

Design and treatment diets

Forty-eight multiparous Holstein cows at the Michigan State University Dairy Field Laboratory were used in a randomized complete block design experiment with a 2x2 factorial arrangement of treatments with 12 cows per treatment. Cows were blocked by date of parturition (within 90 d), BCS (up to 1 unit difference using a 5-point scale, where 1 = thin

and 5 = fat; Wildman et al. (1982)), and previous lactation 305-d mature-equivalent milk production (MEq; within 5,500 kg). The BCS used to block cows was the last measurement before parturition. Cows within each block were randomly assigned to treatment on expected parturition date. Treatment diets were offered from d 1 to 29 postpartum (postpartum period; PP). Treatments contained 20% or 26% fNDF and 0% or 2% saturated free FA supplement (FAT; Energy Booster 100 ®; 96.1% FA: 46.2% C18:0 and 37.0% C16:0; Milk Specialties Global, Eden Prairie, MN). Desired fNDF content of the treatment diets were attained by altering proportions of forages (alfalfa and corn silages and alfalfa hay) and concentrates (corn grain and soybean meal). Starch content was ~24% for the low fNDF diets and ~17.5% for the high fNDF diets, and dietary CP content was held constant across diets. The FA supplement was added at 2% of diet DM, replacing 2% of soyhulls in the 0% FAT diet. Treatment diets were mixed daily in a tumble-mixer and were fed from the morning following parturition. From d 30 to 71 postpartum (carryover period), all cows were offered a common diet, mixed daily in a mixer wagon. The ingredient and nutrient composition of the diets fed as TMR, including close up ration for reference, are described in Table 4.1. All rations were formulated to meet or exceed cows predicted requirements for protein, minerals, and vitamins according to NRC (2001).

Data and sample collection

All samples and body measurements were collected or recorded on the same day of the week during the entire experiment (days 5, 12, 19, 26, 33, 40, 47, 54, 61, and 68 postpartum), so all collection days are ±3 d relative to the first day on the treatment diet. Milk yield and feed offered and refused were recorded daily throughout the entire experiment.

Table 4.1. Ingredient and nutrient composition of close up, treatment, and carryover diets

		Treatment diet[1]				
		20% fNDF		26% fNDF		
Item	Close up diet	0% FAT	2% FAT	0% FAT	2% FAT	Carryover diet
Ingredients, % of DM						
Corn silage	42.5	21.9	21.7	28.2	28.4	29.9
Grass hay	25.4	-	-	-	-	-
Alfalfa silage	-	16.5	16.7	22.0	21.6	17.6
Chopped alfalfa hay	-	4.93	4.96	6.25	6.23	4.51
Dry ground corn	7.95	24.4	24.2	12.7	12.5	23.7
Soybean meal	14.0	12.4	12.8	11.1	11.6	9.58
Cottonseed w/lint	-	-	-	-	-	6.60
SoyChlor®[2]	2.29	-	-	-	-	-
SoyPlus®[2]	-	4.90	4.90	4.86	4.87	3.67
Soyhulls	-	9.55	7.49	9.44	7.55	0.00
Vitamin-mineral mix[3,4,5]	7.84	5.42	5.41	5.43	5.42	4.45
Saturated free FA[6]	-	-	1.90	-	1.91	-
Nutrient composition						
DM, %	57.6	57.3	57.1	51.5	51.6	53.7
OM, % of DM	92.1	93.2	93.3	92.7	92.8	92.2
NDF, % of DM	40.9	31.0	29.8	35.8	34.5	29.5
Forage NDF, % of DM	35.9	20.0	19.9	25.9	25.8	22.7
Starch, % of DM	18.1	24.2	23.8	17.6	17.3	25.6
CP, % of DM	13.6	17.4	17.4	17.4	17.3	17.7
EE, % of DM	2.39	2.83	4.61	2.74	4.53	3.92
Gross energy, Mcal/kg of DM	ND[7]	4.39	4.49	4.42	4.52	ND
Particle size distribution,[8] % of TMR (as DM) retained on sieves						
Upper sieve, particles >19 mm	24.9	7.16	8.40	10.7	10.8	13.1
Middle sieve, particles >8 mm	31.8	35.2	32.3	38.8	39.8	38.4
Bottom sieve, particles >1.18 mm	37.4	42.5	42.0	37.6	36.3	38.4
Bottom pan, particles <1.18 mm	5.92	15.2	17.3	12.9	13.1	10.1

[1]Treatment diets were either 20 or 26% forage NDF (fNDF) and 0 or 2% saturated free FA supplement (FAT), and were fed from d 1 to 29 postpartum. Close up diet was fed from d -14 of expected calving date until calving date. Carryover diet was fed from d 30 to 71 postpartum.
[2]West Central Soy, Ralston, IA.
[3]Vitamin-mineral mix for the close up diet contained (DM basis): 54.8% SoyChlor®, 13.9% limestone, 10.0% rumen-protected choline, 8.8% Ca 23%: P 18%, 4.2% magnesium sulfate, 1.8% salt, 1.8% yeast, 4.4% trace minerals and vitamins, and 0.3% selenium yeast 600 (600 mg of Se/kg).
[4]Vitamin-mineral mix for the treatment diets contained (DM basis): 27.9% molasses, 15.3% limestone, 12.2% sodium bicarbonate, 11.8% blood meal, 8.7% dicalcium phosphate, 6.1% trace minerals and vitamins, 5.7% rumen-protected choline, 4.4% magnesium sulfate, 3.9% salt, 2.7% animal fat, 0.9% yeast, and 0.4% selenium yeast 600 (600 mg of Se/kg).
[5]Vitamin-mineral mix for the carryover diet contained (DM basis): 30.1% limestone, 25.3% sodium bicarbonate, 10.1% salt, 7.1% urea, 6% potassium chloride, 6% dicalcium phosphate, 5.7% animal fat, 5.7% magnesium sulfate, 3.9% trace minerals and vitamins, and 0.2% selenium yeast 600 (600 mg of Se/kg).
[6]Energy Booster 100® (Milk Specialties Global, Eden Prairie, MN): 96.1% FA (46.2% C18:0, 37.0% C16:0, 3.96% C18:1 9c, 2.66% C14:0, and others <2% each).
[7]Not determined.
[8]Particle size of TMR was evaluated with the Penn State Forage Particle Separator.

Samples of all diet ingredients (0.5 kg) and orts from each cow (~12.5%) were collected weekly during the entire experiment and stored in plastic bags at -20°C until processed. On d 5, 12, 19, and 26 of PP, fecal samples (500 g) were collected every 6 h, representing every 6 h of a 24-h period to account for diurnal variation, for nutrient digestibility analysis. Feces were stored in a sealed plastic cup at -20°C until dried. During the entire experiment, milk samples were collected weekly at each milking and stored with preservative at 4°C for component analysis (Universal Lab Services, East Lansing, MI). An additional milk sample was collected at each milking on d 5, 12, 19, and 26 of PP and stored without preservative at -20°C for determination of FA profile. Body weight and BCS were recorded weekly from d -9 of expected parturition day and during the entire experiment. Body condition was scored by 3 trained investigators on a 5-point scale, as described by Wildman et al. (1982).

Sample analysis

Feed, orts, and fecal samples were dried in a 55°C forced-air oven for 72 h to determine DM content. Before drying, ingredients from the close up and carryover diets were composited; concentrates were composited every 4 wk and forages biweekly. All feed ingredients of the treatment diets were analyzed by week for nutrient composition. Orts were dried to calculate DMI on collection days, but only orts collected during the PP were processed further and analyzed for nutrient composition. Once dried, samples of feed ingredients, and orts and feces collected during the PP period, were ground in a Wiley mill (1-mm screen; Arthur H Thomas Co., Philadelphia, PA) and analyzed for ash, NDF, indigestible NDF, CP, and starch. All samples taken during PP were also analyzed for gross energy. Feed ingredients collected during the PP were composited by month and analyzed for

ether extract. Feces were composited by cow by day on an equal DM basis before analysis. All nutrients are expressed as percentages of DM, determined by drying at 105°C in a forced-air oven for more than 8 h. Ash content was determined after 5 h of oxidation at 500°C. Content of NDF was determined according to Mertens (2002). Indigestible NDF, which was used as an internal marker to estimate fecal output and nutrient digestibility (Cochran et al., 1986), was estimated as NDF residue after 240 h in vitro fermentation (Goering and Van Soest, 1970); flasks were reinoculated at 120 h to ensure a viable microbial population. Ruminal fluid for the in vitro incubations was collected from a nonpregnant dry cow fed dry hay only. Crude protein was determined according to Hach et al. (1987). Starch was gelatinized with sodium hydroxide and hydrolyzed using an enzymatic method (Karkalas, 1985); glucose was then measured using a glucose oxidase method (PGO Enzyme Product No. P7119; Sigma Chemical Co., St. Louis, MO) and by determination of absorbance with a microplate reader (SpectraMax 190; Molecular Devices Corp., Sunnyvale, CA). Ether extract was determined according to AOAC International (2005; method 920.39). Gross energy was assayed by bomb calorimeter (Parr Instrument Inc., Moline, IL). Oven-dried samples of all six TMR fed during this experiment (close up diet, treatment diets, and carryover diet) were composited by month and evaluated for particle size distribution using the Penn State Forage Particle Separator in duplicate (Lammers et al., 1996).

Milk samples stored with preservative were analyzed for fat, true protein, lactose, MUN, and SCC by infrared spectroscopy (AOAC International, 1997), by the Michigan Herd Improvement Association (Universal Lab Services). Milk samples stored without preservative were composited by milk fat yield and centrifuged at 17,800 × g for 30 min at 4°C to collect the fat cake. Lipids were extracted according to Hara and Radin (1978) and

FAME prepared according to Christie (1989). Quantification of FAME was performed using a GC-2010 Plus gas chromatograph (Shimadzu, Kyoto, Japan) as described by Lock et al. (2013). A total of approximately 80 individual FA were quantified per sample and used for summations. Yields of individual FA in milk fat were calculated by correcting for glycerol content according to Schauff et al. (1992), and other milk lipid classes according to Glasser et al. (2007).

Statistical analysis

Data were analyzed separately for PP (from 1 to 29 d postpartum) and for the carryover period (from 30 to 71 d postpartum). All weekly data were analyzed using the MIXED procedure of SAS v.9.2 (SAS Institute, Inc. Cary, NC) according to the following model with repeated measures:

$$Y_{ijklm} = \mu + B_i + C(B_jK_kS_l)_j + K_k + S_l + K_kS_l + T_m + K_kT_m + S_lT_m + K_kS_lT_m + J + e_{ijklm}$$

where μ = overall mean, B_i = random effect of block (i= 1 to 12), $C(B_jK_kS_l)_j$ = random effect of cow (j = 1 to 4) within block and treatment diet, K_k = fixed effect of fNDF (k = 1 to 2), S_l = fixed effect of FAT (l = 1 to 2), K_kS_l = interaction between fNDF and FAT, T_m = fixed effect of week (m = 1 to 4), K_kT_m = interaction between fNDF and week, S_lT_m = interaction between FAT and week, $K_kS_lT_m$ = interaction between fNDF, FAT, and week, J = random effect of Julian date, e_{ijklm} = residual error. Unless otherwise specified, first-order autoregressive was the covariate structure used for analysis because it resulted in the lowest Bayesian information criterion for most of the variables measured. Interactions with time

were removed from the model when non significant and a reduced model was used to determine treatment effects. However, all interactions were included in the tables for informational purposes.

Treatment differences within week were analyzed using the GLIMMIX procedure of SAS v 9.2 (SAS Institute) and the SLICE option. The model included the random effects of block and cow nested within block and treatment and the fixed effects of fNDF and FAT and their interaction. The Bonferroni adjustment was applied to decrease the probability of type I error when multiple comparisons were done. Cumulative milk yield and DMI and BW and BCS changes were analyzed using the MIXED procedure of SAS v 9.2 (SAS Institute) with the same model used in the GLIMMIX procedure.

Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. Significance was determined at $P \leq 0.05$ for main effects and $P \leq 0.10$ for interactions. Tendencies were determined at $P \leq 0.10$ for main effects and $P \leq 0.15$ for interactions. All cows were in apparent good health at the beginning of the study, and treatment groups were not different in terms of 305-d MEq ($P = 0.48$), BW ($P = 0.39$), or BCS ($P = 0.33$) pre-calving (Table 4.2). One of the cows on the 26% fNDF 2% FAT diet had a displaced abomasum and underwent surgery on d 59 of the study. Therefore, the data of the last two weeks and the cumulative milk yield and cumulative DMI of the carryover period were excluded from the statistical analyses. All other data were included. Table 4.3 summarizes all health incidents during treatment and carryover periods for reference.

Table 4.2. Least square means for blocking parameters 305-d mature-equivalent milk yield (305-d MEq), BCS (5-point scale), and BW per treatment group[1] pre-calving

	20% fNDF		26% fNDF			
	0% FAT	2% FAT	0% FAT	2% FAT	SE	*P*-value
305-d MEq,[2] kg	13,558	12,894	14,081	13,323	543	0.48
BCS	3.40	3.53	3.19	3.45	0.14	0.33
BW, kg	847	792	795	790	26.8	0.39

[1]Treatment diets were either 20 or 26% forage NDF (fNDF) and 0 or 2% saturated free FA supplement (FAT), and were fed from d 1 to 29 postpartum. Carryover diet was fed from d 30 to 71 postpartum.

Table 4.3. Health incidents during the treatment and carryover periods within treatment diet[1]

	20% fNDF		26% fNDF	
	0% FAT	2% FAT	0% FAT	2% FAT
During treatment period				
Fever with no apparent cause (>39.5°C)	1	0	0	0
Ketosis	2	2	3	5
Lameness	0	0	0	1
Mastitis	2	0	0	0
Metritis	1	0	0	0
Milk fever	1	0	0	0
Retained placenta	0	2	2	1
Udder edema	0	0	0	1
During carryover period				
Displaced abomasum	0	0	0	1
Lameness (unknown origin)	1	0	1	0
Lameness (traumatic origin)	1	0	0	0
Mastitis	1	2	1	0

[1]Treatment diets were either 20 or 26% forage NDF (fNDF) and 0 or 2% saturated free FA supplement (FAT), and were fed from d 1 to 29 postpartum.

RESULTS

Dry matter intake

During the PP, the high fNDF diets decreased DMI by 2.0 kg/d ($P < 0.01$) compared with the low fNDF diets, while 2% FAT increased DMI by 1.4 kg/d ($P = 0.04$) compared with 0% FAT (Table 4.4). However, these treatments interacted over time; the high fNDF diet with 0% FAT decreased DMI compared with the other diets and this difference increased

Table 4.4. Effects of dietary forage NDF (fNDF) content and a saturated free FA supplement (FAT) on dry matter intake and production of dairy cows during the treatment period (d 1 to 29 postpartum)

Item	20% fNDF		26% fNDF		SEM	Significance, *P*-value					
	0% FAT	2% FAT	0% FAT	2% FAT		fNDF	FAT	fNDF x FAT	fNDF x Time	FAT x Time	fNDF x FAT x Time
DMI, kg/d	23.6	24.2	20.8	23.0	0.74	<0.01	0.04	0.25	0.79	0.61	<0.01
Cumulative DMI, kg	705	706	599	661	21.0	<0.001	0.12	0.12	NA[1]	NA	NA
Yield, kg/d											
Milk	51.2	45.3	48.2	47.8	1.89	0.90	0.10	0.16	0.09	0.72	0.22
Fat	2.19	2.03	2.22	2.31	0.13	0.19	0.73	0.29	0.39	0.25	0.51
Protein	1.63	1.51	1.49	1.53	0.05	0.23	0.43	0.13	0.26	0.77	0.11
Lactose	2.47	2.20	2.29	2.31	0.09	0.67	0.16	0.11	0.10	0.58	0.33
3.5% FCM	57.7	52.4	56.9	58.1	2.75	0.35	0.43	0.21	0.21	0.30	0.39
ECM	56.8	51.6	55.1	56.4	2.49	0.52	0.43	0.18	0.19	0.32	0.28
Cumulative milk yield, kg	1,453	1,310	1,375	1,351	51.8	0.72	0.12	0.26	NA	NA	NA
Feed efficiency[2]	2.50	2.22	2.76	2.57	0.13	0.01	0.05	0.71	0.11	0.14	0.10
Milk composition, %											
Fat	4.35	4.52	4.66	4.89	0.19	0.06	0.25	0.86	0.89	0.71	0.74
Protein	3.27	3.43	3.14	3.25	0.09	0.08	0.13	0.80	0.24	0.79	0.68
Lactose	4.83	4.84	4.74	4.82	0.04	0.19	0.22	0.34	0.80	0.19	0.90
MUN, mg/dL	14.0	14.8	15.8	14.5	0.56	0.10	0.67	0.03	0.71	0.14	0.92
SCC, x1,000/mL	135	99.5	61.9	98.6	34.5	0.27	0.98	0.28	0.27	0.28	0.16
BW,[3] kg	739	718	690	700	23.1	0.15	0.79	0.46	0.06	0.70	0.84
BW change (pre-calving - d 26)	-110	-84.9	-126	-107	10.7	0.06	0.03	0.79	NA	NA	NA
BCS	2.86	3.16	2.55	2.86	0.15	0.02	0.02	0.97	0.02	0.15	0.69
BCS change (pre-calving - d 26)	-0.816	-0.490	-0.951	-0.903	0.09	<0.01	0.04	0.13	NA	NA	NA

[1]Not applicable.
[2]3.5% FCM, kg/d / DMI, kg/d.
[3]Because of infinite likelihood with first-order auto-regressive, the variance-covariance structure used to analyze BW was unstructured.

throughout PP (interaction $P < 0.01$; Figure 4.1). Both the low fNDF diet with 0% FAT and the high fNDF diet with 2% FAT increased DMI at a higher rate than the other two diets during PP (interaction $P < 0.01$) and had consistently greater DMI throughout the carryover period, with greater DMI for the low fNDF diet with 0% FAT than the high fNDF diet with 2% FAT throughout the carryover period (interaction $P = 0.10$; Table 4.5).

During the PP, the high fNDF diets decreased cumulative DMI compared with the low fNDF diets ($P < 0.001$), but the decrease tended to be greater for 0% FAT (106 kg, 15%) than for 2% FAT (45 kg, 6.4%; interaction $P = 0.12$). The FAT treatment increased DMI and cumulative DMI for the high fNDF diet, but decreased DMI (interaction $P = 0.10$) and cumulative DMI (interaction $P = 0.07$) for the low fNDF diet for the entire carryover period, although there was a tendency for less difference among treatments as time progressed (interaction $P = 0.13$).

Yields of milk and milk components

During the PP, FAT supplementation tended to decrease milk yield by 3.1 kg/d ($P = 0.10$), but did not affect yields of 3.5% FCM, ECM, or cumulative milk (Table 4.4). Lower fNDF decreased milk yield early but increased milk yield late in the PP (interaction $P = 0.09$). However, fNDF did not affect cumulative milk yield or yields of 3.5% FCM or ECM during the treatment period. During the carryover period, the 2% FAT treatment tended to decrease milk yield and cumulative milk yield more for the low fNDF diet (8.0 kg/d and 358 kg, respectively) than for the high fNDF diet (1.3 kg/d and 56 kg, respectively; interaction $P \leq 0.15$; Table 4.5; Figure 4.2). Similar interactions were observed for FCM and ECM: while 2% FAT decreased FCM 7.6 kg/d and ECM 7.2 kg/d for the low fNDF diet, it slightly

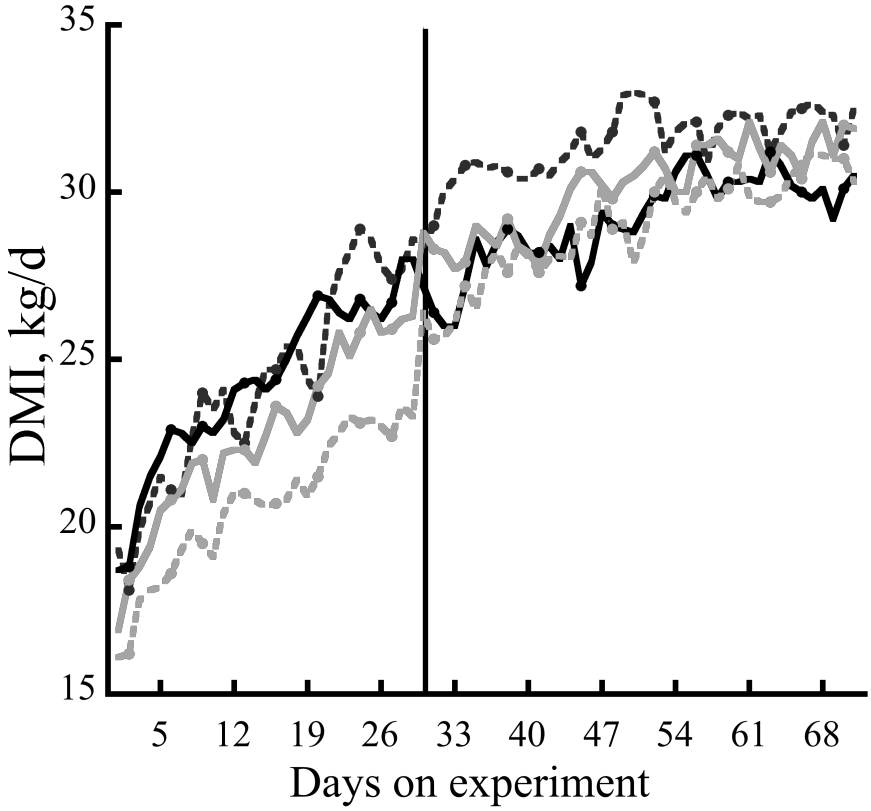

Figure 4.1. Effects of dietary forage NDF (fNDF) content and a saturated free FA supplement (FAT) on dry matter intake (kg/d) over time during the treatment and carryover periods. Treatment diets were: 20% fNDF 0% FAT (black, broken line), 20% fNDF 2% FAT (black, solid line), 26% fNDF 0% FAT (grey, broken line), and 26% fNDF 2% FAT (grey, solid line). Daily averages for treatment groups were calculated with the raw data of 12 cows per treatment diet during both the treatment and carryover periods (n = 11 for 26% fNDF 2% FAT diet during the carryover period). The line on d 30 indicates the start of the carryover period, when all cows were fed a common diet with no supplemental fat added.

Table 4.5. Effects of dietary forage NDF content (fNDF) and a saturated free FA supplement (FAT) fed during the immediate postpartum period on dry matter intake and production of dairy cows when fed a common diet during the carryover period (d 30 to 71 postpartum)

	20% fNDF		26% fNDF			Significance, *P*-value					
Item	0% FAT	2% FAT	0% FAT	2% FAT	SEM	fNDF	FAT	fNDF x FAT	fNDF x Time	FAT x Time	fNDF x FAT x Time
DMI, kg/d	31.5	29.4	29.1	30.2	0.94	0.41	0.62	0.10	0.73	0.12	0.13
Cumulative DMI, kg	1,321	1,224	1,218	1,266	39.8	0.44	0.53	0.07	NA[1]	NA	NA
Yield, kg/d											
Milk	58.4	50.4	58.0	56.7	2.32	0.20	0.05	0.15	0.56	0.58	0.73
Fat	2.06	1.80	2.03	2.10	0.10	0.20	0.34	0.10	0.34	0.45	0.22
Protein	1.62	1.44	1.57	1.58	0.05	0.47	0.11	0.09	0.31	0.53	0.18
Lactose	2.89	2.52	2.84	2.80	0.11	0.29	0.07	0.15	0.38	0.65	0.76
3.5% FCM	58.7	51.1	58.0	58.5	2.47	0.17	0.16	0.10	0.37	0.89	0.44
ECM	57.3	50.1	56.4	56.9	2.27	0.20	0.15	0.09	0.36	0.96	0.42
Cumulative milk yield, kg	2,484	2,126	2,430	2,374	99.7	0.34	0.05	0.14	NA	NA	NA
Feed efficiency[2]	1.88	1.75	2.01	1.95	0.08	0.03	0.19	0.68	0.63	0.55	0.57
Milk composition, %											
Fat	3.56	3.61	3.51	3.71	0.13	0.82	0.29	0.54	0.46	0.05	0.22
Protein	2.79	2.89	2.72	2.80	0.05	0.11	0.09	0.75	0.72	0.90	0.54
Lactose	4.95	4.99	4.90	4.95	0.04	0.26	0.32	0.98	0.12	0.57	0.78
MUN, mg/dL	16.4	16.5	16.8	16.2	0.58	0.88	0.50	0.33	0.98	0.13	0.20
SCC, x1,000/mL	89.4	114	102	127	76.0	0.86	0.73	1.00	0.20	0.43	0.77
BW,[3] kg	741	715	658	675	21.8	<0.01	0.82	0.24	0.29	0.07	0.59
BW change (d 68 - d 33)	-1.70	13.8	1.70	7.17	6.08	0.77	0.07	0.38	NA	NA	NA
BCS[3]	2.50	2.90	2.02	2.34	0.16	<0.001	0.02	0.78	0.10	0.51	0.80
BCS change (d 68 - d 33)	-0.135	-0.073	-0.205	-0.198	0.07	0.19	0.63	0.70	NA	NA	NA

[1]Not applicable.
[2]3.5% FCM, kg/d / DMI, kg/d.
[3]Because of infinite likelihood with first-order auto-regressive, the variance-covariance structure used to analyze BW and BCS was unstructured and compound symmetry, respectively.

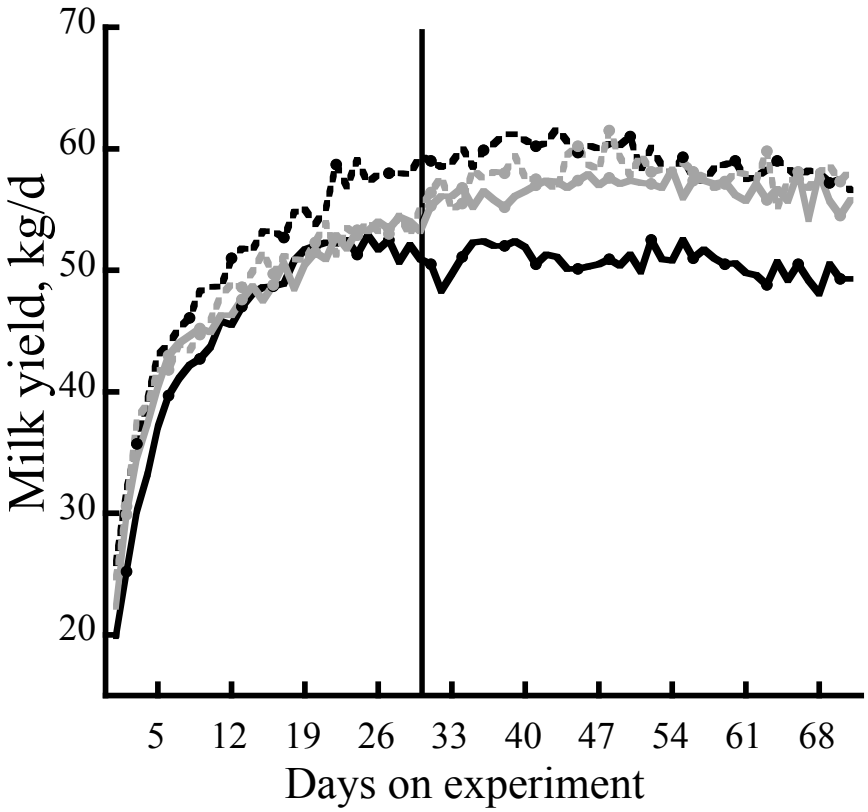

Figure 4.2. Effects of dietary forage NDF (fNDF) content and a saturated free FA supplement (FAT) on milk yield over time during the treatment and carryover periods. Treatment diets were: 20% fNDF 0% FAT (black, broken line), 20% fNDF 2% FAT (black, solid line), 26% fNDF 0% FAT (grey, broken line), and 26% fNDF 2% FAT (grey, solid line). Daily averages for treatment groups were calculated with the raw data of 12 cows per treatment diet during both the treatment and carryover periods (n = 11 for 26% fNDF 2% FAT diet during the carryover period). The line on d 30 indicates the start of the carryover period, when all cows were fed a common diet with no supplemental fat added.

increased FCM and ECM by ~ 0.5 kg/d for the high fNDF diet (interactions $P \leq 0.10$). Across fNDF contents of the diets, FAT supplementation during the PP decreased milk yield by 4.7 kg/d and cumulative milk yield by 207 kg (both $P = 0.05$) during the carryover period. In contrast, fNDF content of diets fed during PP did not have an overall effect on yields of milk, 3.5% FCM, ECM, or cumulative milk yield during the carryover period.

During PP, lower fNDF diets tended to increase milk protein and fat concentrations ($P < 0.10$) but did not affect milk lactose concentration (Table 4.4). Fat supplementation during the PP had no effects on milk protein, fat, or lactose concentrations during the treatment period. However, during the carryover period, 2% FAT increased milk fat concentration during most of the period (interaction $P = 0.05$) and tended to increase milk protein concentration ($P = 0.09$), while dietary fNDF content had no effect on milk fat or protein concentrations (Table 4.5). Compared with high fNDF, low fNDF decreased milk lactose concentration initially when cows were fed the common diet, but the difference between treatments tended to decrease over time (interaction $P = 0.12$) with no overall effect through the period. Lower fNDF treatment decreased MUN concentration for 0% FAT (15.8 vs.14.0 mg/dl), but had little effect for 2% FAT (14.5 vs. 14.8 mg/dl) during the PP (interaction $P = 0.03$). The 2% FAT treatment tended to decrease MUN over time compared with 0% FAT during the treatment period (interaction $P = 0.14$), but differences diminished as the carryover period progressed (interaction $P = 0.13$).

During the treatment period, low fNDF with 0% FAT increased milk protein yield compared with the other treatments, but the differences among treatments decreased as time progressed (interaction $P = 0.11$; Table 4.4). The lower fNDF diets increased yield of milk lactose throughout the PP at a faster rate compared with the high fNDF diets (interaction $P =$

0.10) with no overall effect because lactose yield was lower for the low fNDF diets than for the high fNDF diets at the beginning of the period, but higher at the end. Fat supplementation during the PP decreased yields of fat and protein during the carryover period only for the low fNDF treatment by 0.26 and 0.18 kg/d, respectively (interaction $P \leq 0.10$; Table 4.5). During the carryover period, FAT tended to decrease lactose yield overall ($P = 0.07$), but more so in the low fNDF diet (interaction $P = 0.15$).

Body condition score and BW

During the PP, low fNDF diets and 2% FAT supplementation decreased BCS loss; the effect of FAT supplementation tended to be more pronounced for the low fNDF diet than the high fNDF diet (interaction $P = 0.13$; Table 4.4). Therefore, FAT supplementation and lower fNDF increased BCS by 0.3 units (both $P = 0.02$). Treatment differences increased over time through the period with a greater BCS loss for 0% FAT compared with 2% FAT (interaction $P = 0.15$) and for low fNDF compared with high fNDF (interaction $P = 0.02$). The effect of FAT treatment on BCS was sustained through the carryover period, during which BCS was 0.36 units higher ($P = 0.02$) for the 2% FAT treatment compared with the 0% FAT treatment (Table 4.5). Treatment differences for fNDF continued to increase through the carryover period (interaction $P = 0.10$), which resulted in 0.52 units higher BCS for the low fNDF treatment ($P = 0.001$). Although there were no overall effects of fNDF content on BW during the PP, the high fNDF treatment decreased BW at a greater rate than the low fNDF treatment (interaction $P = 0.06$) during this period and decreased BW compared with the low fNDF treatment (61.5 kg; $P < 0.01$) in the carryover period. Overall, and during PP, FAT supplementation decreased BW loss, while higher fNDF diets tended to

increase BW loss. During the carryover period, FAT supplementation tended to decrease BW loss regardless of dietary fNDF content.

Feed efficiency

Lower fNDF (2.36 vs. 2.67, $P = 0.01$) and FAT supplementation (2.40 vs. 2.63, $P = 0.05$) decreased feed efficiency (FE; 3.5% FCM, kg/DMI, kg) through the treatment period (Table 4.4). However, FAT supplementation in the low fNDF diet decreased FE greatly compared with the other diets early in the period and this difference in FE became smaller as time progressed (interaction $P = 0.10$). Feed efficiency during the carryover period was not affected by FAT supplementation during PP, but lower fNDF during PP continued to reduce FE during the carryover period (1.82 vs. 1.98; $P = 0.03$, Table 4.5).

Total-tract digestibility during PP

Overall, fat supplementation increased gross energy digestibility and total tract OM digestibility ($P \leq 0.05$). The effect of fat supplementation on OM digestibility was mainly because of its effect on the low fNDF diet (interaction $P < 0.08$; Table 4.6). Both higher fNDF and 2% FAT increased digestibility of NDF and CP, but the significant overall effect of FAT on NDF digestibility was entirely due to its effect in the low fNDF diet (interaction $P = 0.04$). An interaction between fNDF and FAT with time was detected for total tract digestibility of starch ($P = 0.02$); while the high fNDF diet with 2% FAT decreased starch digestibility over time, the low fNDF diet with 2% FAT increased it. Overall, higher fNDF and 2% FAT decreased starch digestibility (both $P \leq 0.05$).

Milk FA yields and profile during PP

Diets did not affect yields of palmitic acid or mixed source FA in milk (Table 4.7; Table 4.8). Overall, high fNDF diets decreased de novo but increased preformed FA yields, compared with low fNDF diets (both $P = 0.02$). Higher fNDF tended to increase stearic acid yield in milk ($P = 0.06$), and this effect was more pronounced earlier in the treatment period (interaction $P = 0.07$). Supplementation of FAT did not affect yields of stearic acid or preformed FA during the PP overall, but increased stearic acid yield later in the period (interaction $P < 0.01$). Diets without FAT tended to increase preformed FA early in the treatment period, but this effect disappeared over time (interaction $P = 0.11$). Fat supplementation increased proportion of mixed source FA ($P < 0.01$), tended to decrease proportion of preformed FA ($P = 0.06$), but did not affect proportion of FA from de novo synthesis in milk (Table 4.7). High fNDF diets decreased proportions of FA from mixed source and de novo synthesis, but increased proportion of preformed FA in milk (all $P \leq 0.05$).

Table 4.6. Effects of dietary forage NDF (fNDF) content and a saturated free FA supplement (FAT) on total-tract digestibility of nutrients of dairy cows during the treatment period (d 1 to 29 postpartum)

	20% fNDF		26% fNDF			Significance, *P*-value					
Item	0% FAT	2% FAT	0% FAT	2% FAT	SEM	fNDF	FAT	fNDF x FAT	fNDF x Time	FAT x Time	fNDF x FAT x Time
Total tract digestibility, %											
DM	64.5	66.1	64.9	64.9	0.65	0.36	0.07	0.09	0.82	0.69	0.21
OM	65.9	67.6	66.3	66.4	0.63	0.33	0.04	0.08	0.74	0.76	0.24
NDF	40.1	44.0	45.6	45.6	1.12	<0.001	0.03	0.04	0.66	0.93	0.83
CP	63.5	65.3	65.3	66.4	0.81	0.04	0.04	0.65	0.87	0.38	0.21
Starch	95.1	94.9	94.4	93.4	0.31	<0.001	0.05	0.18	0.29	0.62	0.02
Gross energy	62.5	64.2	63.2	63.4	0.71	0.97	0.05	0.17	0.71	0.79	0.32

Table 4.7. Effects of dietary forage NDF (fNDF) content and a saturated free FA supplement (FAT) on yields and profile of milk FA summed by source of dairy cows during the treatment period (d 1 to 29 postpartum)

	20% fNDF		26% fNDF			Significance, *P*-value					
Item	0% FAT	2% FAT	0% FAT	2% FAT	SEM	fNDF	FAT	fNDF x FAT	fNDF x Time	FAT x Time	fNDF x FAT x Time
Yield,[1] g/d											
By source[2]											
De novo	404	398	343	363	20.1	0.02	0.72	0.52	0.75	0.77	0.26
Mixed	620	614	622	673	37.5	0.39	0.52	0.42	0.42	0.21	0.77
Preformed	1022	895	1113	1138	76.2	0.02	0.47	0.28	0.36	0.11	0.64
Profile,[1] %											
By source											
De novo	20.2	21.7	17.0	16.9	0.95	0.0001	0.45	0.39	0.95	0.12	0.50
Mixed	30.4	32.6	29.9	31.0	0.53	0.05	<0.01	0.29	0.50	0.43	0.32
Preformed	49.5	45.7	53.2	52.1	1.31	<0.001	0.06	0.29	0.71	0.19	0.65

[1]A total of approximately 80 individual FA were quantified and used for calculations (summation by source).
[2]De novo FA originate from mammary de novo synthesis (< 16 carbons), preformed FA originate from extraction from plasma (> 16 carbons), and mixed FA originate from both sources (C16:0 plus C16:1 *cis*-9).

Table 4.8. Effects of dietary forage NDF (fNDF) content and a saturated free FA supplement (FAT) on selected individual milk FA yields[1] of dairy cows during the treatment period (d 1 to 29 postpartum).

Item	20% fNDF		26% fNDF		SEM	Significance, *P*-value					
	0% FAT	2% FAT	0% FAT	2% FAT		fNDF	FAT	fNDF x FAT	fNDF x Time	FAT x Time	fNDF x FAT x Time
Selected FA, [1] g/d											
C4:0	71.8	64.8	75.2	78.9	4.15	0.04	0.69	0.20	0.97	0.22	0.10
C6:0	39.2	37.6	34.8	36.2	2.08	0.16	0.94	0.47	0.99	0.95	0.07
C8:0	20.4	20.1	16.6	16.8	1.22	<0.01	0.98	0.81	0.91	0.79	0.12
C10:0	44.1	44.8	31.6	32.5	2.88	<0.0001	0.76	0.98	0.62	0.42	0.30
C12:0	46.5	48.5	33.5	34.7	2.95	<0.0001	0.56	0.89	0.53	0.39	0.37
C14:0	171	173	143	155	8.83	0.01	0.42	0.54	0.71	0.56	0.47
C16:0	574	576	572	626	33.7	0.45	0.38	0.41	0.44	0.22	0.78
C16:1 *cis*-9	45.8	38.3	49.4	46.5	4.22	0.11	0.16	0.53	0.26	0.23	0.73
C18:0	230	222	240	273	16.4	0.06	0.42	0.19	0.07	<0.01	0.50
C18:1 *trans*-4	0.219	0.211	0.203	0.222	0.01	0.78	0.58	0.16	0.63	0.49	0.62
C18:1 *trans*-5	0.192	0.198	0.184	0.206	0.01	0.98	0.13	0.41	0.85	0.30	0.70
C18:1 *trans*-6 to 8	4.31	4.75	4.29	5.08	0.25	0.54	0.02	0.49	0.23	0.15	0.84
C18:1 *trans*-9	3.07	3.81	3.25	4.46	0.18	0.01	<0.0001	0.15	0.46	<0.01	0.28
C18:1 *trans*-10	7.26	8.94	9.14	7.89	1.36	0.76	0.87	0.29	0.63	0.63	0.64
C18:1 *trans*-11	14.9	14.0	18.1	16.5	1.36	0.02	0.31	0.78	0.49	0.17	0.80
C18:1 *trans*-12	5.21	5.01	5.26	5.50	0.29	0.35	0.96	0.44	0.16	0.13	0.81
C18:1 *cis*-9	566	462	635	628	49.8	0.01	0.21	0.28	0.53	0.19	0.67
C18:1 *cis*-11	19.9	15.5	21.0	19.8	1.67	0.07	0.06	0.26	0.09	0.72	0.69
C18:1 *cis*-12	5.85	4.97	5.87	6.13	0.40	0.15	0.44	0.16	0.22	0.25	0.75
C18:1 *cis*-13	3.77	3.02	4.42	3.90	0.43	0.04	0.09	0.76	0.17	0.53	0.62
C18:1 *cis*-14/*trans*-16	4.52	4.11	4.61	4.37	0.26	0.49	0.20	0.71	0.08	0.06	0.82
C18:2 *cis*-9, *cis*-12	52.1	43.6	48.9	48.9	2.71	0.69	0.12	0.12	0.20	0.32	0.55
C18:2 *cis*-9, *trans*-11	6.46	5.70	7.62	6.33	0.50	0.04	0.02	0.54	0.50	0.13	0.74
C18:2 *trans*-9, *cis*-11	0.099	0.073	0.137	0.075	0.03	0.44	0.09	0.49	0.72	0.31	0.19
C18:2 *trans*-10, *cis*-12	0.050	0.048	0.040	0.031	0.01	0.32	0.69	0.82	0.16	0.99	0.71
C18:3 *cis*-9, *cis*-12, *cis*-15	8.16	6.97	8.90	8.06	0.45	0.04	0.02	0.68	0.48	0.26	0.65

[1]A total of approximately 80 individual FA were quantified, but only select FA are reported in the table

DISCUSSION

Interaction between fNDF content and FAT supplementation

Dietary treatments used in this experiment are similar to those evaluated by Weiss and Pinos Rodriguez (2009) for cows starting 3 wk into lactation, but our results were not entirely consistent with theirs. Regardless of dietary fNDF content, FAT supplementation increased DMI, decreased BW loss, and tended to decrease BCS loss and milk yield during the treatment period. Furthermore, treatment effect on BCS loss tended to be more pronounced when FAT was supplemented in the low fNDF diet than in the high fNDF diet. Therefore, during the PP, FAT supplementation favored energy partitioning to body reserves and not milk production, especially in the low fNDF diet. Interestingly, the diets fed during the immediate postpartum period had a tremendous carryover effect during early lactation, when cows were fed a common diet: the low fNDF with 2% FAT diet decreased 3.5% FCM ~7.5 kg/d during the entire carryover period compared with the other three diets. Overall, FAT supplementation tended to decrease milk yield with no effect on 3.5% FCM and ECM during the treatment period and decreased milk yield during the carryover period, mainly due to the low fNDF with 2% FAT diet. Even though milk yield was numerically lower for the low fNDF with 2% FAT diet during the first days PP (Figure 4.2), cows seemed to reach a lower peak milk yield that happened earlier in lactation compared with the other groups and this effect was sustained after cows were switched to a common diet during the carryover period. The 2% FAT and low fNDF diets continued to have higher BCS during the carryover period, but BCS change during this period was not affected by diet, and therefore, the effect on overall BCS observed was due to differences obtained during the treatment period. When comparing our results with those from early lactation cows reported by Weiss and Pinos

Rodriguez (2009), we find that the same fat supplement increased energy partitioned to body condition and not milk for high fNDF diets in both experiments but results differed between experiments when fat was supplemented in the low fNDF diets. In their experiment, supplementation of FA in the low fNDF diet partitioned energy to milk rather than body reserves, which is the opposite of what happened during the treatment period in our experiment. In addition, FA supplementation in the low fNDF diet increased DMI early but decreased it later in the treatment period compared with the low fNDF diet with supplemental fat in their experiment (Weiss and Pinos-Rodriguez, 2009). Moreover, and consistent with our results, they showed that fat supplementation increased DMI in the high fNDF diet during most of the treatment period, compared with high fNDF diet with no supplemental fat. Even though cows used by Weiss and Pinos Rodriguez (2009) had lower peak milk yield than cows used in this experiment (~50 vs. ~60 kg/d) and their low fNDF content was lower than what we used (17 vs. 20% fNDF), the most likely reason for the discrepancy in results is the different physiological state of the cows used (early lactation vs. immediate postpartum cows).

Other studies have reported supplementation of saturated prilled FA at different levels of dietary forage and results are inconsistent. Jerred et al. (1990) added a saturated prilled fat supplement at 5% of dietary DM to diets varying in F:C from day 5 postpartum for 100 d and did not detect interactions between forage level and fat supplementation for DMI, milk yield, 4% FCM yield, BW change, or any other response measured. In contrast, Grum et al. (1996) fed diets with two different levels of concentrate (resulting in ~33 vs. ~28% NDF diets) with or without fat supplementation (3% saturated prilled FA supplement plus 10% whole raw soybeans) to early and mid lactation cows averaging ~28 kg of milk/d and detected an

interaction between concentrate level and fat addition for DMI, but not for milk yield or 4% FCM. The interaction indicated that supplemental fat increased DMI when fed in the high NDF diet (20.7 vs. 19.2 kg/d) in agreement with our results, but decreased DMI when fed in the low NDF diet (19.4 vs. 20.2 kg/d), which is inconsistent with our results. However, the amount of fat supplemented was greater and of mixed source.

Dry matter intake

The negative effect of diets with higher fNDF on DMI during the postpartum and early lactation periods has been documented and reviewed previously (Allen, 1996). Rabelo et al. (2003) reported that a higher forage diet (30% NDF) tended to decrease DMI compared with a lower forage diet (25% NDF; 15.3 vs. 16.5 kg/d; $P = 0.10$) during the first 20 d postpartum. Forage fiber clears from the rumen more slowly than other diet fractions and is therefore more filling over time in the rumen. Signals from ruminal distension can dominate control of feed intake when the filling effect of the diet is high enough. Although feed intake is likely controlled primarily by mechanisms related to oxidation of fuels in the liver in early postpartum (Allen and Piantoni, 2013), rumen distension likely dominated control of intake for the high fNDF treatment in the current experiment. During the treatment period and regardless of dietary fNDF content, supplemental FAT increased DMI and tended to decrease plasma NEFA concentration (695 vs. 827 μEq/L; $P = 0.06$; data shown in Piantoni et al., submitted). Furthermore, the effect on plasma NEFA concentration was more pronounced at the beginning of the treatment period (interaction $P = 0.05$). The increase in DMI observed with fat supplementation might be related to a decreased flux of fuels to the liver that could have potentially decreased satiety and improved DMI (Allen et al., 2009).

Saturated fat supplementation during the PP

Production responses to saturated fat supplementation postpartum have been inconsistent. Moallem et al. (2007) fed the same FA supplement used in this experiment in a 17% fNDF diet and showed that the FA supplement increased DMI (25.1 vs. 24.3 kg/d; $P <$ 0.5) in agreement with our results, but also increased milk yield (44.4 vs. 42.3 kg/d) and BCS loss (0.96 vs. 0.55 units) compared with a control diet with no supplemental fat (all $P < 0.05$). However, these diets were fed prepartum (from 256 d pregnant) to 100 days postpartum, at approximately half the inclusion rate used in the current experiment, and the effects on milk yield and DMI were reported as least squares means for the whole 100 d in lactation. Therefore, the effect of fat supplementation during the first 30 d postpartum on production performance cannot be discerned. In addition, Beam and Butler (1998) supplemented the same prilled FA supplement used in the current experiment at 2.6% of diet DM in a ~33% NDF diet and reported no effect on DMI ($P = 0.13$) during the first 6 wk PP, when compared with a control diet with no supplemental fat added. However, fat supplementation decreased DMI compared with the control diet when only the first 4 wk PP were considered (15.5 vs. 17.3 kg/d; $P < 0.05$). In addition, an interaction between diet and week was detected for yields of 4% FCM and milk: fat supplementation decreased yields of 4% FCM and milk during the first 3 wk PP, but increased them during the last 2 wk of the experiment ($P < 0.10$).

Feed efficiency

The FAT treatment decreased FE overall during the treatment period, but especially in the low fNDF diet, which decreased FE earlier but not later in the treatment period compared with the other diets. The decrease in FE was therefore related to the reduction in

mobilization of body reserves. In contrast, the higher fNDF diets increased FE overall, but especially earlier in the treatment period, and this was related to increased energy partitioned to milk production, with increased BCS loss and decreased DMI. For cows past peak lactation, saturated fat supplements have increased FE, either by decreasing DMI with no effect on milk yield (Lock et al., 2013; Rico et al., 2014) or by increasing milk yield with no effect on DMI (Wang et al., 2010; Piantoni et al., 2013). In early lactation cows, Weiss and Pinos Rodriguez (2009) showed that fat supplemented in the low fNDF diet increased FE because of an increase in milk yield, but when supplemented in the high fNDF diet decreased FE because of an increase in body condition. In postpartum cows, a decrease in FE might be desirable if milk production is maintained and DMI is increased, which would indicate a decrease in mobilization of body reserves. In the current experiment, during the treatment period, fat supplementation decreased FE regardless of fNDF content of the diet, and this was related to a greater DMI and a decrease in BCS loss.

Milk components and FA

Higher forage diets with higher fNDF and lower starch contents often increase milk fat concentration (Jerred et al., 1990). In the current experiment, higher fNDF tended to increase milk fat concentration and tended to interact with FAT and time to affect milk protein yield and low fNDF with 0% FAT had greater milk protein yield compared with the other diets throughout the treatment period. An increase in milk protein yield might be from greater rumen microbial protein reaching the duodenum for absorption because of the greater supply of ruminally fermentable energy. We did not detect an effect of FAT supplementation on milk fat percent or milk fat yield during the treatment period, in contrast to other

experiments supplementing the commercial FA used in our experiment (Wang et al., 2010). Moreover, FAT treatment decreased yields of preformed FA in milk during the first week but not later during the treatment period, and the secretion pattern of preformed FA in milk followed that of plasma NEFA concentrations (Piantoni et al., submitted), consistent with the concept that greater milk fat output following parturition is from mobilization of adipose reserves (Kronfeld, 1965). In contrast, high fNDF diets increased yields of preformed FA in milk and decreased de novo FA yields, which might be related to the increased BCS loss and decreased DMI, respectively, observed with these diets.

Total-tract digestibility of nutrients

Effects of saturated FA supplements on digestibility of dietary components are inconsistent across experiments. In our experiment, FAT supplementation increased NDF digestibility by 9.7% in the low fNDF diet, but had no effect in the high fNDF. In agreement, a 99% pure palmitic acid supplement fed in a 19% fNDF diet at 2% of diet DM increased NDF digestibility by 9.2% ($P < 0.001$) when compared with a diet with no supplemental fat in cows past peak lactation (Piantoni et al., 2013). We showed that palmitic acid increased plasma concentration of cholecystokinin, a gut peptide responsible for decreasing abomasal motility, and speculated that the increase in NDF digestibility observed was related to increased retention time in the rumen (Piantoni et al., 2013). In addition, Piantoni et al. (accepted) showed that a 98% pure stearic acid supplement fed in a 24.5% fNDF diet at 2% of diet DM tended to increase NDF digestibility by 3.1% ($P = 0.10$) when compared with a diet with no supplemental fat in cows past peak lactation. Also consistent with our results, supplementation of prilled FA did not affect nutrient digestibility when supplemented in a

high forage diet (~42% NDF) in late lactation cows (Schauff and Clark, 1989). However, a saturated FA supplement had no effect on NDF digestibility when fed to cows past peak lactation in a 17% fNDF diet (Harvatine and Allen, 2006b) or across fNDF contents when fed to cows in the postpartum period and in early lactation (Jarred et al., 1990), which is inconsistent with out results. Furthermore, a saturated FA supplement increased digestibility of OM and CP when supplemented to cows past peak lactation in a higher forage diet (33% NDF) but decreased them when supplemented in a lower forage diet (28% NDF; Grum et al., 1996). Effects of saturated FA supplementation on nutrient digestibility are likely dependent upon FA composition and its interaction with dietary components, which will likely affect gut peptide release and their effects on retention time of digesta in the rumen.

CONCLUSIONS

Supplementation of FAT during the immediate PP favored energy partitioning to body reserves rather than milk yield, especially in the lower fNDF diet. The high fNDF diet with supplemental FAT increased DMI and tended to decrease BCS compared with the same diet without FAT. The low fNDF diet with supplemental FAT increased DMI and digestibility of OM and tended to decrease BCS loss but reduced milk yield compared with the other diets. Regardless of fNDF content, supplemental FAT during the PP increased DMI, decreased BCS loss, but tended to decrease milk yield, and therefore, decreased FE. Supplementation of FAT did not affect yields of 3.5% FCM, ECM, and milk fat during the PP. When cows were fed a common diet during the carryover period, the low fNDF with 2% FAT treatment that was fed during the PP period continued to decrease milk yield and maintained higher BCS compared to the other three diets, but did not affect DMI, BCS loss,

or FE. In general, FAT supplementation alleviated the deleterious effects of feeding a high fNDF diet on DMI during the first 30 days postpartum. However, supplementing FAT in the lower fNDF diet during the immediate postpartum period limited milk yield in the carryover period.

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CHAPTER 5

SATURATED FAT SUPPLEMENTATION INTERACTS WITH DIETARY FORAGE NDF CONTENT DURING THE IMMEDIATE POSTPARTUM PERIOD IN HOLSTEIN COWS: ENERGY BALANCE AND METABOLISM

ABSTRACT

Forty-eight multiparous cows were used in a randomized complete block design experiment with a 2x2 factorial arrangement of treatments to determine the interaction between a highly saturated free FA supplement (FAT) and dietary forage NDF (fNDF) content on energy balance and metabolic responses in postpartum cows. Treatment diets were offered from 1 to 29 d postpartum and contained 20% or 26% fNDF and 0% or 2% FAT (Energy Booster 100; 96.1% FA: 46.2% C18:0 and 37.0% C16:0). Overall, low fNDF vs. high fNDF and 2% FAT vs. 0% FAT increased digestible energy intake (DEI) (67.5 vs. 62.2 Mcal/d and 68.1 vs. 61.6 Mcal/d, respectively). The low fNDF diet with FAT increased energy balance compared with the other treatments early during the treatment period, but treatment differences diminished over time. Overall, low fNDF vs. high fNDF diets and 2% FAT vs. 0% FAT improved energy balance (-13.0 vs. -16.3 Mcal/d and -12.0 vs. -17.3, respectively) decreasing efficiency of utilization of DEI for milk (Milk NE_L/DEI; 0.575 vs. 0.634 and 0.565 vs. 0.643). Low fNDF diets increased plasma insulin (308 vs. 137 µg/mL) and glucose concentrations (50.5 vs. 45.7 mg/dL) and decreased plasma non-esterified FA (606 vs. 917 µEq/L) and ß-hydroxybutyrate (9.29 vs. 16.5 mg/dL) concentrations and liver triglyceride content. Compared with 0% FAT, 2% FAT decreased plasma NEFA concentration during the first week postpartum (706 vs. 943 µEq/L) and tended to decrease plasma non-esterified FA overall throughout the treatment period, but did not affect liver

triglyceride content. During a glucose tolerance test, 2% FAT increased plasma insulin concentration more in the low fNDF diet (84.5 vs. 44.6 μIU/mL) than in the high fNDF diet (40.4 vs. 38.0 μIU/mL). After glucose infusion, 2% FAT increased insulin area under the curve by 64% when included in the low fNDF diet, but only by 5.1% when included in the high fNDF diet. Even though 2% FAT did not affect weekly plasma insulin concentration, it increased plasma insulin baseline concentration prior to the tolerance tests. Supplementation of FAT and low fNDF diets increased DEI and improved energy balance, but decreased apparent efficiency of utilization of DEI for milk production. Fat supplementation affected energy partitioning, increasing energy balance and decreasing body condition score loss, especially in the lower fNDF diet. The decrease in body condition score loss observed was likely related to an increase in plasma insulin concentration. Feeding FAT in a low fNDF diet during the first 29 d postpartum might have primed the cows to limit fat mobilization at the expense of milk.

INTRODUCTION

Adaptation to the rapid increase in energy demand at parturition is challenging for dairy cows. Body reserves are mobilized around parturition because plasma insulin concentration (Zachut et al., 2013) and insulin sensitivity of extra-hepatic tissues are low (Bell, 1995). Increased mobilization of body reserves results in increased plasma NEFA concentration (Gonzalez et al., 2011). Control of feed intake is likely dominated by hepatic oxidation of NEFA during the transition period (Allen et al., 2009), and increased NEFA supply to the liver can decrease energy intake and rumen fill, increasing the risk of displaced abomasum and acidosis (Allen and Piantoni, 2013). Elevated plasma NEFA concentrations

can also alter immune function and might increase the risk of infectious diseases such as metritis (Lacetera, et al., 2004; Sordillo et al., 2009). High plasma NEFA concentrations also increase risk of hepatic lipidosis (Rabelo et al., 2005), which can potentially affect liver function (Gonzalez et al., 2011), decreasing gluconeogenesis, and milk yield.

To support energy demands at parturition and decrease mobilization of body reserves, diets with a higher energy density could be used. Higher dietary starch content or supplemental fats have been used to increase energy density of diets. However, not only the amount but also the form of the energy are important and can have different effects on performance. For example, a diet with lower forage to concentrate ratio (F:C) has the potential to increase energy intake and milk yield (Rabelo et al., 2003), decrease liver triglyceride (TG) content and plasma BHBA concentration, and increase plasma glucose and insulin concentrations during the first 3 wk postpartum (PP) when compared with a higher F:C diet (Rabelo et al., 2005). In contrast, a saturated free FA supplement fed during the first 6 wk PP had no effect on net energy intake, energy balance (EBal), or milk yield when compared with a control diet with no supplemental fat (Beam and Butler, 1998). Moreover, supplemental fat did not affect plasma insulin, glucose, and NEFA concentrations during the first 4 weeks PP (Beam and Butler, 1998).

There is limited research studying the interaction between forage NDF (fNDF) and highly saturated fats during the PP period. Weiss and Pinos-Rodriguez (2009) showed that a saturated FA supplemented (2.3% of DM) in a low fNDF diet increased milk yield, but when supplemented in a high fNDF diet it increased BCS in early lactation cows (21 to 126 DIM). Unfortunately, only production responses were measured in that experiment, and therefore, the mechanism by which this interaction affected energy partitioning remains unknown.

Moreover, since dietary treatments started early in lactation and not after parturition, it is not clear if these diets would have the same effect in immediate PP cows. Earlier, Jerred et al. (1990) fed a saturated prilled fat (at 5% of DM) in diets with different F:C to cows from 5 to 100 DIM and observed no interaction between F:C and fat supplementation and no overall effect of the fat supplement on EBal or energy intake. However, fat supplementation increased energy intake and EBal during the first weeks of the experiment. Inconsistent results between Jerred et al. (1990) and Weiss and Pinos-Rodriguez (2009) might be related to the different DIM of the cows at the start of the experiments (5 vs. 21 DIM) and to the inclusion rates used (5% vs. 2.3% of diet DM).

Based on the aforementioned findings, our objectives were to determine the interaction between a highly saturated FA supplement and dietary fNDF content on energy intake, EBal, and metabolic responses of dairy cows in the immediate PP period. We hypothesized that the saturated FA supplement will increase EBal and plasma insulin concentration and decrease plasma NEFA and BHBA concentrations and liver TG content in the higher fNDF diet, and that it will decrease EBal and plasma insulin concentration and increase liver total lipids content when added to the lower fNDF diet, consistent with the production results reported by Weiss and Pinos-Rodriguez (2009).

MATERIALS AND METHODS

This article is the second of two articles from an experiment that evaluated the use of a highly saturated (≥ 85% saturated) free FA supplement in rations with two fNDF contents in the immediate PP period. This article discusses the effect of these diets on energy intake, EBal, plasma metabolites and hormones, responses to glucose and insulin tolerance tests, and

liver lipids and glycogen contents. The companion paper describes treatment effects on DMI, yield of milk and milk components, and digestibility of nutrients during the immediate PP period, and DMI and yields of milk and milk components during a carryover period, when all cows received a common diet.

Animal housing and care

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University (East Lansing). The experiment started on September 30th, 2011 and finished on May 1st, 2012. All cows were housed in the same tie-stall barn, assigned by parturition order, throughout the entire treatment period. Cows were fed once daily (1000 h) at 120% of expected intake and milked twice daily (0400 and 1430 h). The amounts of feed offered and orts were weighed for each cow daily. Standard reproduction and health herd checks and breeding practices were maintained during the study.

Design and treatment diets

Forty-eight multiparous Holstein cows at the Michigan State University Dairy Field Laboratory were used in a randomized complete block design experiment with a 2x2 factorial arrangement of treatments with 12 cows per treatment. Cows were blocked by date of parturition (within 90 d), BCS (up to 1 unit difference using a 5-point scale, where 1=thin and 5=fat; Wildman et al., 1982), and previous lactation 305-d mature-equivalent milk production (MEq; within 5,500 kg). The BCS used to block cows was the last measurement before parturition. Cows within each block were randomly assigned to treatment based on expected parturition date. All cows were in apparent good health at the beginning of the

study, and treatment groups were not different in terms of 305-d MEq ($P = 0.48$), BW ($P = 0.39$), or BCS ($P = 0.33$) pre-calving (Piantoni et al., submitted). Treatment diets were offered from 1 to 29 d PP. Treatments contained 20% or 26% fNDF and 0% or 2% saturated free FA supplement (FAT; Energy Booster 100®; 96.1% FA: 46.2% C18:0 and 37.0% C16:0; Milk Specialties Global, Eden Prairie, MN). Desired fNDF content of the treatment diets were attained by altering proportions of forages (alfalfa and corn silages and alfalfa hay) and concentrates (corn grain and soybean meal). The FA supplement was added at 2% of diet DM, replacing 2% of soyhulls in the 0% FAT diets. Starch content was ~24% for the low fNDF diets and ~17.5% for the high fNDF diets. Treatment diets were mixed daily in a tumble-mixer and fed beginning the morning following parturition. The ingredient and nutrient composition of the diets fed as TMR, including close up ration for reference, as well as a summary of all health incidents during the treatment period were reported in the companion article (Piantoni et al., submitted).

Data and sample collection

All samples and body measurements were collected or recorded on the same day of the week during the entire treatment period (d 5, 12, 19, 26 PP), so all collection days are ±3 d relative to the first day on the treatment diet. All data (milk yield, feed offered and refused, BW, and BCS) were recorded and samples (milk, feces, feed ingredients, and orts) were collected and stored as described by Piantoni et al. (submitted). Body condition was scored by 3 trained investigators on a 5-point scale, as described by Wildman et al. (1982).

Blood samples were collected weekly by venipuncture of coccygeal vessels within one hour prior to feeding on days 5, 12, 19, and 26 PP. Blood was collected into 2 evacuated

tubes, one containing potassium EDTA and the other containing potassium oxalate with sodium fluoride as a glycolytic inhibitor. Both were centrifuged at 2,000 x g for 15 min immediately after sample collection, and plasma was harvested and stored at -20°C until analysis. A sample containing EDTA was also preserved with benzamidine (0.05 M final concentration), a proteolytic inhibitor, to reduce glucagon degradation.

Glucose and insulin tolerance tests

A glucose tolerance test (GTT) was conducted 13±3 d PP according to Bradford and Allen (2007) and an insulin tolerance test (ITT) 14±3 d PP according to Smith et al. (2007). To minimize stress, all cows were fitted with a single jugular catheter (left or right jugular vein). Catheterization was performed 2 d prior to the GTT, according to Bradford et al. (2006). Catheter patency was checked daily until removed after the ITT. On the days of the tests, cows were blocked from feed at 0700 and were not allowed access until tests were completed. For the GTT, a sterile solution of 50% dextrose (wt/vol) was administered by intrajugular bolus at a dose of 1.67 mmol glucose/kg of BW over the course of 5 min. Blood samples were collected from the jugular vein 10 min prior to infusion, immediately before infusion, and every 10 min through 120 min, and at 150 min and 180 min post-infusion. For the ITT, insulin (0.1 IU/kg of BW) was infused over the course of 1 min. Blood was sampled at −30, −20, −10, 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25, 30, 45, 60, 90, 120, and 130 min relative to the insulin injection. Catheters were flushed with 25 mL of sterile 4.2% Na citrate after glucose (GTT) and insulin (ITT) infusions and with 5 to 10 mL after each blood collection. Samples were processed as described above, within 1 h of collection.

Liver biopsies

Liver tissue was collected by biopsy before feeding 19±3 d PP for determination of glycogen, triglyceride, and total lipid contents. After local anesthetization with 2% lidocaine hydrochloride, a skin incision was performed and the biopsy instrument (14-gauge Vet-Core biopsy needles, Global Veterinary Products; New Buffalo, MI) inserted between the 10th and 11th ribs on a line between the olecranon and the tuber coxae on the right side. Ten samples of ~20 mg each were collected, snap-frozen in liquid nitrogen, and stored at -80°C until further processing.

Sample analysis

Feed, orts, and fecal samples were processed and analyzed as described in Piantoni et al. (submitted). Nutrient digestibility was determined as described in Piantoni et al. (submitted), using indigestible NDF as the internal marker. Energy intakes and balance were calculated weekly using equations (NRC, 2001) according to Harvatine and Allen (2006).

Weekly plasma samples were analyzed in duplicate. Commercial kits were used to determine plasma concentrations of insulin (Bovine insulin ELISA, #10-1201-01, Mercodia, Uppsala, Sweden; intraassay CV: 6.5%, interassay CV: 4.8%), glucagon (Glucagon RIA kit no. GL-32K; Millipore Corp., St. Charles, MA; intraassay CV: 4.3%, interassay CV: 5.9%), NEFA (NEFA-HR (2) kit; Wako Chemicals USA Inc.; Richmond, VA; intraassay CV: 1.6%, interassay: 6.2%), TG (L-Type Triglyceride M kit, Wako Chemicals USA, Inc.; intraassay CV: 7.2%, interassay: 10.9%), and BHBA (kit no. 2240, Stanbio Laboratory, Boerne, TX; intraassay CV: 2.7%, interassay: 3.9%). Plasma glucose concentration was analyzed using a glucose oxidase method (PGO Enzyme Product No. P7119; Sigma Chemical Co., St. Louis,

MO; intraassay CV: 1.4%, interassay CV: 1.3%). Plasma samples from the GTT and ITT were analyzed in singlicate. Plasma insulin concentration was determined with a commercial kit (Coat-A-Count RIA kit; Siemens Healthcare Diagnostics, Deerfield, IL; GTT interassay CV: 11%; ITT interassay CV: 10%) and plasma glucose concentration was analyzed as described above for the weekly samples (GTT interassay CV: 2.0%; ITT interassay CV: 2.9%). Area under the curve for glucose and insulin was calculated using the trapezoidal rule.

Liver samples were analyzed for glycogen, total lipid, and TG contents. Liver glycogen content was determined according to Hawk and Bergeim (1926), as modified by Bernal-Santos et al. (2003). Prior to glycogen determination, liver samples were freeze-dried and weighed to calculate liver DM. Total liver lipids were extracted according to Bligh and Dyer (1959). Liver TG content was determined as described by Rice et al. (2010). Absorbance was determined with a micro-plate reader (SpectraMax 340; Molecular Devices Corp., Sunnyvale, CA). Liver glycogen, total lipids, and TG are expressed as percent of liver DM.

Statistical Analysis

All weekly data were analyzed using the MIXED procedure of SAS v.9.2 (SAS Institute, Inc. Cary, NC) according to the following model with repeated measures:

$$Y_{ijklm} = \mu + B_i + C(B_jK_kS_l)_j + K_k + S_l + K_kS_l + T_m + K_kT_m + S_lT_m + K_kS_lT_m + J + e_{ijklm}$$

where μ = overall mean, B_i = random effect of block (i= 1 to 12), $C(B_jK_kS_l)_j$ = random effect of cow (j = 1 to 4) within block and treatment diet, K_k = fixed effect of fNDF (k = 1 to 2), S_l

= fixed effect of FAT (l = 1 to 2), K_kS_l = interaction between fNDF and FAT, T_m = fixed effect of week (m = 1 to 4), K_kT_m = interaction between fNDF and week, S_lT_m = interaction between FAT and week, $K_kS_lT_m$ = interaction between fNDF, FAT, and week, J = random effect of Julian date, e_{ijm} = residual error. First-order autoregressive was the covariate structure used for analysis because it resulted in the lowest Bayesian information criterion for most of the variables measured. Interactions with time were removed from the model when non significant and a reduced model was used to determine treatment effects. However, all interactions were included in the tables for informational purposes.

Treatment differences within week were analyzed using the GLIMMIX procedure of SAS v 9.2 (SAS Institute) and the SLICE option. The model included the random effects of block and cow nested within block and treatment and the fixed effects of fNDF and FAT and their interaction. The Bonferroni adjustment was applied to decrease the probability of type I error when multiple treatment means were compared. Liver parameters, responses to the glucose and insulin tolerance tests, and BW and BCS changes were analyzed using the MIXED procedure of SAS v 9.2 (SAS Institute) with the same model used in the GLIMMIX procedure.

Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. Significance was determined at $P \leq 0.05$ for main effects and $P \leq 0.10$ for interactions. Tendencies were determined at $P \leq 0.10$ for main effects and $P \leq 0.15$ for interactions. All data were included in the statistical analyses.

RESULTS

Energy intake and EBal

Overall, low fNDF and 2% FAT increased digestible energy intake (DEI; both $P \leq 0.01$; Table 5.1). Digestible energy intake increased over time for all four diets, and was highest for the low fNDF diet with 2% FAT and lowest for the high fNDF diet with 0% FAT during the entire treatment period (Figure 5.1A). The low fNDF diet with 0% FAT increased DEI at a higher rate during the last week of treatment (interaction $P = 0.03$). The low fNDF diet with 2% FAT increased EBal during the entire PP compared with the other three diets, but differences among treatments decreased over time (interaction $P = 0.10$; Table 5.1; Figure 5.1B). Overall, low fNDF diets and 2% FAT increased EBal (both $P < 0.05$) and decreased efficiency of utilization of DEI (both $P < 0.05$; Table 5.1). The low fNDF with 2% FAT tended to decrease BCS loss compared with the other diets (interaction $P = 0.13$; Table 5.1; Figure 5.1C), and 2% FAT decreased BW loss compared with 0% FAT (Table 5.1; Figure 5.1D).

Plasma metabolites and hormones

The low fNDF treatment increased plasma insulin, insulin to glucagon ratio, and glucose concentrations (all $P \leq 0.001$) and decreased plasma NEFA and BHBA concentrations (both $P < 0.001$), and tended to increase TG concentrations ($P = 0.06$) compared with the high fNDF treatment (Table 5.2). At the beginning of the PP, low fNDF diets increased glucagon concentration compared with high fNDF, but this difference disappeared as time progressed (interaction $P = 0.01$). Overall, 2% FAT increased plasma TG concentration ($P < 0.01$) and tended to decrease plasma NEFA concentration ($P = 0.06$);

Table 5.1. Effects of dietary forage NDF (fNDF) content and a saturated free FA supplement (FAT) on DMI, milk yield, energy intake, energy balance, and efficiency of energy utilization of dairy cows during the postpartum period (d 1 to 29 postpartum; n = 48)

	20% fNDF		26% fNDF			Significance, *P*-value					
Item	0% FAT	2% FAT	0% FAT	2% FAT	SEM	fNDF	FAT	fNDF x FAT	fNDF x Time	FAT x Time	fNDF x FAT x Time
DMI, kg/d	23.6	24.2	20.8	23.0	0.74	<0.01	0.04	0.25	0.79	0.61	<0.01
Milk yield, kg/d	51.2	45.3	48.2	47.8	1.89	0.90	0.10	0.16	0.09	0.72	0.22
Energy Intake, Mcal/d											
DE[1]	65.0	70.0	58.1	66.2	2.16	<0.01	<0.01	0.40	0.76	0.69	0.03
ME[2]	55.0	59.9	49.3	56.7	1.88	0.01	<0.001	0.45	0.75	0.72	0.04
NE_L[3]	34.2	37.7	30.7	35.7	1.20	0.01	<0.001	0.49	0.75	0.75	0.04
Production											
NE_L Maintenance,[4] Mcal/d	11.4	11.0	10.7	10.9	0.28	0.14	0.65	0.33	0.05	0.78	0.79
Milk NE_L,[5] Mcal/d	39.4	35.9	38.0	39.2	1.71	0.55	0.49	0.16	0.20	0.30	0.30
BW change[6]	-110	-84.9	-126	-107	10.7	0.06	0.03	0.79	NA	NA	NA
BCS change[6]	-0.816	-0.490	-0.950	-0.903	0.09	<0.01	0.04	0.13	NA	NA	NA
Energy Balance,[7] Mcal/d	-16.4	-9.54	-18.1	-14.5	1.76	0.04	<0.01	0.33	0.31	0.40	0.10
Efficiency											
NE_L Milk/DE Intake	0.622	0.527	0.664	0.603	0.03	0.02	<0.01	0.47	0.20	0.08	0.19
NE_LProd.[8]/DE Intake	0.805	0.687	0.853	0.772	0.03	0.01	<0.001	0.47	0.24	0.06	0.17

[1]DE Intake = Gross energy intake (Mcal/d) x Gross energy digestibility.
[2]ME Intake = MEp (metabolizable energy at production levels of intake; Mcal/kg of DM; calculated according to NRC, 2001) x DMI (kg/d)
[3]NE_L Intake was calculated from DE through ME according to NRC (2001).
[4]NE_L Maintenance (Mcal/d) = 0.08 Mcal/kg x BW $(kg)^{0.75}$ (NRC, 2001).
[5]Milk NE_L (Mcal/d) = Milk yield (kg/d) x [(fat % x 0.0929) + (true protein % x 0.0563) + (lactose % x 0.0395)] (NRC, 2001).

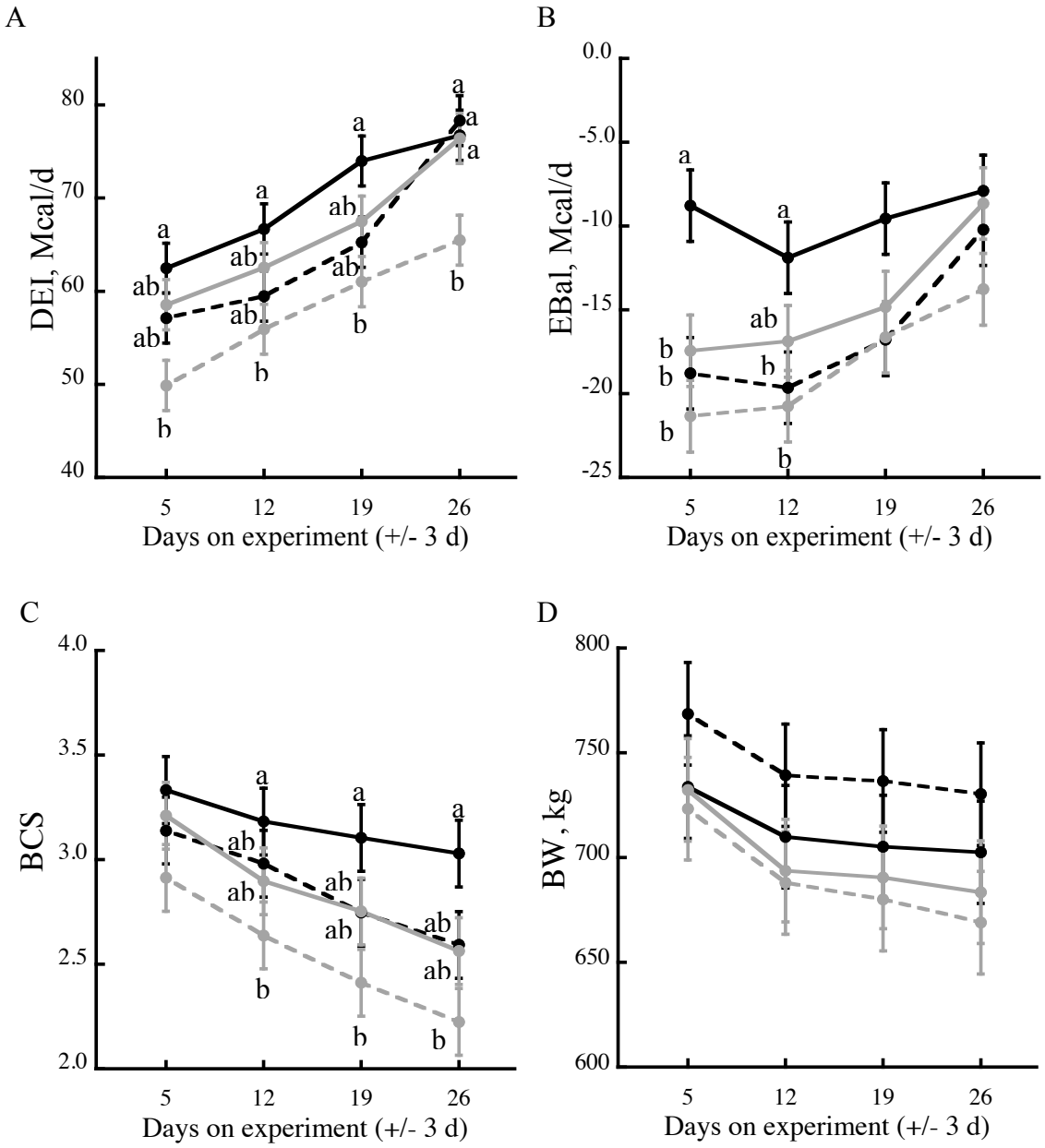

Figure 5.1. Effects of dietary forage NDF (fNDF) content and a saturated free FA supplement (FAT) on: A) digestible energy intake (DEI), B) energy balance (EBal), C) BCS, and D) BW, over time during the postpartum period. Treatment diets were: 20% fNDF 0% FAT (black, broken line), 20% fNDF 2% FAT (black, solid line), 26% fNDF 0% FAT (grey, broken line), and 26% fNDF 2% FAT (grey, solid line) (n = 48). Different letters within a time point signify significant difference at $P \leq 0.05$. Interactions between fNDF, FAT, and time were detected for DEI and EBal ($P \leq 0.10$). Interactions between FAT and time and fNDF and time for BW and BCS, respectively, were detected (both $P < 0.10$). Data for BCS and BW were presented in the companion paper (Piantoni et al., submitted).

Table 5.2. Effects of dietary forage NDF (fNDF) content and a saturated free FA supplement (FAT) on plasma metabolites and hormones of dairy cows during the postpartum period (d 1 to 29 postpartum; n = 48)

Item	20% fNDF		26% fNDF		SEM	Significance, *P*-value					
	0% FAT	2% FAT	0% FAT	2% FAT		fNDF	FAT	fNDF x FAT	fNDF x Time	FAT x Time	fNDF x FAT x Time
Insulin, µg/mL	262	353	129	145	45.9	<0.001	0.25	0.42	0.32	0.31	0.34
Glucagon, pg/mL	118	126	114	120	4.91	0.30	0.13	0.88	0.01	0.41	0.16
Insulin:glucagon	2.21	2.83	1.12	1.23	0.34	<0.001	0.29	0.45	0.55	0.33	0.35
Glucose, mg/dL	49.9	51.1	45.5	45.8	1.37	0.001	0.62	0.74	0.84	0.72	0.38
NEFA, µEq/L	689	522	965	868	74.0	<0.0001	0.06	0.61	0.66	0.05	0.18
BHBA, mg/dL	8.95	9.62	15.0	17.9	1.99	<0.001	0.32	0.53	0.16	0.16	0.48
Triglycerides, mg/dL	5.81	7.19	5.22	6.33	0.42	0.06	<0.01	0.72	0.55	0.05	0.73

the decrease in NEFA was more pronounced at the beginning than at the end of the treatment period (interaction $P = 0.05$) and the increase in TG was observed during the whole treatment period except for d 12 PP (interaction $P = 0.05$). Compared with 0% FAT, 2% FAT decreased plasma NEFA concentration during the first week postpartum (706 vs. 943 μEq/L; $P < 0.05$). No interactions between dietary fNDF content and FAT were detected.

Glucose and insulin tolerance tests

The low fNDF diets resulted in greater response to the GTT performed on d 13 PP than the high fNDF diets (Table 5.3). The low fNDF treatment increased plasma glucose baseline values and maximum concentration of glucose after glucose infusion and decreased the time required for plasma glucose concentration to return to baseline values ($P < 0.01$), decreasing plasma glucose area under the curve (AUC; $P = 0.03$) compared with high fNDF diets. During the GTT, 2% FAT increased the time required for glucose to reach baseline concentration in the high fNDF diet and decreased it in the low fNDF diet (interaction $P = 0.07$) but had no other effects on glucose responses. Low fNDF diets and 2% FAT increased maximum plasma insulin concentration after glucose infusion ($P \leq 0.05$) and the rate at which this maximum concentration was reached (both $P = 0.02$) but FAT effects were greater in the low fNDF diets than in the high fNDF diets (both interactions $P < 0.10$). Both low fNDF diets and 2% FAT increased baseline insulin concentration on the day of the GTT (both $P < 0.05$). Low fNDF increased insulin AUC after glucose infusion ($P = 0.01$), while 2% FAT only tended to increase it ($P < 0.10$). Fat supplementation tended to interact with dietary fNDF content for insulin secretion: 2% FAT increased insulin AUC by 65% when included in the low fNDF diet, but only by 5.5% when included in the high fNDF diet

(interaction $P = 0.12$).

During the ITT, low fNDF increased baseline plasma glucose concentration and the rate of decrease to its nadir compared with the high fNDF treatment (both $P < 0.05$). Fat supplementation decreased the absolute value of the AUC for glucose after insulin infusion ($P = 0.02$). The low fNDF diet with 2% FAT increased baseline plasma insulin on the day of the ITT compared with the other diets (interaction $P = 0.07$); overall, both the low fNDF diets and 2% FAT increased baseline plasma insulin concentration (both $P \leq 0.05$). During the ITT, 2% FAT decreased insulin time to baseline in the low fNDF but increased it in the high fNDF treatment (interaction $P < 0.01$).

Liver glycogen, total lipid, and TG contents

Dietary fNDF content and FAT did not interact to affect liver parameters measured on day 19 PP (Table 5.4). High fNDF diets tended to increase BCS loss ($P = 0.07$) and increased DM percent and liver total lipids content (both $P < 0.05$). High fNDF diets increased TG content by 42% compared with the low fNDF diets ($P < 0.05$). Even though 2% FAT tended to increase both BW and BCS loss by day 19 PP ($P < 0.10$), it did not affect DM percent or liver total lipids and TG contents. Liver glycogen content was not affected by treatment. Liver TG were positively related to plasma NEFA concentration at day 19 PP ($R^2 = 0.52$; $P < 0.0001$; Figure 5.2A), and negatively related to the BCS change observed from parturition until day 19 PP ($R^2 = 0.25$; $P < 0.001$; Figure 5.2B).

Table 5.3. Effects of dietary forage NDF (fNDF) content and a saturated free FA supplement (FAT) on glucose and insulin responses to glucose (13±3 d postpartum) and insulin (14±3 d postpartum) tolerance tests in dairy cows (n = 48)

Item	20% fNDF		26% fNDF		SEM	Significance, *P*-value		
	0% FAT	2% FAT	0% FAT	2% FAT		fNDF	FAT	fNDF x FAT
Glucose tolerance test								
Glucose								
Baseline, mg/dL	49.5	50.1	45.1	43.1	1.80	<0.01	0.70	0.48
Maximum,[1] mg/dL	170	171	161	158	4.52	0.02	0.81	0.72
Rate, min x mg/dL	12.1	12.1	11.6	11.5	0.36	0.14	0.92	0.95
Time to baseline, min	62.5	55.8	67.5	75.0	3.75	<0.01	0.91	0.07
AUC,[2] min x mg/dL	3,488	3,371	3,772	3,965	206	0.03	0.85	0.44
Insulin								
Baseline,[3] µIU/mL	1.57	2.74	1.03	1.52				
Log-transformed	0.195	0.438	0.014	0.183	0.09	0.02	0.03	0.68
Maximum, µIU/mL	44.6	84.5	38.0	40.4	10.7	0.02	0.05	0.07
Time to maximum, min	15.0	15.0	14.2	12.5	1.45	0.23	0.55	0.55
Rate, min x µIU/mL	3.07	5.61	2.66	3.04	0.64	0.02	0.02	0.08
Time to baseline, min	78.3	75.8	80.0	75.0	3.83	0.91	0.33	0.74
AUC, [2] min x µIU/mL	1,575	2,586	1,243	1,307	320	0.01	0.08	0.12
Insulin tolerance test								
Glucose								
Baseline, mg/dL	53.6	51.1	48.3	45.9	1.57	<0.01	0.14	0.97
Minimum, mg/dL	25.0	26.9	23.6	24.4	1.41	0.17	0.34	0.72
Time to minimum, min	37.9	34.6	40.0	42.1	3.32	0.16	0.85	0.42
Rate, min x mg/dL	-0.821	-0.769	-0.648	-0.568	0.08	0.02	0.40	0.86
Time to baseline, min	110	98.8	111	105	6.92	0.59	0.21	0.72
AUC, [2] min x mg/dL	-2,034	-1,561	-1,834	-1,518	167	0.47	0.02	0.64
Insulin								
Baseline,[3] µIU/mL	1.39	2.56	1.14	1.24				
Log-transformed	0.142	0.409	0.057	0.094	0.07	<0.01	0.02	0.07
Maximum, µIU/mL	712	777	637	707	92.9	0.43	0.46	0.97
Time to maximum, min	2.50	2.50	2.50	2.71	0.10	0.32	0.32	0.32
Rate, min x µIU/mL	284	309	254	259	35.0	0.25	0.66	0.77
Time to baseline, min	118	85.0	101	115	6.76	0.32	0.17	<0.01
AUC, [2] min x µIU/mL	9,518	9,066	8,368	9,056	745	0.37	0.85	0.38
BW change, 12 d - pre-calving	-98.5	-61.7	-86.6	-86.2	10.9	0.55	0.09	0.09
BCS change, 12 d - pre-calving	-0.427	-0.330	-0.497	-0.521	0.08	0.09	0.63	0.43

[1]Time to maximum plasma glucose concentration was 10 min for all cows.
[2]Area under the curve, calculated with the trapezoidal rule.
[3]For interpretation purposes, means were back-transformed from the log-transformed means showed in the following row.

Table 5.4. Effects of dietary forage NDF (fNDF) content and a saturated free FA supplement (FAT) on liver glycogen, triglyceride, and total lipid contents of dairy cows 19±3 d postpartum (n = 48)

Item	20% fNDF		26% fNDF		SEM	Significance, *P*-value		
	0% FAT	2% FAT	0% FAT	2% FAT		fNDF	FAT	fNDF x FAT
Liver								
DM, %	29.2	28.9	31.7	31.9	1.29	0.03	0.95	0.85
Total lipids, % of DM	30.3	30.0	40.4	38.4	3.69	0.02	0.76	0.82
Triglycerides, % of DM	7.47	6.01	10.4	8.69	1.39	0.04	0.24	0.91
Glycogen, % of DM	2.70	2.19	1.86	1.87	0.41	0.14	0.53	0.51
BW change, 19 d - pre-calving	-104	-65.7	-94.9	-89.4	12.3	0.54	0.07	0.17
BCS change, 19 d - pre-calving	-0.629	-0.410	-0.708	-0.639	0.09	0.07	0.09	0.37

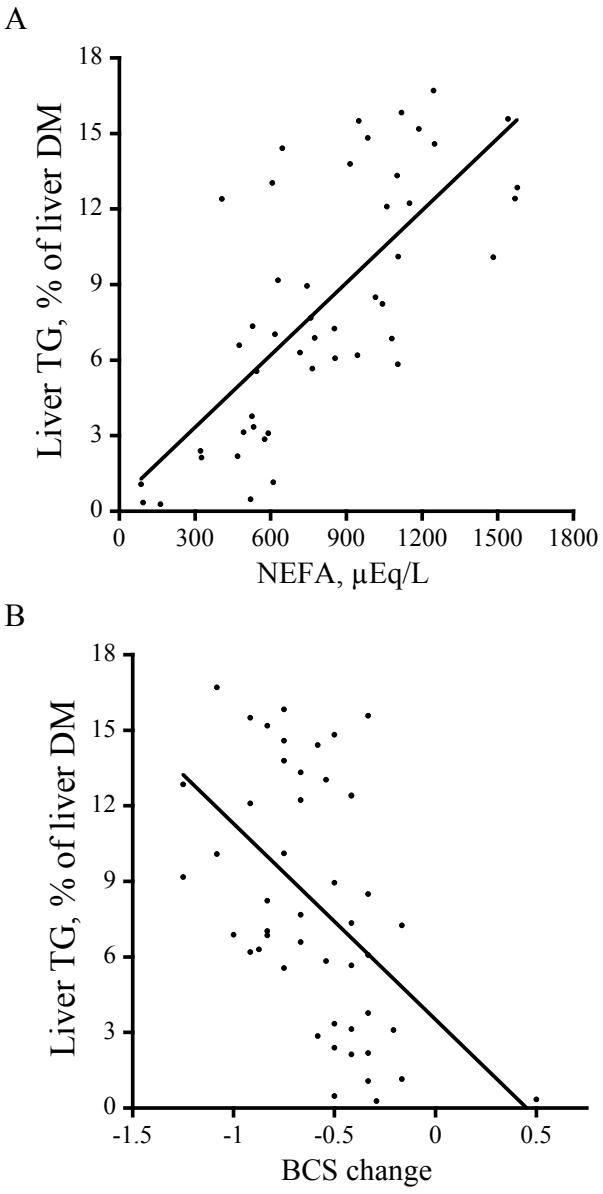

Figure 5.2. Relationship between liver triglyceride (TG) content and plasma NEFA concentration and BCS change between prepartum and 19±3 d postpartum (n = 48). Panel A: Relationship between liver TG content and plasma NEFA concentration [Liver TG (% of liver DM) = 0.459 + 0.010 x NEFA (µEq/L); $R^2 = 0.52$; $P < 0.0001$]. Panel B: Relationship between liver TG content and BCS change [Liver TG (% of liver DM) = 3.51 – 7.78 x BCS change; $R^2 = 0.25$; $P < 0.001$].

DISCUSSION

Digestible energy intake and EBal

Previous research suggests that feeding low forage/high starch diets or saturated fats during the first weeks PP might improve net energy intake and EBal (Jerred et al., 1990). However, other experiments in the literature vary not only in level of dietary forage and type of supplements fed, but also in days on treatment and stage of lactation of cows being supplemented (e.g. pre- and PP, only PP, early lactation), making it difficult to compare results. For example, Rabelo et al. (2003) showed that a lower F:C diet (25% NDF) did not affect energy intake or EBal when fed during the first 70 d PP compared with a higher F:C diet (30% NDF). Nevertheless, in that same experiment the lower F:C diet tended to increase DMI, and therefore, increased energy intake during the first 3 wk PP, which is consistent with Jerred et al. (1990) and our results with the lower fNDF diets during the first weeks PP. Unfortunately, Rabelo et al. (2003) did not calculate EBal for the first 3 wk PP. In terms of fat supplementation, Moallem et al. (2007b) fed a saturated fat during the pre and PP periods and reported that fat supplementation did not affect predicted energy intake or milk yield, but decreased predicted EBal PP compared with a diet with no supplemental fat. Consistent with these results, Beam and Butler (1998) showed that a saturated free FA supplement fed only during the immediate PP period did not affect predicted energy intake during the first 6 wk of lactation. However, in the current experiment we observed an increase in energy intake as well as EBal when feeding FAT during the first weeks of the PP, regardless of dietary fNDF. Weiss and Pinos-Rodriguez (2009) reported an interaction among saturated free FA supplementation, dietary fNDF content, and time in early lactation cows: diets supplemented with saturated fat increased predicted net energy intake in both high and low fNDF diets

before cows reached peak milk, but lower fNDF diets increased predicted net energy intake after peak lactation. Overall, that experiment showed that fat supplementation and lower fNDF diets increased net energy intake from 21 to 126 d PP. Experiments mentioned indicate that feeding a higher energy dense diet do not always increase energy intake and improve EBal and that results observed in early lactation may not apply to cows in the immediate PP period.

Unfortunately, DEI was generally predicted from the diet and not actually measured in previous experiments with PP cows. Considering how variable DM digestibility among cows can be (Piantoni et al., 2013), predicting EBal using energy concentrations predicted from dietary composition is inadequate, and therefore, digestibility of nutrients was evaluated weekly in the current experiment. In the current experiment, FAT supplementation increased measured DEI and EBal regardless of fNDF content of the diet. However, FAT decreased BCS loss but did not affect milk NE_L output during the PP, and therefore, FAT supplementation decreased the efficiency of utilization of DEI for milk NE_L. Consequently, fat supplementation during the immediate PP increased energy partitioned to body reserves and not milk production, regardless of dietary fNDF content, but more so in the low fNDF diet. In contrast, high fNDF diets decreased DEI and EBal, but did not affect milk NE_L, increasing BCS loss, and therefore, efficiency of utilization of NE_L. Our hypothesis was based upon previous research with cows in early lactation in which treatments were initiated 21 d PP (Weiss and Pinos-Rodriguez, 2009); since production results in the current experiment with PP cows differ from those, our hypothesis was not confirmed.

Plasma metabolites and hormones

The effect of FAT on energy partitioning was consistent with a tendency for lower plasma NEFA concentrations, which suggests reduced lipolysis and fat mobilization. The difference in plasma NEFA concentration between 0% and 2% FAT was greatest during the first week PP, when NEFA concentrations are highest in plasma (Contreras et al., 2010). Results are in contrast with previous studies in which saturated fat supplementation had no effect on plasma NEFA concentrations during the PP period (Beam and Butler, 1998; Moallem et al., 2007a). Although we did not detect an effect of FAT supplementation on weekly plasma insulin concentrations, 2% FAT increased baseline plasma insulin concentration by more than 50% compared with 0% FAT for the GTT and ITT, and more so in the low fNDF diet for the ITT, consistent with the effect of FAT on energy partitioning. The insulinotropic effect of long-chain saturated FA such as palmitic and stearic acids has been previously demonstrated in in vitro studies with perfused pancreas of fasted rats (Stein et al., 1997) and in vivo in cows (Harvatine and Allen, 2006). Inconsistency in plasma insulin concentrations between weekly samples and baseline values for GTT and ITT could be explained by the different times of the day relative to feeding in which these samples were taken: weekly samples were taken within one hour before feeding, while samples for baseline values for GTT and ITT were taken ~3 h before feeding. The effect of high fNDF diets on energy partitioning was supported by increased plasma NEFA and BHBA concentrations and decreased plasma insulin and glucose concentrations and insulin to glucagon ratio. The effects of fNDF treatment on glucose, insulin, and BHBA plasma concentrations are consistent with those reported by Rabelo et al. (2005) in PP cows. However, dietary NDF content had no effect on plasma NEFA concentration in that experiment.

Liver total lipid, TG, and glycogen contents

A higher dietary starch content during the PP period, when intense lipomobilization in dairy cows usually occurs, tended to decrease liver TG content at day 21 PP (Rabelo et al., 2005), despite the lack of an effect on BW loss (Rabelo et al., 2003). In agreement with Rabelo et al. (2005), our results indicate that lower fNDF diets reduced liver total lipid and TG contents, which in our case, were related to a tendency for a decrease in BCS loss. Results in the literature are inconsistent regarding the effect of fat supplementation on liver parameters. Supplementing a saturated fat during the transition period decreased liver TG content, plasma NEFA concentrations, and plasma aspartate amino-transferase activity, an indicator of liver health, in PP cows compared with a control diet with no supplemental fat (Karcagi et al., 2009). In contrast, supplementation of a saturated fat during the transition period did not affect liver TG contents, BW, or BCS during this period, even though it tended to decrease plasma NEFA concentration PP (Ballou et al., 2009). In our experiment, 2% FAT tended to decrease BCS loss from parturition to 19 d PP, but did not decrease total liver lipid or TG content. Interestingly, a lipogenic diet fed during the transition period increased liver TG during the PP period but had no effect on BW loss compared with a glucogenic diet (van Knegsel et al., 2007). A higher starch diet is therefore more likely to decrease liver total lipids and TG than supplemental fat, which is consistent with our results.

Insulin and glucose tolerance tests

Insulin resistance of extra-hepatic tissues develops during late pregnancy and persists during early lactation, and this is accompanied by a decrease in pancreatic insulin secretion (Bell, 1995; Zachut et al., 2013). To our knowledge, there are no experiments that have

evaluated the effects of saturated fat supplementation and diets with different fNDF contents on responses to insulin or glucose tolerance tests in PP cows. In our experiment, after the glucose infusion, the low fNDF diet with 2% FAT increased plasma insulin concentration to the greatest extent and at a faster rate compared with the other diets, which could indicate superior pancreatic insulin secretion capacity in these cows. Also, the lower fNDF diet with 2% FAT increased AUC for insulin the most during the GTT. The effects of fat supplementation in the lower fNDF diet on insulin response were likely responsible for plasma glucose concentrations reaching baseline values faster compared with the other diets during the GTT. Consistent with our results, Pires et al. (2007) reported that maximum insulin and insulin AUC were higher in nonlactating, nongestating cows infused with tallow than in cows infused with saline during a GTT.

During the ITT, FAT supplementation decreased the absolute value of the glucose response AUC, which suggests increased resistance to insulin in extra-hepatic tissues, and therefore, less glucose utilization. Interestingly, intravenous infusions of tallow also reduced glucose response during an ITT, compared with saline infusions in nonlactating nonpregnant cows (Pires et al., 2007) as well as in nonlactating late-gestation cows (Salin et al., 2012). Also similar to what was observed in our experiment, Pires et al. (2007) showed that tallow infusion increased basal plasma insulin concentrations, but did not affect insulin response during the ITT. Responses to GTT and ITT observed by Pires et al. (2007) and Salin et al. (2012) indicate that tallow infusion may have caused insulin resistance in cows, and researchers concluded that this was likely due to increased plasma NEFA concentrations. Even though in our experiment FAT supplementation elicited similar glucose and insulin responses to GTT and ITT to those observed by Pires et al. (2007), they were not associated

with higher plasma NEFA concentrations measured within one hour prior to feeding. Saturated FA increase adipose tissue expansion, inducing insulin resistance, and impairing insulin signaling in laboratory animals (Kennedy et al., 2009). These effects on metabolism are consistent with our results with the tolerance tests, and might also explain why insulin baseline concentrations were enhanced, since plasma insulin concentrations generally increase to compensate for increased insulin resistance. However, cows with greater insulin resistance might be expected to have greater response in milk yield, which is opposite to what happened when cows were supplemented fat in the lower fNDF diet. Although supplemental fat might have increased insulin resistance of tissues, the elevated plasma insulin concentration when combined with the higher starch (low fNDF) diet might have reduced net lipolysis and loss of body reserves at the expense of milk yield.

During the GTT, low fNDF diets, with greater starch content, resulted in greater insulin secretion compared with high fNDF diets. This was demonstrated by increased insulin maximum, the rate at which insulin increased, and insulin AUC. During the GTT, glucose AUC was lower for lower fNDF diets, indicating faster clearance from blood likely related to increased insulin sensitivity of tissues and not greater secretion in milk, since milk production in these cows was not different from those on the higher fNDF diet. Moreover, during the ITT, low fNDF diets decreased plasma glucose concentration at a faster rate than high fNDF diets, also indicating higher insulin responsiveness from insulin responsive tissues.

CONCLUSIONS

In a companion article we showed that feeding 2% FAT in a lower fNDF diet during the first 29 d PP affected energy partitioning, decreasing BCS loss in the PP period and 3.5%

FCM yield in the carryover period. Data presented in this article indicate that effects on energy partitioning observed in the lower fNDF diet with 2% FAT might be related to an increase in plasma insulin concentration. Even though feeding a highly saturated FA supplement in the PP period increased EBal and DEI, especially in the lower fNDF diet, it did not affect liver TG content. A lower fNDF diet during the PP period not only improved EBal and plasma metabolic and hormonal profile, but also decreased liver TG content. Both 2% FAT supplementation and lower fNDF diets increased insulin secretion, and insulin resistance of tissues was likely increased by 2% FAT and decreased by lower fNDF. Feeding a highly saturated FA supplement during the PP period in a low fNDF diet might have primed the cows to limit fat mobilization apparently at the expense of milk, making it difficult to justify their use in similar diets during this period.

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CHAPTER 6

CONCLUSIONS AND FUTURE DIRECTIONS

We conclude that supplementation of saturated fats might benefit lactating dairy cows in some cases, but results are dependent upon fat supplements fed, diet, stage of lactation, and milk yield of cows. Currently, there are inadequate data available to determine the effects of individual FA and their interaction with dietary ingredients and different stages of lactation or level of milk production on cow performance. The work presented in this dissertation provides knowledge on how certain fats could be used in lactating dairy cow rations, but also raises more questions about how to feed them and if it is even profitable with current supplement and milk prices. Still more work is required to evaluate the effects of supplementation of pure FA supplements and a mixture of these on digestion, and metabolic and production responses in lactating dairy cows. Interactions among level of milk production/stage of lactation, dietary forage level or other ingredients, and fat supplementation are of great interest and its importance was confirmed with results presented in this work. Further work is needed to clarify these interactions as well as the marginal economic return, if any, of specific fat supplements for different situations. Marginal economic return will likely be highly variable and will depend on interacting factors affecting production responses as well as feed and milk component prices.

The following experiments would be beneficial:

1) Determine the effects of palmitic and stearic acids at two concentrations of fNDF of the diet on productive performance, feeding behavior, and total tract digestion of nutrients.

2) Conduct dose response experiments with different FA.

3) Evaluate apparent digestibility of different fat supplements.

4) Evaluate interactions among different dietary ingredients and fat supplements.

5) Conduct feeding behavior studies to gain insight into why certain FA supplements (e.g. C18:0 in past-peak cows and Energy Booster 100® in post-partum cows) increase DMI.

6) Periodically perform marginal economic analyses considering not only production responses, but also effects on EBal, BCS, and reproductive performance, and evaluate profitability of fat supplementation in dairy operations.

Importantly, these experiments should be done with cows in different physiological states (e.g., immediately postpartum, peak lactation, late lactation). Also, experiments should include a control diet with no supplemental fat, because unfortunately much research has been published comparing fat sources to each other with no proper control diet. The long-term goal of these experiments would be to answer questions such as:

- At which stages of lactation are responses to FA supplementation greatest and/or profitable?
- Is there an optimum content of FA in dairy diets and does it vary depending on other interacting factors?
- Can we modulate response to FA supplementation by altering concentration of different dietary ingredients (e.g., insulin secretion or sensitivity)?

The results from these experiments will help nutritionists and farmers determine whether they should feed fat, and if so, which fat should they feed, at which inclusion rate, in what kinds of diets, and to which groups of cows. Moreover, it would help determine if feeding fats is an economically sound decision to increase profitability of the dairy operation.