CHANGING THE DIETARY RATIO OF FATTY ACIDS UNDER DIFFERENT PHYSIOLOGICAL CONDITIONS ALTERS ENERGY PARTITIONING OF DAIRY COWS

By

Jonas de Souza

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ABSTRACT

CHANGING THE DIETARY RATIO OF FATTY ACIDS UNDER DIFFERENT PHYSIOLOGICAL CONDITIONS ALTERS ENERGY PARTITIONING OF DAIRY COWS

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Fat supplements are often used in an attempt to increase energy intake, yields of milk and milk components, and body reserves of dairy cows. However, different fatty acids (FA) have different metabolic fates and therefore it is critical to understand how FA may affect energy partitioning and milk production. Importantly, physiological state (i.e. lactation stage, lactation number, production level) plays an important role in the efficiency of nutrient utilization and may interact with different nutrition strategies affecting metabolic and production responses. Our research examined the effects of varying the dietary ratio of FA under different physiological conditions on nutrient digestion, energy partitioning, and production responses of dairy cows. In the first research chapter, we evaluated the effects of varying the ratio of dietary palmitic (C16:0), stearic (C18:0), and oleic (*cis*-9 C18:1) acids on post-peak dairy cows. Among the combinations of C16:0, C18:0, and *cis*-9 C18:1 evaluated, FA supplements with more C16:0 increased energy output in milk, whereas FA supplements with more *cis*-9 C18:1 increased energy storage in body reserves. Increasing C18:0 in a FA supplement reduced FA digestibility and did not increase energy intake, which most likely explains its lower performance compared with the other FA treatments. In the second research chapter, we determined the long-term effects of C16:0 supplementation on primiparous and multiparous post-peak dairy cows. Our results demonstrated that supplementation with C16:0 consistently increased DMI, energy intake, milk yield, milk fat content and yield, energy-corrected milk (ECM), and NDF digestibility in both primiparous and multiparous cows. In addition, C16:0 supplementation increased body

weight (BW) change in primiparous cows but not in multiparous cows. In the third and fourth research chapters, we determined the effects of timing of C16:0 supplementation on production and metabolic responses of early lactation dairy cows. Our results demonstrated that feeding a C16:0 supplement to early lactation cows consistently increased the yield of ECM compared with a non-fat control diet regardless of the timing of supplementation. C16:0 supplementation also increased NDF digestibility, energy intake, and milk energy output. When fed in the immediate postpartum period, C16:0 increased negative energy balance, plasma non-esterified FA, and BW and BCS loss, and decreased plasma insulin. In the fifth chapter, we evaluated responses of lactating dairy cows with different levels of milk production to alterations in the dietary ratio of C16:0 and *cis*-9 C18:1. Our results indicated that high producing dairy cows (averaging 60 kg/d) responded better to FA supplements containing more *cis*-9 C18:1, while lower producing cows (averaging 45 kg/d) responded better to FA supplements containing more C16:0. Regardless of production level, increasing *cis*-9 C18:1 increased total FA digestibility, BW and BCS change, with no effect on DMI. In our last research chapter, we determined the effects of altering the dietary ratio of C16:0 and *cis*-9 C18:1 on metabolic and production responses of early lactation dairy cows during the immediate postpartum period and the carryover effects of the treatment diets in early lactation. We observed that feeding FA supplements containing C16:0 and *cis*-9 C18:1 during the immediate postpartum period increased milk yield and ECM, tended to increase DMI and reduce BW loss compared with a non-fat control diet. Additionally, the yield of milk and milk components and ECM were higher during the carryover period for cows that received FA-supplemented diets compared with control during early postpartum period. Overall, our results indicate that different combinations of FA should be used according to production level and stage of lactation in dairy cows.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

AOAC	Association of Official Agricultural Chemists
BCS	body condition score
BH	Biohydrogenation
BHB	β-hydroxybutyrate
BW	body weight
Ca	calcium
C16:0	palmitic acid
C18:0	stearic acid
cis-9 C18:1	oleic acid
CON	control treatment
CP	crude protein
CS	cottonseed based diet
d	day (s)
DE	digestible energy
DIM	days in milk
DM	dry matter
DMI	dry matter intake
ECM	energy-corrected milk
FA	fatty acid(s)
FAYR	FA yield response
FAME	fatty acid methyl ester(s)

FCM	fat-corrected milk
FID	flame-ionization detector
FR	Fresh period (1-24 DIM)
g	gram(s)
GE	gross energy
GLC	gas-liquid chromatography
h	hour(s)
iNDF	Indigestible NDF
kg	kilogram(s)
M	moles per liter
MBW	metabolic body weight
Mcal	Mega calories
ME	metabolized energy
MFD	milk fat depression
min	minutes(s)
mL	milliliter(s)
mm	millimeter(s)
μm	micrometer(s)
MUFA	monounsaturated FA
n	number
N_2	nitrogen gas
NDF	neutral detergent fiber
NE	net energy

NEFA	non-esterified fatty acid
NE_L	net energy for lactation
NRC	National Research Council
OBCFA	odd- and branched-chain fatty acid
PA	palmitic acid
PA+OA	palmitic and oleic acids
PA+SA	palmitic and stearic acids
PK	Peak period (25 -67 DIM)
pMY	preliminary milk yield
PUFA	poly-unsaturated fatty acid
R^2	coefficient of determination
SAS	Statistical Analysis System
SE	standard error
SEM	standard error of the mean
SFA	saturated fatty acid
SH	Soyhulls based diet
TAG	Triglyceride (s)
TMR	total mixed ration
Trt or Treat	P-value associated with the treatment effect
UFA	unsaturated fatty acid

CHAPTER 1

INTRODUCTION

Nowadays, the yield of milk components is the principal driver of variation in producer milk price, which underlies the importance on focusing on increasing the yields of these components. Compared to other milk components, milk fat is typically the most easily manipulated by nutrition and management. At the same time, avoiding excessive body weight loss in early lactation as well as recovering of body condition in post-peak cows is important to improve reproductive performance and farm profitability, while ensuring that it does not result in excessive body condition in later lactation. Therefore, understanding the effects of different fat sources on milk production and energy partitioning is crucial. While in general fat supplementation has been shown to increase the yield of milk, milk fat and reproductive performance, great variation has been reported for different fat types, and indeed the same supplement across different diets and studies (Rabiee et al., 2012; Rodney et al., 2015). Reasons for variability across experiments could be from use of different types of fat supplements, level of FA supplementation, interactions with other diet ingredients, and the physiological state of cows.

The importance of individual fatty acids (FA) on a diet extends beyond their energy contribution to potentially metabolic and physiological effects. We propose that the FA profile of a fat supplement is most likely the major factor affecting the response to it. Recently, the effects of individual FA on digestibility, metabolism, and production responses of dairy cows has received renewed attention. In this regard, palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (*cis*-9 C18:1) usually comprise most FA present in milk fat and adipose tissue of dairy cows. These FA have different functions and fates in metabolism, but they may also interact with

each other by competition or complementary mechanisms under different physiological conditions. Importantly, since these FA are also the most abundant FA in feeds and FA supplements to feed cows, determining an optimal dietary ratio among these FA may optimize their utilization. Therefore, understanding the differential responses of adipose and mammary tissues to diet with different combinations of FA under different physiological conditions is important.

To our knowledge, few studies were designed to evaluate the effects of different FA ratios on the production and metabolic responses of dairy cows. It is critical to understand how energy-dense diets based on supplemental fat affect energy partitioning in order to improve our understanding on what (s) FA and when fed supplemental fat in dairy diets. Increased dairy farm profitability due to supplemental FA would depend on the cost of the supplement relative to other diet ingredients, the value of the production responses in relation to milk price, and other intangibles factors related to body reserves, 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. Our objective was to determine the effects of varying the dietary ratio of FA under different physiological conditions on energy partitioning, nutrient digestibility, and production responses of dairy cows. The interconnected relationship between digestion of FA, storage of FA in adipose tissue, and milk fat synthesis in the mammary gland requires consideration of how specific FA impacts these locations.

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CHAPTER 2

LITERATURE REVIEW

Importance of Milk Components

Currently, the Federal Milk Order Program uses multiple component pricing to value milk. The yields of milk fat and protein are the major contributors to the price that producers receive for milk. In an economic analysis assessing the value of milk components, a 5% increase in fat yield, protein yield, and milk yield increased net farm income by 13%, 15%, and 2%, respectively (St-Pierre, 2017). This reinforces the importance of focusing on increasing the yield of milk components and not milk yield per se to maximize milk price and income. Additionally, milk fat is typically the most variable milk component and it is easier to influence (both positively and negatively) through dietary manipulations than milk protein. Therefore, nutrition strategies focused on increasing milk fat yield have the potential to enhance farm profitability.

Rumen Metabolism of FA

Dietary FA are derived from forages, grains, byproducts, and fat supplements fed to lactating dairy cattle. Most dietary lipids are in the form of triglycerides (TAG), glycolipids, or phospholipids (Lock et al., 2005). Dietary FA composition has less influence on the milk FA composition of ruminant animals than non-ruminant animals. Although major dietary FA are unsaturated FA (UFA), the FA reaching the intestine are mostly saturated (SFA) due to the lipid metabolism in the rumen (Harfoot and Hazlewood, 1997). UFA are toxic to many rumen bacteria, thus an extensive metabolism of dietary lipids occurs in the rumen that has a major

impact on the profile of FA available for absorption and tissue utilization (Palmquist et al., 2005).

Rumen bacteria, rather than protozoa and fungi, are the main microbes that perform hydrolysis in the rumen (Harfoot and Hazlewood, 1997). Microbial lipases release FA from their glycerol backbone through hydrolysis (Jenkins, 1993). After hydrolysis, rumen bacteria biohydrogenate UFA to form SFA through isomerization and hydrogenation and produce many different intermediates (Harfoot and Hazlewood, 1997; Shingfield and Wallace, 2014). The primary dietary UFA sources for biohydrogenation (BH) are *cis*-9, *cis*-12 C18:2, *cis*-9, *cis*-12, *cis*-15 C18:3, and the extents of rumen BH for these FA range from 70-95% and 85-100%, respectively (Jenkins et al., 2008). Conditions that might affect the extent of BH and increase passage of UFA to the duodenum are increased rumen concentration of UFA, decreased rumen pH, and the presence of ionophores (Jenkins and Harvatine, 2014). Therefore, C18:0 rather than UFA, is the predominant FA available for absorption by the dairy cow in typical feeding situations (Bauman and Lock, 2006; Boerman et al., 2015). Due to the continued passage of digesta leaving the rumen, some BH intermediates and dietary UFA escape the rumen and are available for absorption in the small intestine as well.

Additionally, rumen bacteria are able to synthesize FA. Odd- and branched-chain FA (OBCFA) in milk primarily originate from rumen bacterial membrane lipids, and therefore milk OBCFA can be used as a tool to predict rumen bacteria populations and rumen fermentation (Fievez et al., 2012). Cellulolytic bacteria contain higher proportions of even and odd- iso FA, in contrast to amylolytic bacteria, which are more enriched by anteiso and linear odd-chain FA (Vlaeminck et al., 2006).

Effect of FA on NDF Digestibility

The amount of FA included in a diet is relatively low for lactating dairy cattle, and changes in FA digestibility, therefore, may have minimal effects on overall DM digestibility and digestible energy intake. Changes in intake and digestibility of other nutrients, such as NDF, due to FA supplementation may affect positively or negatively the digestible energy value of the fat supplement (Boerman et al., 2015). The concept that FA supplementation negatively impacts NDF digestibility is widely accepted. Several studies in the 1950s addressed the effects of added vegetable oils on fiber digestion in the rumen indicating negative effects of oils on cellulose digestibility (Palmquist and Jenkins, 2017). The potential reduction in fiber digestibility when oil is supplemented is thought to be from one or a combination of four proposed mechanisms: 1) coating of the fiber with fat preventing microbial interaction; 2) the toxic effect of UFA on certain microbial populations; 3) inhibition of microbial activity on cell membranes by FA; and 4) reduced cation availability due to the formation of insoluble complexes with FA (Palmquist and Jenkins, 1980).

From the growing interest in the use of lipids to enhance diet energy density, along with the desire to minimize negative effects of UFA on rumen fermentation and digestion the concept of "bypass fats" or "rumen-protected fats" emerged (Palmquist and Jenkins, 2017). When calcium salts of palm FA and prilled fat supplements were included up to 3.5% of diet DM in dairy cow diets, no effects were observed for DM or NDF digestibility (Grummer, 1988). Recently, Weld and Armentano (2017) performed a meta-analysis to evaluate the effects of FA supplementation on DMI and NDF digestibility of dairy cows. Supplementation of FA supplements high in medium chain FA (12 and 14-carbons) decreased both DMI and NDF digestibility. Addition of vegetable oil decreased NDF digestibility by 2.1 percentage units, but

did not affect DMI. Also, feeding saturated prilled fat (combinations of C16:0 and C18:0) did not affect DMI, but increased NDF digestibility by 0.22 percentage units. Overall, the authors concluded that the addition of a fat supplement, in which the FA are 16-carbons or greater in length, has minimal effects on NDF digestibility.

Additionally, recently studies feeding C16:0 supplement to dairy cows reported increases in NDF while DMI was not affected (Warntjes et al., 2008; Piantoni et al., 2013; de Souza et al., 2017; Rico et al., 2017a). A recent meta-analysis has analyzed available individual cow data from 6 studies that fed a C16:0-enriched supplement to dairy cows, and observed that NDF digestibility was positively impacted by total C16:0 intake (Figure 2.1; de Souza et al., 2016) and DMI was not affected. This suggests that the increase in NDF digestibility when C16:0 supplements are fed to dairy cows is not explained through a decrease in DMI. Although reasons for this effect need to be further determined, potential explanations may involve changes in gut peptides that are related to gastrointestinal motility and direct effect of FA on microbial populations. Piantoni et al. (2013) related the increase in NDF digestibility with an increase in retention time driven by enhanced in CCK secretion. Alternatively, bacteria typically synthesize C16:0 de novo to produce phosphatidic acid, the precursor for FA components in membranes of *Butyrivibrio* bacteria (Hackmann and Firkins, 2015). However, if dietary C16:0 could be incorporated into rumen bacterial membranes, considerable ATP would be spared which may favor bacterial growth (Vlaeminck et al., 2006), potentially increasing NDF digestibility.

Metabolism of FA in the Intestine

Under typical feeding situations, C18:0 is the predominant FA available for absorption by the dairy cow, regardless of the diet fed. There is no significant absorption or modification of

long and medium chain FA in the omasum or abomasum, therefore, the lipid material available for absorption in the small intestine is similar to that leaving the rumen (Moore and Christie, 1984). This material consists of approximately 80-90% free FA attached to feed particles (Doreau and Chilliard, 1997). The remaining lipid components are microbial phospholipids and some small amounts of TAG and glycolipids from residual feed material. These esterified FA are hydrolyzed by intestinal and pancreatic lipases (Doreau and Ferlay, 1994). In ruminants, micelle formation is the key to digestion and, therefore, key to efficient FA absorption (Davis, 1990). The important feature of micellular solutions is their ability to dissolve (solubilize) the water-insoluble FA by incorporating appropriately shaped and charged molecules either into the core or the outer sheath of the bile salt molecules that comprise the micellular matrix (Freeman, 1984). Both the bile and pancreatic secretions are required for this process; bile supplies bile salts and lecithin, and pancreatic juice provides enzymes to convert lecithin to lysolecithin and bicarbonate to raise the pH. Lysolecithin, together with bile salts, desorb the FA from feed particles and bacteria, allowing the formation of the micelle (Lock et al., 2005). The critical role of lysolecithin and bile salts in this process is demonstrated in studies where FA absorption was virtually eliminated when bile secretion into the duodenum was blocked in sheep (Moore and Christie, 1984). Once micelles are formed they facilitate the transfer of water-insoluble lipids across the unstirred water layer of intestinal epithelial cells of the jejunum, where the FA and lysolecithin are absorbed.

Absorption of FA into intestinal epithelial cells is an energy-independent process that is facilitated by the maintenance of a concentration gradient into the cells (Drackley, 2000). In intestinal cells, free FA are combined with glycerol to form TAG and packaged with cholesterol, phospholipids, and apoproteins into lipoproteins as chylomicrons or VLDL (Bauchart, 1993).

The size of these lipoproteins precludes their direct transfer into the venous bloodstream and therefore they are first secreted into the lymph to be delivered into the bloodstream close to the heart for transport to other organs (Moore and Christie, 1984). Free polyunsaturated FA (PUFA) in intestinal epithelial cells are preferentially incorporated into phospholipids and cholesterol esters as a way to prevent PUFA from being oxidized as fuels (Moore and Christie, 1984). Also, short and medium chain (<14 carbon) are not re-esterified in the intestine and may enter into circulation as free FA (Bauchart, 1993).

Usually, FA digestibility decreases as the flow of FA increases to the intestine (Figure 2.2). The flow of C18:0 has a critical impact on total FA digestibility as observed in a recent meta-analysis and meta-regression examining the intestinal digestibility of long-chain FA in lactating dairy cows (Boerman et al., 2015). When the authors compared the digestibility of 16- and 18-carbon FA to the digestibility of C18:0 in diets supplemented with fat across the entire data set, they observed modest differences between C18:0 and UFA. Implications for differences among FA was highlighted when they generated best- fit equations for the relationship between flow and digestibility of FA (Boerman et al., 2015). A negative relationship between the total flow and digestibility of FA was observed, and the decrease in total FA digestibility appears to be driven by the digestibility of C18:0 because of the pronounced negative relationship between the duodenal flow and digestibility of C18:0 (Figure 2.2). The exact mechanisms for the reduction in digestibility are not understood; however, potential causes include limits in lysolecithin or competition for absorption sites (Drackey, 2000).

Recently, FA digestibility research has utilized and focused on C16:0 and C18:0 supplements. Boerman et al. (2017) fed increasing levels of a C18:0 supplement (93% C18:0) to dairy cows and observed no positive effect on production responses, which was likely associated

with the pronounced decrease in total FA digestibility as FA intake increased (Figure 2.3). Similarly, Rico et al. (2017a) fed increasing levels of a C16:0 supplement (87% C16:0) to dairy cows and even though a positive effect was observed on production response up to 1.5% diet DM, a decrease in total FA digestibility as FA intake increased was observed (Figure 2.3). Considering that the range on FA intake was similar across both studies, the decrease in total FA digestibility was more pronounced when there was increased intake/rumen outflow of C18:0 rather than C16:0. Potential causes of these differences include the lower solubility of C18:0 than C16:0, which would be more dependent on emulsification for absorption. With respect to *cis*-9 C18:1, results indicate that this FA has greater digestibility that C18:0 and C16:0 (Boerman et al., 2015), and has been proposed to have important amphiphilic properties when fed as a Ca-salt to ruminants (Moate et al., 2004). Also, Freeman (1969) examined the amphiphilic properties of polar lipid solutes and found that *cis*-9 C18:1 had a positive effect on the micellar solubility of C18:0. Considering that most FA leaving the rumen are saturated and the predominant FA available for absorption is C18:0, it is not surprising that the ruminant has evolved such an efficient system involving amphiphilic compounds for solubilizing FA, especially SFA.

Metabolism of FA in the Liver

There is conflicting evidence as to the rate at which individual FA are used by the liver. Bell (1981) reviewed literature data and suggested minimal hepatic utilization of C18:0. Similarly, Mashek and Grummer (2003) showed that the net uptake of C18:0 was lower than that of C16:0, *cis*-9 C18:1, *cis*-9, *cis*-12 C18:2, *cis*-9, *cis*-12, *cis*-15 C18:3 in perfused liver of goats. Authors concluded that liver uptake of all FA tested, except for C18:0, was similar. In contrast, there is a substantial incorporation of radiolabelled C18:0 into ketones, suggesting no

impairment to uptake (Pethick et al., 1983). In transition cows, the liver may take up more C16:0 and *cis*-9 C18:1 since the concentration of these FA increased in liver TAG post-calving compared to pre-calving values mainly due to these being the major FA in adipose tissue (Douglas et al., 2007). Similarly, Prom et al. (2017) reported that feed restriction induced-negative energy balance increased the proportion of FA in TAG and increased C16:0 and *cis*-9 C18:1 in all liver lipid fractions (NEFA, cholesterol and phospholipids).

The liver takes up free FA from blood in proportion to their concentration in plasma (Emery et al. 1992). According to Drackley (2000) within the hepatocytes, long-chain FA (greater that 14 carbons) are activated by acyl-CoA synthetase found in the outer mitochondrial membrane. Short- and medium-chain FA (lower than 12 carbons) pass through the mitochondrial membrane and are activated by acyl-CoA synthetase found within the mitochondria. Under conditions of increased FA uptake, the liver often produces large amounts of the ketone bodies acetoacetate and β-hydroxybutyrate, in the process known as ketogenesis. The two main factors regulating the degree to which FA are oxidized by the liver are the supply of FA to the liver via lipolysis and the partitioning within hepatocytes between mitochondrial oxidation and microsomal esterification.

Ruminants have a very low rate of VLDL export compared with rats, despite similar rates of esterification of FA to TAG (Drackley, 2000). Whether this limitation is in VLDL synthesis or secretion is unknown (Bauchart, 1993). Based on available evidence, it appears that the rate of synthesis or assembly of VLDL is more likely to be limiting than is the secretory process per se (Drackley, 2000). Consequently, extensive body fat mobilization usually results in accumulation of TAG within the liver, potentially resulting in fatty liver (Bauchart, 1993).

Metabolism of FA in the Mammary Gland

Milk FA originate from two sources: < 16 carbon FA are synthesized de novo in the mammary gland and > 16 carbon FA are extracted from plasma as preformed FA. The mixed FA (16-carbon FA) can be derived from either de novo or preformed sources. Acetate and β-hydroxybutyrate, formed by rumen fermentation of carbohydrates, represent the major carbon sources for FA synthesized de novo in the mammary gland (Bauman and Griinari, 2003). In plasma, FA absorbed from the intestine are transported in lipoproteins and FA mobilized from body tissues are transported as NEFA (Bauman and Griinari, 2003). Microbial synthesis of OBCFA in the rumen and absorption of biohydrogenation intermediates also contribute to the diversity of FA secreted in milk fat.

De novo FA synthesis

To produce milk FA from 4 to 16 carbons in length in the mammary gland, the main pathway involves acetate being converted to acetyl CoA by acetyl CoA synthetase. Next, acetyl-CoA carboxylase (ACC) converts acetyl-CoA to malonyl-CoA in an irreversible reaction (Bauman and Davis, 1974). The production of malonyl-CoA is considered the rate-limiting step for de novo synthesis of milk FA and the activity of ACC is considerably lower than the activity of other FA synthesis enzymes. β-hydroxybutyrate can also contribute carbons for initiating milk FA synthesis. In fact, Lin and Kumar (1972) indicated that lactating mammary glands utilize butyryl-CoA more efficiently than acetyl-CoA as a ‘‘primer’’ for FA synthesis. Further, these authors showed that butyryl-CoA can also be synthesized from acetyl-CoA by, essentially, a reversal of β-oxidation in the mammary glands of rabbits, rats and cows. These results agree with the large incorporation of β-hydroxybutyrate as the methyl terminal C_4 up to 50% of FA synthesized de novo by the lactating mammary gland (Palmquist et al., 1969). Propionate and

other branch chain volatile FA can be used as a primer for milk FA synthesis leading to the synthesis of odd and branch FA (Palmquist, 2006). Smith et al. (1974) indicated using radiolabeled precursors that the total carbon contribution for de novo milk FA synthesis was 42.0, 9.4 and 48.6% for acetate, β-hydroxybutyrate and other plasma precursors, respectively. Acetate and β-hydroxybutyrate account for all carbons in C4-C12 milk FA, 75% of C14:0 and 50% of C16:0 (Smith et al., 1974). It is important to point out that although several precursors can initiate FA synthesis, Acetyl-CoA is the principal building block that is used by the complex of FA synthase (FAS) generating palmitate.

Besides a carbon source, FA synthesis requires NADPH. In humans, some of the NADPH required is generated in the first two oxidative steps in the pentose phosphate pathway by glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, respectively. This provides perhaps one-half of the reducing equivalents required for FA synthesis. The activities of both citrate lyase and malic enzyme increase with high carbohydrate diets in non-ruminants. The activities of these latter enzymes are low in ruminants (Bauman et al., 1970), probably reflecting the greater availability of acetate as a lipogenic precursor in these species or the absence of the need to transport these units from the mitochondrion to the cytosol, or both. In ruminants, a major metabolic difference is the limited carbon from glucose to contribute to FA synthesis (Palmquist, 2006). This phenomenon is usually accounted by the low activity of ATP citrate lyase and malate dehydrogenase. In ruminants, most of glucose is derived from gluconeogenesis, while acetate, and other main fuel molecules produced in the rumen, compromising the precursors for the initiation of lipogenesis in both adipose tissue and the mammary gland.

Preformed FA

A second source of FA to the mammary gland is long chain FA from the diet and other tissues. The TAG contained within chylomicrons and VLDL in plasma are the primary source of milk FA >16 carbons in length taken up by the mammary gland (Palmquist, 2006) with NEFA also contributing FA to milk fat when concentrations of plasma NEFA are high, usually occurring during periods of negative energy balance in early lactation (Miller et al., 1991). After absorption in the small intestine, these preformed FA are esterified to glycerol, forming relatively inert TAG. These TAG are then packaged into TAG-rich lipoproteins that usually comprise chylomicrons or VLDL (Smith et al., 2006). Due to the large size of chylomicron and VLDL particles, they have little capacity to move across capillaries (Young and Zechner, 2013). Therefore, the movement of lipids in the cells depends on hydrolysis of the TAG within these particles, a process that is carried out by lipoprotein lipase (LPL) along the luminal surface of capillary endothelial cells (Smith et al., 2006). This process removes around 90% of the TAG from the particles, generating remnant lipoproteins that are largely taken up and removed by the liver (Drackley, 2000). Therefore, FA enter the cells either as FA released from the TAG-rich lipoproteins or FA within the albumin-FA pool. Free FA and diacylglycerol are taken up by mammary epithelial cells and used for TAG synthesis in the mammary gland.

Triglyceride synthesis

Milk fat is composed of 95% triglycerides, 2% diacylglycerol and small concentrations of phospholipids, cholesterol esters, and free FA (Jensen, 2002). The primary pathway used for synthesis of TAG in the mammary gland is the *sn*-glycerol 3 phosphate pathway where both de novo and preformed FA are incorporated into the glycerol-3 phosphate backbone (Dils, 1983). Glycerol phosphate acyl transferase (GPAT) is responsible for adding a fatty acyl-CoA to the *sn*-

1 position of glycerol-3 phosphate and acyl glycerol phosphate acyl transferase (AGPAT) adds the second fatty acyl-CoA to the *sn*-2 position. The final fatty acyl-CoA is added to the *sn*-3 position by diglyceride acyl transferase (DGAT) forming the TAG.

The location of FA in the glycerol backbone is not random with individual FA being preferentially located at different *sn*-positions (Jensen, 2002). Interestingly, SFA are predominantly esterified at the *sn*-1 position and UFA at the *sn*-2 position (Jensen, 2002). Since C16:0 is the end product of de novo synthesis, it is a potential key FA in this process. A higher preference (8 to 10 fold) was shown for C16:0 as a substrate for GPAT than for C18:0 and *cis*-9 C18:1 in mammary gland of dairy cows (Kinsella and Gross, 1973). Also, short- and medium-chain FA are preferentially esterified to the *sn*-3 position. Over 98% of C4:0 and 93% of C6:0 are esterified on the *sn*-3 position (Jensen, 2002). The *sn*-2 position contains greater than 50% of all C10:0 to C14:0 milk FA. Distribution of C16:0 is fairly uniform between the *sn*-1 and *sn*-2 position while C18:0 is primarily esterified to *sn*-1 with a smaller proportion esterified to sn-3. *cis*-9 C18:1 is esterified to either the *sn*-1 or *sn*-3 position of TAG (Jensen, 2002).

Importantly, this control of FA placement within TAG provides the mammary gland with plasticity to secrete TAG into droplets that can be incorporated into milk and be fluid at body temperature (Dils, 1986; Jensen, 2002). Therefore, the control of melting point of milk fat is relatively constant even with greater variation in FA that differ in melting point. The mechanisms that the mammary gland uses to control melting point of TAG include: increasing unsaturated FA by desaturation, the synthesis of short- chain FA, and preferentially positioning short-chain FA at the *sn*-3 position of the glycerol backbone (Dils, 1986).

Metabolism of FA in Adipose Tissue

Lipogenesis

In ruminants, acetate, and to a lesser extent propionate and butyrate (main fuel molecules produced in the rumen) are the precursors for the initiation of lipogenesis in adipose tissue (Palmquist, 2006). A second greater source of FA in adipose tissue is preformed FA from the diet and circulation. Saturated FA are predominantly esterified at the *sn*-1 position and unsaturated FA at the *sn*-2 position (Dircks and Sul, 1999). It is believed that the substrate selectivity of mitochondrial GPAT plays a key role in this non-random distribution of FA, but the mechanisms have not yet been elucidated (Ahmadian et al., 2007). Although C16:0 is the major FA produced by lipogenesis in the adipose tissue, C18:0 can be synthesized from the elongation of C16:0 (Colleman and Lee, 2004). The high concentration of *cis*-9 C18:1 in adipose tissue arises from the activity of stearoyl-CoA desaturase (Smith et al., 2006). Stearoyl-CoA desaturase is a key enzyme in the synthesis of UFA by insertion of a *cis*-double bond in the carbon-9 position of FA substrates (Kim and Ntambi, 1999). C16:0 and C18:0 are the preferred substrates, which are converted to *cis*-9 C16:1 and *cis*-9 C18:1, respectively.

The primary purposes of the adipose tissue in the synthesis and storage of TAG in periods of energy excess, and hydrolysis of those to generate FA for use by other organs during periods of energy deprivation (Ahmadian et al., 2007). Generally, insulin controls TAG synthesis in adipose tissue by increasing FA uptake and regulating its conversion to glycerol-3-phosphate via glycolysis (Nye et al., 2008). Glucose metabolism via glycolysis generates dihydroxyacetone phosphate, which can be reduced to glycerol-3-phosphate for TAG synthesis in these tissues (Ahmadian et al., 2007). The metabolic pathway glyceroneogenesis, (i.e. the synthesis of glyceride-glycerol from sources other than glucose) is an important source of carbon during

starvation in non-ruminants (Nye et al., 2008) and ruminants (Palmquist, 2006). Glyceroneogenesis in adipose tissue occurs due to both pyruvate carboxylase and the cytosolic isoform of phosphoenolpyruvate carboxykinase (PEPCK-C; Ballard et al., 1967). The metabolic significance of glyceroneogenesis is that any compound that can enter the citric acid cycle and form oxalacetate can contribute to TAG synthesis, which is important for TAG synthesis in low carbohydrate diets (Chen et al., 2005).

Lipolysis

When energy is limited, adipose tissue releases FA from TAG through lipolysis. In general, lipolysis can be broadly divided into two categories: basal and demand lipolysis. While basal lipolysis rate is positively associated with adipocyte size and increases steadily throughout lactation, demand lipolysis is regulated hormonally in response to energy demands (Contreras et al., 2017). Regardless of the type of lipolysis, TAG within the adipocyte lipid droplet are broken down by the action of three different lipases: adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL), and monoglyceride lipase (MGL). The hydrolysis of TAG is initiated by ATGL, followed by HSL that hydrolyzes diacylglycerol with the formation of monoacylglycerol, and finally, MGL produces the final free FA acid molecule and glycerol (Contreras et al., 2018; Zechner et al., 2009). Free FA are released into the bloodstream or maybe re-esterified in the lipogenic pathway.

In dairy cows, compared with other stages of lactation, periparturient period adipose tissue lipolytic responses are enhanced due to hormonal changes associated with parturition and the onset of lactation (Contreras et al., 2017). The most important hormonal adaptation related to lipolysis is the reduction in plasma insulin and increase of insulin resistance in peripheral tissues (Bell, 1995). Adipocytes are one of the most insulin responsive cell types (Kahn and Flier,

2000). In adipocytes, insulin stimulates glucose transport and lipogenesis, promotes the uptake of FA from systemic circulation, and inhibits lipolysis (Contreras et al., 2018). Additionally, increases in the plasma concentrations of growth hormone, prolactin, and angiopoietin-like 4 around parturition and during the first weeks of lactation enhances lipolysis rate in adipocytes by reducing insulin sensitivity, limiting FA uptake, and increasing their response to catecholamines (Bell, 1995; Contreras et al., 2017).

Lipogenesis and lipolysis are continuous processes occurring simultaneously within adipocytes (Contreras et al., 2018). Net FA flux across the adipocyte membrane denotes the absolute change per day in the total mass of FA within the adipocyte. This may be net positive, if adipose tissue mass is increasing, net negative, if adipose tissue mass is decreasing, or net neutral, if adipose tissue mass is constant (Smith et al., 2006). Therefore, it is important to consider that synthesis and degradation of FA occur simultaneously in adipose tissue and that the net FA flux will depend upon physiological conditions. In addition, as adipocytes increase in size, both synthesis and lipolysis become more active (Young et al., 2013). Larger adipocytes synthesize TAG more rapidly than smaller adipocytes, but they also release FA more rapidly than smaller ones (Jamdar, 1978). Therefore, they are able to clear a greater mass of TAG from the blood in the post-absorptive period, but so necessarily will release more FA too (Smith et al., 2006).

Impact of Individual FA on Production Responses

The effect of individual FA on production responses of dairy cows has recently received renewed attention. In the 1960's Steele and co-workers performed a series of studies using relatively pure sources of C16:0 and C18:0 and their findings suggested that C16:0

supplementation induces a higher milk fat response (concentration and yield) when compared to C18:0 supplementation. More recent work from Enjalbert et al. (1998) suggests that the uptake efficiency of the mammary gland is higher for C16:0 than for C18:0 and *cis*-9 C18:1. Similarly, a series of studies have examined the effect of individual SFA on production and metabolic responses of lactating cows (Lock et al., 2013; Piantoni et al., 2013; Rico et al., 2014; Piantoni et al., 2015a; de Souza et al., 2017; Rico et al., 2017a). These results indicate that C16:0 supplementation has the potential to increase yields of milk and milk fat as well as the conversion of feed to milk, independent of production level when it was included in the diet for soyhulls or C18:0.

Rico et al. (2017a) fed increasing levels of a C16:0 supplement (87% C16:0) to dairy cows and observed a quadratic response with a positive effect on milk fat yield, 3.5% fat-corrected milk and feed efficiency up to 1.5% diet DM (Table 1). Furthermore, using a random regression model to analyze available individual cow data from 10 studies, de Souza et al. (2016) observed that energy partitioning toward milk was increased linearly with C16:0 intake, as a result of a linear increase in milk fat yield and 3.5% fat-corrected milk with increasing intake of C16:0.

Piantoni et al. (2015a) reported that C18:0 increased DMI and yields of milk and milk components, with increases more evident in cows with higher milk yields, indicating that there was significant variation in response. Reasons why only higher yielding cows responded more positively to C18:0 supplementation than lower yielding cows remains to be determined. However, when C16:0 and C18:0 supplementation were directly compared, the yield of milk fat and 3.5% FCM increased with C16:0 regardless of level of milk production (Table 1, Rico et al., 2014). In a recent dose response study feeding a C18:0 supplement (93% C18:0) increased DMI

but had no effect on the yields of milk or milk components when compared to non-FA supplemented control diet, which is probably associated with the decrease in FA digestibility (Boerman et al., 2017).

In early lactation cows, Beam and Butler (1998) fed a saturated FA supplement (~ 40% C16:0 and 40% C18:0) and observed that FA supplementation decreased DMI and did not affect yields of milk and ECM in the first 4 weeks after calving. Piantoni et al. (2015b) fed a similar saturated FA supplement (~ 40% C16:0 and 40% C18:0) and observed that FA supplementation during the immediate postpartum period (1-29 DIM) favored energy partitioning to body reserves rather than milk yield, especially in the lower forage diet. The high forage diet with supplemental FA increased DMI and tended to decrease BCS loss compared with the same diet without FA supplementation. Also, regardless of forage level, feeding supplemental FA increased DMI, decreased BCS loss, but tended to decrease milk yield. When cows were fed a common diet during the carryover period, the low forage diet with FA supplementation fed during the immediate postpartum continued to decrease milk yield and maintained higher BCS compared with the other treatments. Weiss and Pinos-Rodriguez (2009) fed a similar saturated FA supplement (~ 40% C16:0 and 40% C18:0) to early-lactation cows (21 to 126 DIM) and observed that when a high-forage diet was supplemented with FA, the increased NE_L intake was partitioned toward body energy reserves as measured by higher BCS with no change in milk yield. However, when a low-forage diet was supplemented with FA, milk yield increased (2.6 kg/d) with no change in BCS.

Importance of Controlling Energy Partitioning During Lactation

The mechanisms controlling energy partitioning in lactating dairy cows are not well understood; therefore, we currently cannot accurately predict the optimal dietary nutrient

composition for cows in different lactation stages. Fat is typically the most variable component in milk and is affected by several factors (Jensen, 2002). Also, fat is the milk component with the highest energy content and its production constitutes the major 'energetic investment' of milk synthesis, accounting for over one-half of the milk energy output (NRC, 2001). Milk fat synthesis is especially responsive to nutrition, with milk fat depression (MFD) being one of the more challenging problems that dairy consultants and farmers must solve. During MFD, there is a decreased priority for milk production and an increased priority for storage of energy in adipose tissue (Van Soest, 1963). Therefore, a repartitioning of energy to body weight gain occurs during diet-induced MFD (Griinari and Bauman, 2006).

Using NRC (2001) equations, every 0.25 percentage unit change in milk fat concentration results in ~ 3% increase/reduction in milk energy output (assuming no change in milk yield). If milk energy output is reduced then spared energy can be used for other purposes and storage. For cows in positive energy balance, a reduction in milk fat synthesis may result in more rapid gain in BW and BCS, thereby reducing efficiency of nutrient use for milk synthesis. On the other hand, an increase in milk energy output not followed by energy intake may result in body reserve mobilization. Ideally, adipose tissue reserves that are mobilized in early lactation when cows are in a lipolytic state are replenished as lactation proceeds. However, the inability to recover body reserves leading to inadequate body condition at parturition can limit milk yield and increase risk of reproductive failure (Roche et al., 2009). Conversely, cows that gain excessive body condition in mid and late lactation are at high risk for culling or an extended calving interval during the next lactation due to increased risk of metabolic disorders and reproductive failure (NRC, 2001; Roche et al., 2009).

Therefore, an important goal for diet formulation and nutritional management of lactating cows is to achieve optimum milk energy output as well as body condition. Since fat supplements are often used to increase energy intake, yields of milk and milk components, and body reserves in dairy cows, it is critical to understand how different FA may affect energy partitioning. Understanding how different FA can impact energy partitioning in lactating dairy cows could aid the development of dietary strategies to reduce excessive body condition and minimize variation among cows.

FA Effects on Energy Partitioning

Individual FA can have an impact on energy partitioning of dairy cows, and this will depend on the individual FA as well as on characteristics of the diet (Bauman et al., 2011). A classic example is dietary-induced MFD, in which a decrease in milk fat concentration and yield, may redirect nutrients to the adipose tissue (Griinari and Bauman, 2006). These changes in energy partitioning during MFD have been associated with a shift in ruminal biohydrogenation pathways and increased production of several *trans* FA intermediates. 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. These finding suggested that the increase in BW 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. Conversely, Urrutia and Harvatine (2017) observed reduced lipogenic capacity of adipose tissue explants without changes in gene expression of key lipogenic enzymes during 4-d of *trans*-10,

cis-12 C18:2 infusion in low-producing cows, which suggested that energy partitioning under MFD-induced conditions may be affected by physiological state.

Additionally, FA could also affect energy partitioning through an increase in plasma insulin concentration or modulation of insulin resistance, but results have been inconsistent. Previous studies reported that UFA increased insulin secretion in a perfused pancreas in rats (Stein et al., 1997), while in dairy cows increasing amounts of dietary UFA increased (Liu et al., 2015) or decreased plasma insulin (Choi and Palmquist, 1996). Chilliard (1993) suggested that the inconsistent insulin responses to fat supplementation might be related to their effect on DMI, which dietary ingredient was removed when fat is supplemented, and/or to the glucose sparing effect that fats might have if they decrease milk fat synthesis.

Recently, research has indicated that feeding C16:0 increased milk energy output in post-peak cows (i.e. de Souza et al., 2017; Rico et al., 2017a). A recent meta-regression also indicated that energy partitioning towards milk (as % of energy intake) was positively associated with C16:0 intake (Figure 2.4). One mechanism proposed to explain these results suggests that C16:0 supplementation induces insulin resistance mediated through ceramides reducing the utilization of glucose by adipose and muscle tissues (Mathews et al., 2016). Circulating ceramides are positively associated with the availability of NEFA in plasma, with very long chain ceramides being the most responsive (Rico et al., 2017b). Recent data suggests that plasma C24:0-ceramide is inversely associated with glucose clearance rates following an insulin challenge postpartum (Rico et al., 2017b). Also, feeding C16:0 in early lactation rapidly increased circulating ceramide, especially C24:0-ceramide (Davis et al., 2017). Because the availability of lipolysis-derived C16:0 declines with the progression of lactation, feeding C16:0 supplements may

increase the availability of C16:0 in circulation to tissues thereby sustaining nutrient partitioning towards the mammary gland.

A previous study has also indicated that FA supplements differing in degree of saturation may affect energy partitioning of dairy cows differently. Liu et al. (2015) reported that feeding an UFA supplement (soybean oil) increased plasma insulin and energy partitioning towards body reserves, whereas a SFA supplement (C16:0 supplement) increased energy partitioning towards milk. Additionally, in a recent meta-regression based on individual cow data from Michigan State University, energy partitioning towards body reserves (as % of energy intake) was positively associated with *cis*-9 C18:1 intake (Figure 2.4). Since *cis*-9 C18:1 can be extensively converted to C18:0 in the rumen, we are unsure if these results are related to *cis*-9 C18:1 intake or 18-carbon FA intake. Importantly, these results support the concept that there is a strong relationship between milk fat synthesis and energy partitioning and that different FA may be, at least partly, the mediator of changes in metabolism in adipose tissues and the mammary gland of lactating dairy cows.

Role of Physiological State on Production Responses

Reasons for variability across experiments to different types of FA supplements could be associated with the physiological state of cows. Production level is well established as a potential factor that interacts with nutrition on production responses of dairy cows (e.g. Harvatine and Allen, 2005; Piantoni et al., 2015a). Palmquist and Jenkins (1980) reported that cows with low production did not respond to fat supplementation compared with cows with high production potential in their feeding trials. Recently, supplementation with C16:0 has been shown to increase milk yield, milk fat yield, and feed efficiency regardless of level of milk production

(Piantoni et al., 2013, Rico et al., 2014). In contrast, DMI and milk production increased with C18:0 supplementation and the results were more pronounced for higher-producing cows than for lower-producing cows (Piantoni et al., 2015a).

Previous research has also suggested that primiparous cows might have greater production responses to supplemental fat since they have additional energy requirements for growth as well as for milk production compared to multiparous cows. Grummer et al. (1995) found that supplemental tallow increased milk yield in primiparous compared with multiparous cows by 1.5 kg/d after 7 wk of lactation. Holter et al. (1992) observed that milk yield increased in primiparous but not in multiparous cows when Ca-salts of palm FA was fed for 16-wks. Conversely, Drackley et al. (2003) did not observe interactions between parity and white grease supplementation (3% diet DM) on production responses of mid lactation cows.

The impact of lactation stage is probably the most important factor that may affect energy partitioning. There are marked changes in lipid metabolism during pregnancy and lactation in most mammals. Endocrine profiles change (Bauman, 2000) and lipolysis and lipogenesis are regulated to increase lipid reserves during pregnancy, and, subsequently, these reserves are utilized following parturition and the initiation of lactation (Roche et al., 2009). Therefore, the response of dairy cows to similar nutrition strategies during different stages of lactation may be distinctive.

Conclusion

Although the effects of individual FA have been described in previous studies, there remain substantial gaps in our knowledge concerning the interactions among different FA, diets, and the physiological state of cows. Also, it is crucial to understand how energy-dense diets

based on supplemental fat affect energy partitioning. Cows that gain excessive or inadequate body condition are at high risk for culling or an extended calving interval during the next lactation because of increased risk of metabolic disorders and reproductive failure. We propose that the FA profile of a fat supplement is most likely the major factor affecting the response to it. In this regard, C16:0, C18:0, and *cis*-9 C18:1 usually comprise most FA present in milk fat and adipose tissue of dairy cows. These FA have different functions in metabolism, but they may also interact with each other by competition or complementary mechanisms under different physiological conditions. Understanding how and which FA affect energy partitioning in lactating dairy cows should allow the development of nutritional management strategies that reduce the risk of over conditioning cows and improve milk component yields and milk income and possibly reproductive performance.

Our central objective was to determine and understand the different responses of adipose and mammary tissues to diet with different combinations of FA under different physiological conditions. We determined the effects of varying the ratio of dietary C16:0, C18:0, and *cis*-9 C18:1 on production and metabolic responses of post-peak cows. Also, we evaluated the effects of varying the ratio of dietary C16:0, and *cis*-9 C18:1 on production and metabolic responses of early lactation cows, mid lactation cows, and with cows at different production levels. These research chapters although broad in objectives all deal with increasing our understanding of metabolism and digestion of FA and the effect of different ratios of FA on energy partitioning of dairy cows.

APPENDIX

APPENDIX

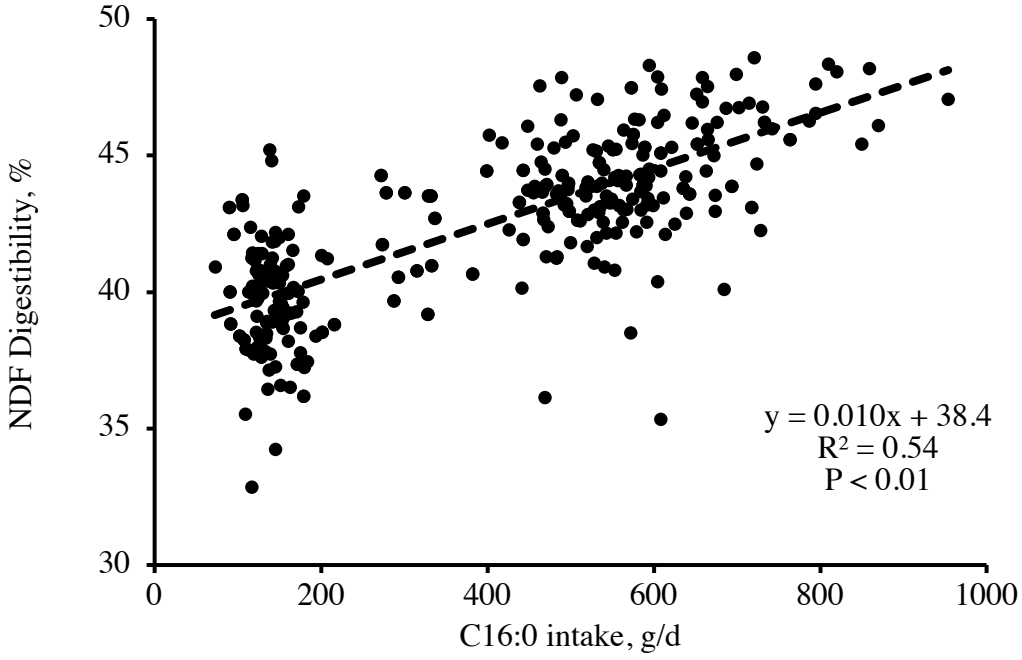

Figure 2.1. Relationship between C16:0 intake and NDF digestibility of dairy cows fed C16:0-enriched FA supplements.
Results in Panel A represent a combined data set evaluated using a random regression model from 6 studies feeding C16:0-enriched supplements on NDF digestibility of dairy cows (de Souza et al., 2016).

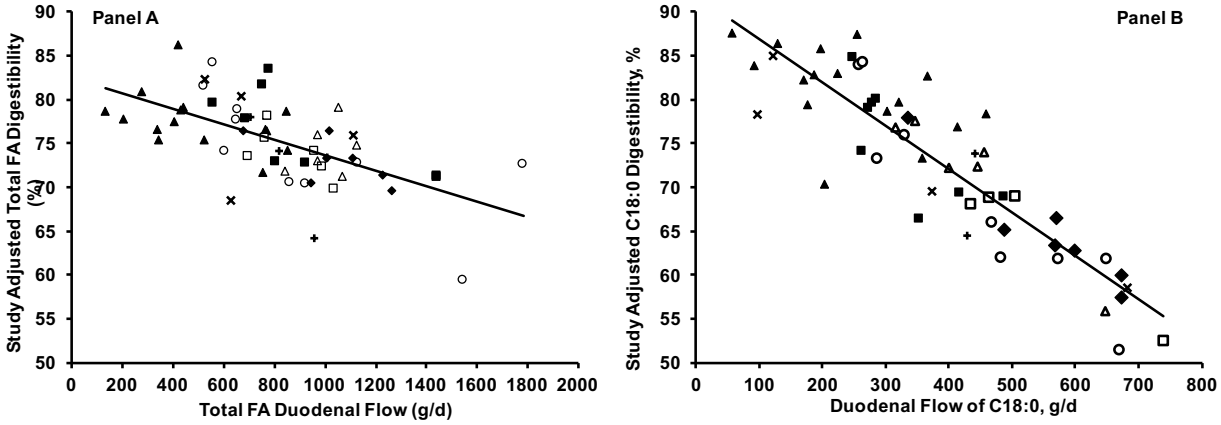

Figure 2.2. Relationship between study adjusted apparent total FA intestinal digestibility and total FA duodenal flow (Panel A) and study adjusted C18:0 apparent intestinal digestibility and duodenal flow of C18:0 (Panel B).
Results from a meta-analysis using 15 published studies that measured duodenal flow and intestinal digestibility of FA in dairy cows (Boerman et al., 2015). Control treatments represented by black triangles; animal-vegetable fat treatments represented by black diamonds; calcium salt treatments represented by black squares; tallow treatments represented by open circles; vegetable oil treatments represented by open triangles; seed meal treatments represented by open squares; whole seed treatments represented by black addition sign; and other treatments represented by black multiplication sign.

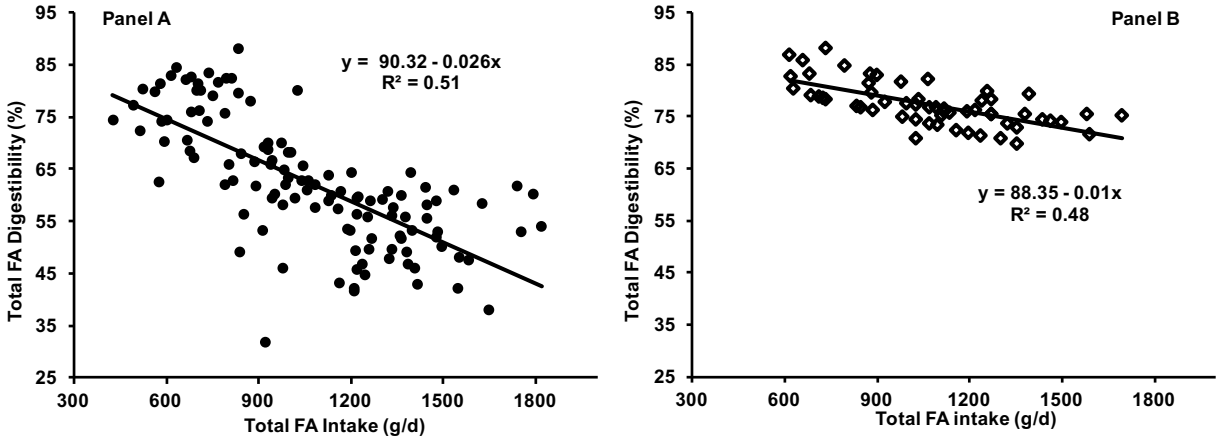

Figure 2.3. Relationship between total FA intake and apparent total-tract FA digestibility of dairy cows supplemented with either a C18:0-enriched supplement (Panel A) or a C16:0-enriched supplement (Panel B).
Results in Panel A utilized 32 mid-lactation cows receiving diets with increasing levels (0 to 2.3% dry matter) of a C18:0-enriched supplement (85% C18:0) in a 4 X 4 Latin square design with 21-d periods (Boerman et al., 2017). Results in Panel B utilized 16 mid-lactation cows receiving diets with increasing levels (0 to 2.25% dry matter) of a C16:0-enriched supplement (87% C16:0) in a 4 X 4 Latin square design with 14-d periods (Rico et al., 2017a).

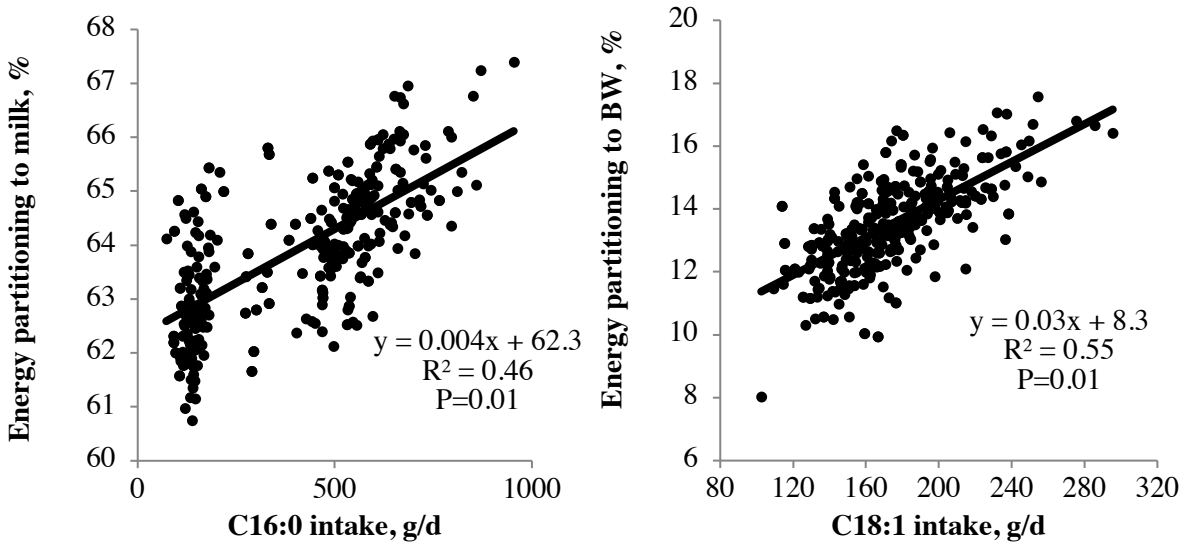

Figure 2.4. Individual FA intake on energy partitioning.
Meta-regression of the effects of C16:0 and C18:1 intake on energy partitioning towards milk or BW. Individual data points (381) were compiled from 7 studies supplementing different FA to post-peak dairy cows.

Table 2.1. Summary of DMI, milk production and composition, BW, and BCS for cows supplemented with C16:0 and C18:0 supplements.

	Piantoni et al. (2013)[1]			Piantoni et al. (2015)[2]			Rico et al. (2014)[3]		
Variable	Control	C16:0	SEM	Control	C18:0	SEM	C16:0	C18:0	SEM
DMI, kg/d	27.8	27.8	0.54	25.2^{n}	26.1^{m}	0.42	32.1	32.3	0.44
Milk yield, kg/d	44.9^{b}	46.0^{a}	1.7	38.5^{n}	40.2^{m}	0.71	46.6	45.8	2.02
Fat yield, kg/d	1.45^{b}	1.53^{a}	0.05	1.35^{n}	1.42^{m}	0.03	1.68^{y}	1.59^{z}	0.05
Milk fat, %	3.29^{b}	3.40^{a}	0.11	3.60	3.59	0.12	3.66^{y}	3.55^{z}	0.09
Protein yield, kg/d	1.38	1.41	0.04	1.14^{n}	1.19^{m}	0.02	1.50	1.49	0.05
Milk Protein %	3.11	3.09	0.05	3.00	2.99	0.05	3.24	3.29	0.05
3.5% FCM	42.9^{b}	44.6^{a}	1.35	38.6^{n}	40.5^{m}	0.76	47.5^{y}	45.6^{z}	1.64
3.5% FCM/DMI	1.54^{b}	1.60^{a}	0.03	1.53	1.55	0.04	1.48^{y}	1.40^{z}	0.05
Body weight, kg	722	723	14.7	727	730	12.8	720	723	13.6
BCS	2.99	2.93	0.15	2.67	2.67	0.11	2.93^{z}	2.99^{y}	0.11

[1]Treatments were either a control diet (with 2% of diet DM as added soyhulls) or a C16:0-supplemented diet (with 2% of diet DM as C16:0). Means within a row with different superscripts ($^{a, b}$) differ ($P < 0.05$).

[2]Treatments were either a control diet (with 2% of diet DM as added soyhulls) or a C18:0-supplemented diet (with 2% of diet DM as C18:0). Means within a row with different superscripts ($^{m, n}$) differ ($P < 0.05$).

[3]Treatments were either a C16:0-supplemented diet (with 2% of diet DM as C16:0) or a C18:0-supplemented diet (with 2% of diet DM as C18:0). Means within a row with different superscripts ($^{y, z}$) differ ($P < 0.05$).

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CHAPTER 3

ALTERING THE RATIO OF DIETARY PALMITIC, STEARIC, AND OLEIC ACIDS IN DIETS WITH OR WITHOUT WHOLE COTTONSEED AFFECTS NUTRIENT DIGESTIBILITY, ENERGY PARTITIONING, AND PRODUCTION RESPONSES OF DAIRY COWS

The objective of our study was to evaluate the effects of varying the ratio of dietary palmitic (C16:0), stearic (C18:0), and oleic (*cis*-9 C18:1) acids in basal diets containing soyhulls or whole cottonseed on nutrient digestibility, energy partitioning and production responses of lactating dairy cows. We observed that diet with whole cottonseed increased milk fat yield and energy partitioning to BW, without reducing milk energy output. Among the combinations of C16:0, C18:0 and *cis*-9 C18:1 evaluated, fat supplements with more C16:0 increased energy output in milk, while fat supplements with more *cis*-9 C18:1 increased energy storage as body weight. The fat supplement with more C18:0 reduced nutrient digestibility, which most likely explains its lower performance compared with the other treatments.

For a full text of this work see: J. de Souza, C.L. Preseault, and A.L. Lock. J. Dairy Sci. 101: 172 – 185.

CHAPTER 4

LONG-TERM PALMITIC ACID SUPPLEMENTATION INTERACTS WITH PARITY IN LACTATING DAIRY COWS: PRODUCTION RESPONSES, NUTRIENT DIGESTIBILITY, AND ENERGY PARTITIONING

The objective of our study was to evaluate the effects of long-term palmitic acid (**C16:0**) supplementation and parity on production, nutrient digestibility and energy partitioning of mid-lactation dairy cows. We observed that feeding a C16:0 supplement consistently increased neutral detergent fiber digestibility, milk fat yield, energy-corrected milk, and feed efficiency of mid-lactation dairy cows. In addition, PA supplementation interacted with parity with production responses increased to a greater extent in multiparous than primiparous cows when PA was fed.

For a full text of this work see: J. de Souza, and A. L. Lock. J. Dairy Sci.: 101: 3044 – 3056.

CHAPTER 5

EFFECTS OF TIMING OF PALMITIC ACID SUPPLEMENTATION ON PRODUCTION RESPONSES OF EARLY LACTATION DAIRY COWS

Abstract

The objective of our study was to evaluate the effects of timing of palmitic acid (C16:0) supplementation on production responses of early lactation dairy cows. Fifty-two multiparous cows were used in a randomized complete block design experiment and assigned to either a control diet containing no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving to 24 DIM (fresh period-FR) or from 25 to 67 DIM (peak period-PK). During the FR period, we did not observe treatment differences for DMI, and milk yield. Compared with CON, PA increased the yield of 3.5% FCM by 5.3 kg/d, yield of ECM by 4.70 kg/d, milk fat content by 0.41% units, milk fat yield by 280 g/d, and protein yield by 100 g/d ON. The increase in milk fat associated with our PA treatment during FR period occurred due to an increase in yield of 16-carbon milk FA by 147g/d (derived from both de novo synthesis and extraction from plasma), and an increase in preformed milk FA by 96 g/d. Compared with CON, PA reduced BW by 21 kg, and BCS by 0.09 units and tended to increase BW loss by 0.76 kg/d. While PA compared with CON consistently increased milk fat yield and ECM over time, a treatment by time interaction was observed for BW and BCS due to PA inducing a greater decrease in BW and BCS after the second week of treatments. Feeding PA during PK period increased milk yield by 3.45 kg/d, yield of 3.5% FCM by 4.5 kg/d, yield of ECM by 4.60 kg/d, milk fat content by 0.22% units, milk fat yield by 210 g/d, protein yield by 140 g/d, lactose yield by 100 g/d, while PA tended to reduced BW by 10 kg compared with CON. Also, during PK period we observed an interaction between diet fed in FR and PK period for milk fat yield due to

feeding PA during PK period increased milk fat yield to a greater extent in cows that received the CON diet (+ 240 g/d) rather than PA (+ 180 g/d) diet during FR period. This difference is associated with yield of preformed FA because we observed that feeding PA during PK period increased the yield of preformed milk FA only in cows that received the CON diet during FR period. In conclusion, feeding a C16:0 supplement to early lactation cows consistently increased the yield of ECM in both FR and PK periods compared with a non-fat control diet. For some variables, the effect of feeding C16:0 were affected by timing of supplementation since milk yield increased only during the PK period and BW reduced to a greater extent in the FR period. Regardless of diet fed in FR period, feeding a C16:0 supplement during PK period increased yield of milk and milk components.

Introduction

The high metabolic demand of lactation and reduced DMI during the immediate postpartum period result in a state of negative energy balance in dairy cows (NRC, 2001). Approaches to increase energy intake of postpartum cows include increasing dietary starch content and supplementing fat to increase the energy density of the diet (McCarthy et al., 2015; Piantoni et al., 2015b). However, feeding high starch diets that promote greater ruminal propionate production during early lactation could be hypophagic and therefore further reduce DMI and increase the risk of ruminal acidosis and displaced abomasum (Allen et al., 2009; Allen and Piantoni, 2013). Some authors suggest that caution should be exercised when using supplemental fats to increase the caloric density of diets in early lactation dairy cows, since a high lipid load may affect the endocrine system, feed intake, and increase the risk for metabolic disorders (Kuhla et al., 2016). However, we are increasing our understanding in the effects of different fatty acids (FA) on metabolism and animal responses. For example, unsaturated FA can

depress feed intake (Allen, 2000), increase plasma insulin (de Souza et al., 2018), alter ruminal biohydrogenation and increase energy partitioning to body reserves (Harvatine et al., 2009; de Souza et al., 2018), whereas saturated FA have little effect on DMI (Allen, 2000), and can increase milk energy output (Lock et al., 2013; de Souza et al., 2018). Hence, determining dairy cow responses to specific FA may allow for more precise recommendations.

In general fat supplementation has been shown to increase milk yield (Rabiee et al., 2012) and reproductive performance (Rodney et al., 2015), but great variation has been reported for different fat types, and indeed the same supplement across different diets and studies. Although most commercially available FA supplements have typically contained mixtures of different FA, supplements enriched with individual FA are becoming increasingly available. Determining the effects of individual FA on production responses and metabolism of lactating dairy cows is therefore important. Recently, considerable research has focused on palmitic acid (C16:0) because of its potential to increase milk fat concentration and yield, and the efficiency of milk production compared with a control diet (Lock et al., 2013; de Souza et al., 2017) and with other FA supplements (Rico et al., 2014a; Rico et al., 2014b; de Souza et al., 2018). However, to our knowledge our research, and work by others with C16:0, has evaluated production and metabolic responses of post-peak cows. Thus, this raises a question about the response of early lactation cows to C16:0 supplements and when these supplements should be fed.

With early lactation cows, previous studies suggest that the response to FA supplementation may vary due to the timing when supplemental FA is fed. Of particular importance, Piantoni et al. (2015b) fed a saturated FA supplement (~ 40% C16:0 and 40% C18:0) and observed that FA supplementation during the immediate postpartum period (1-29 DIM) favored energy partitioning to body reserves rather than milk yield, especially in a lower

forage diet. The high forage diet with supplemental FA increased DMI and tended to decrease BCS loss compared with the same diet without FA supplementation. However, Weiss and Pinos-Rodriguez (2009) fed a similar FA supplement (~ 40% C16:0 and 40% C18:0) to early-lactation cows (21 to 126 d postpartum) and observed that when diets were supplemented with FA, energy intake was increased and directed mostly to milk production in the lower forage diet and to body reserves in the high forage diet. Interestingly, these results suggest that energy partitioning due to FA supplementation is affected differently according to the timing of supplementation. Although these results suggest a positive effects of FA supplementation on production responses of early lactation dairy cows, it is possible that the magnitude of response may vary not only due to the FA profile, but also timing when the supplement is fed.

Therefore, the objective of our study was to evaluate the effects of timing of C16:0 supplementation on production responses of early lactation dairy cows. We hypothesized that feeding a C16:0 supplement would increase milk yield and milk fat yield in early lactation cows, but we postulated that the production responses to supplemental fat would be greater if the supplementation starts after the fresh period (~ 3 weeks after parturition).

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 December 18th, 2015 and finished on August 4th, 2016. Cows were fed once daily (9000 h) at 120% and 110% of expected intake during the treatment periods, 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

Fifty-two 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. Cows were blocked by BCS (up to 0.50 unit difference using the 1=thin, 5=fat scale in 0.25 increments), previous lactation 305-ME (within 1,500 kg), and parity (up to 1 lactation difference). 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. Cows were assigned to either a control diet containing no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving [1 to 24 DIM; fresh period (FR)] or after 3 weeks from calving [25 to 67 d; peak period (PK)]. The FR and PK diets fed were adjusted for fiber, CP and starch levels. The FA supplement was added at 1.5% of diet DM, replacing 1.5% of soyhulls in the control diet. Treatment diets were mixed daily in a tumble-mixer and were fed from the morning following parturition. The ingredient and nutrient composition of the diets fed as TMR, including close up ration for reference, are described in Table 5.1. All rations were formulated to meet or exceed cows predicted requirements for 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, so all collection days are ±3 d. Daily milk yield and feed offered and refused were recorded daily throughout the experiment. Samples of all diet ingredients (0.5 kg) and orts from each cow (~12.5%) were collected weekly during the experiment and stored in plastic bags at -20°C until processed. Milk samples were collected twice a week 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, 33, 47 and 61 postpartum, and stored without preservative at -20°C for determination of FA profile. BW was recorded three times per week from d -21 of expected parturition day and throughout the experiment. Body condition was scored weekly 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 and orts samples were dried in a 55°C forced-air oven for 72 h and analyzed for DM content. Before drying, ingredients were composited monthly. Orts were dried to calculate DMI weekly, but only orts collected on d 5, 12, 19, 33, 47 and 61 postpartum were processed further and analyzed for nutrient composition. Once dried, samples of feed ingredients, and orts collected were ground in a Wiley mill (1-mm screen; Arthur H Thomas Co., Philadelphia, PA) and analyzed for ash, NDF, indigestible NDF, CP, starch and FA concentration as described by Boerman et al. (2017). Gross energy was determined by bomb calorimeter (Parr Instrument Inc., Moline, IL). Indigestible NDF was determined after 240 h in vitro fermentation (Goering and Van Soest, 1970).

Milk samples were analyzed for fat, true protein, and lactose concentrations by mid-infrared spectroscopy (AOAC, 1990; method 972.160) (Universal Lab Services, Lansing, MI). Yields of 3.5% FCM, ECM, milk energy, and milk components were calculated using milk yield and component concentrations from each milking, summed for a daily total, and averaged for each week. 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. Milk lipids were extracted, and FA-methyl esters prepared and quantified using GLC according to Lock et al. (2013). Yield

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

Statistical Analysis

Data were analyzed separately for FR (from 1 to 24 d postpartum) and for the PK (from 25 to 67 d postpartum) periods. All weekly data were analyzed using the MIXED procedure of SAS v.9.2 (SAS Institute, Inc. Cary, NC) with repeated measures.

For the FR period, the model used included:

$Yijklm = \mu + Bi + C(BiFk)j + Fk + Tl + Jm + FkTl + eijklm$

Where μ = overall mean Bi = random effect of block, C(BiFk)j = random effect of cow within block and treatment diet, Fk = fixed effect of treatment during the fresh period, Tl = fixed effect of time, Jm = random effect of Julian date, eijklm = residual error.

For the PK period, the model used included:

$Yijklmn = \mu + Bi + C(BiFkLl)j + Fk + Ll + FkLl + Tm + Jn + FkTm + LlTm + FkLlTm + eijklmn$

Where μ = overall mean Bi = random effect of block, C(BjFkLl)j = random effect of cow within block and treatment diet, Fk = fixed effect of treatment during the fresh period, Ll = fixed effect of treatment during the peak period, Tm = fixed effect of time, Jn = random effect of Julian date, eijklmn = residual error.

Unless otherwise specified, first-order autoregressive was the covariate structure used for analysis because it resulted in the lowest BIC for most of the variables measured. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals vs. predicted values. Significance was declared at $P \leq 0.05$ for main

effects and $P \leq 0.10$ for interactions. Tendencies were declared 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-ME ($P = 0.79$), BW ($P = 0.84$), or BCS ($P = 0.43$) pre-calving. Two cows (one CON and one PA treatments) had a displaced abomasum and underwent surgery and were excluded from the statistical analyses.

Results

Diets and Nutrient Composition, and Health Incidents

All cows received the same close-up diet before calving (Table 5.1). During the FR period, the CON diet contained (DM basis) 31.9% NDF, 24.0% forage NDF, 23.5% starch, and 2.99% total FA, while PA diet contained 30.9% NDF, 24.0% forage NDF, 23.5% starch, and 4.48% total FA. During the PK period, diets were adjusted to reduce forage and increase starch content. Therefore, the CON diet contained (DM basis) 30.8% NDF, 21.0% forage NDF, 27.4% starch, and 3.55% total FA, and PA diet contained 29.7% NDF, 21.0% forage NDF, 27.4% starch, and 5.07% total FA. In both FR and PK periods, PA diet mainly increased dietary C16:0, while a slight increase in dietary C18:0 and *cis*-9 C18:1 were also observed compared with CON.

This study was not designed to evaluate treatment effects on health incidents. Therefore, only a summary of health incidents is presented in Table 5.2. Retained placenta was the major health incident observed with 5 and 4 cases detected for CON and PA, respectively, with 6 out of 9 cases occurring in cows that calved during the summer. During the FR period, we observed 4 and 5 cases of ketosis for CON and PA, respectively, while no incidents were detected during the PK period. The major health incident during the PK period was mastitis with 1 and 2 cases for CON and PA, respectively.

Production Responses During FR

During the FR period, DMI and milk yield increased over time for both treatments (Figure 5.1 A and 1B), but we did not observe treatment differences for DMI ($P = 0.92$; Table 5.3) or milk yield ($P = 0.39$). Compared with CON, PA increased the yield of 3.5% FCM by 5.3 kg/d ($P < 0.01$), and the yield of ECM by 4.70 kg/d ($P < 0.01$). The increase in ECM was consistent over time (Figure 5.1 C). PA increased milk fat content by 0.41% units ($P = 0.01$), milk fat yield by 280 g/d ($P < 0.01$), protein yield by 100 g/d ($P = 0.03$), and feed efficiency ($P < 0.01$), compared with CON. We did not observe treatment differences for milk protein content ($P = 0.65$), milk lactose content ($P = 0.46$), and milk lactose yield ($P = 0.43$). Although cumulative milk yield did not differ between treatments ($P = 0.25$), PA increased cumulative yield of milk fat ($P < 0.01$) and protein ($P = 0.05$) compared with CON. Compared with CON, PA reduced BW by 21 kg ($P = 0.05$) and BCS by 0.09 units ($P = 0.04$) and tended to increase BW loss by 0.76 kg/d compared with CON ($P = 0.07$). While PA consistently increased milk fat yield over time (Figure 5.2 A), a treatment by time interaction was observed for BW ($P = 0.05$) and BCS ($P = 0.07$) due to PA inducing a greater decrease in BW and BCS over time (Figure 5.2 B and 2C, respectively).

Production Responses During PK

During the PK, no treatment by time interactions were observed for all variables evaluated ($P > 0.15$; Table 5.4). The interaction between diet fed at FR and PK periods was also not significant for most of variables evaluated ($P > 0.15$). In contrast, feeding PA during the PK period increased milk fat yield to a greater extent in cows that received the CON diet during the FR period (interaction; $P = 0.07$).

During the PK period, the effect of diet fed during the FR period was not significant for most variables evaluated ($P > 0.10$; Table 5.4). In contrast, we observed that cows that received PA during the FR period had lower BW ($P = 0.01$) and tended to reduce BW change ($P = 0.07$) compared with cows that received CON during the FR period.

Feeding PA during the PK period increased milk yield by 3.45 kg/d ($P = 0.01$), the yield of 3.5% FCM by 4.5 kg/d ($P < 0.01$), and the yield of ECM by 4.60 kg/d ($P < 0.01$) compared with CON. We did not observe treatment differences for DMI ($P = 0.68$), milk protein content ($P = 0.22$), and milk lactose content ($P = 0.43$). The increase in milk yield and ECM by PA was consistent over time (Figure 5.1 B and 5.1 C, respectively). PA increased milk fat content by 0.22% units ($P < 0.01$), milk fat yield by 210 g/d ($P < 0.01$), protein yield by 140 g/d ($P = 0.04$), lactose yield by 100 g/d ($P = 0.04$), and feed efficiency ($P < 0.01$) compared with CON. PA increased cumulative yield of yield ($P < 0.01$), milk fat ($P < 0.01$), and protein ($P = 0.05$) compared with CON. In contrast, compared with CON, PA reduced BCS by 0.10 units ($P = 0.05$) and tended to reduced BW by 10 kg ($P = 0.06$).

Milk FA Concentration and Yield During FR

Milk FA are derived from 2 sources: < 16 carbon FA from de novo synthesis in the mammary gland and > 16 carbon FA originating from extraction from plasma. Mixed source FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis in the mammary gland and extraction from plasma. Compared with CON, PA did not affect de novo FA concentration ($P = 0.23$; Table 5.5), tended to reduce preformed FA concentration ($P = 0.07$), and increased mixed source FA ($P < 0.01$). Compared with CON, PA increased milk FA concentration of C16:0 ($P < 0.01$; Table 5.7), but reduced concentration of *cis*-9, *cis*-12, *cis*-15 C18:3 ($P = 0.03$). We observed an interaction between treatment and time for mixed FA concentration ($P < 0.01$) due to PA

increasing mixed FA concentration over time compared with CON (data not shown). On a yield basis, PA increased mixed source FA ($P < 0.01$; Table 5.5) primarily due to the increase in the yield of C16:0 ($P < 0.01$; Table 5.8). Compared with CON, PA did not affect the yield of de novo milk FA ($P = 0.32$), but increased the yield of C4:0 ($P = 0.02$). Additionally, compared with CON, PA increased the yield of preformed milk FA ($P = 0.05$) mainly due to the increase in the yield of yield of C18:0 ($P = 0.03$; Table 5.8) and *cis*-9 C18:1 ($P = 0.05$). We observed a tendency for an interaction between treatment and time for mixed FA yield ($P = 0.12$) due to PA increasing mixed FA yield over time compared with CON (Figure 5.3).

Milk FA Concentration and Yield During PK

We observed a tendency for an interaction between treatment and time for de novo FA yield ($P = 0.03$; Table 5.6) due to PA reducing mixed FA yield compared with CON at wk 7 but not at wks 5 and 9 (Figure 5.3). Additionally, PA tended to increase the yield of C4:0 ($P = 0.10$; Table 5.10) compared with CON.

The interaction between diet fed during FR and PK periods were not significant for the concentration and yield of most FA evaluated ($P > 0.15$; Table 5.6). However, feeding PA during PK period increased the concentration of mixed milk FA to a greater extent in cows that received the PA diet during FR period (interaction; $P = 0.06$). Also, feeding PA during PK increased the yield of preformed milk FA only in cows that received the CON diet during FR period (interaction; $P = 0.06$).

During PK period, PA reduced de novo FA concentration ($P < 0.01$; Table 5.6), tended to reduce preformed FA concentration (P = 0.08), and increased mixed source FA ($P < 0.01$) compared with CON. Compared with CON, PA decreased milk FA concentration of C6:0, C8:0, C10:0, C12:0 C14:0, C18:0, *cis*-9, *cis*-12 C18:2, and *cis*-9, *cis*-12, *cis*-15 C18:3 (all $P < 0.05$;

Table 5.9), while increased the concentration of C16:0 ($P < 0.01$). On a yield basis, PA increased mixed source FA ($P < 0.01$; Table 5.6) primarily due to the increase in the yield of C16:0 ($P < 0.01$; Table 5.10). In contrast, PA did not affect the yield of de novo milk FA ($P = 0.54$) or preformed milk FA ($P = 0.72$).

Discussion

The challenge of meeting nutritional requirements is greater during early lactation and cows generally enter a period of negative energy balance (NRC, 2001). Supplemental fat can be used to increase the energy density of diets and energy intake (Piantoni et al., 2015b). However, the potential response of supplemental fat during early lactation and when supplemental fat should be fed is still not well described and previous results are inconsistent. Grummer (1992) suggested based on studies conducted in early 1990's that supplemental tallow had little benefits on cow performance when fed in the first 5 to 7 wks of lactation, which is likely associated with the high levels of supplemental fat included in the diet (5-6% DM) and reduced DMI. In contrast, recent research has raised interest in the effects of feeding individual FA, extending beyond their energy contribution to include potential metabolic, and physiological effects of individual FA (Palmquist and Jenkins, 2017). Considerable research has evaluated the effects of C16:0 supplements on dairy cow performance and metabolism (e.g. Piantoni et al., 2013; de Souza and Lock, 2018); however, these studies were only conducted in post-peak cows. Therefore, our study evaluated the effects of timing of C16:0 supplementation on production responses of early-lactation cows.

Since feed intake in early postpartum is likely controlled by mechanisms related to oxidation of fuels in the liver (Allen and Piantoni, 2013), some authors suggest that supplementing fat to cows during the immediate postpartum period may depress feed intake

(Kuhla et al., 2016). The effect of fat supplements on DMI is variable and usually depends on the type of fat being fed (Rabiee et al., 2012). With post-peak cows, results from studies with highly enriched (≥ 85%) sources of C16:0 and C18:0 have been variable, but DMI has typically not been reduced (Piantoni et al., 2013; de Souza et al., 2016; Piantoni et al., 2015a) compared with non-FA supplemented diets. In our study, feeding PA during the FR or PK periods did not change DMI, and the increase in DMI over time after parturition was consistent for all treatments. Similarly, feeding saturated FA supplements (combination of C16:0 and C18:0) from calving to 100 DIM usually did not impact DMI in dairy cows (Jerred et al., 1990; Beam and Butler, 1998), while other studies feeding a similar saturated FA supplement reported increased DMI in cows in the immediate postpartum and early lactation periods (Piantoni et al., 2015b; Moallem et al., 2007). Therefore, the effect of saturated FA supplements (C16:0 and combinations of C16:0 + C18:0) on DMI of early lactation cows is minimal.

Interestingly, we observed that feeding PA did not impact milk yield during the immediate postpartum period (FR period), but feeding PA during PK period increased milk yield by 3.4 kg/d compared with CON. Also, there was no interaction between the diet fed in FR and PK periods on milk yield response, so that regardless of the diet that cows received during the FR period, PA consistently increased milk yield when fed during the PK period. A meta-analysis by Onetti and Grummer (2004) observed that fat supplementation increased milk yield and milk fat yield when fed to cows during early-lactation (<120 DIM) but not in mid-lactation (>120 DIM). However, production responses to saturated FA supplementation in the immediate postpartum period have been inconsistent. Beam and Butler (1998) supplemented a saturated FA supplement (C16:0 + C18:0) and reported an interaction between diet and time for milk yield due to supplemental fat decreasing milk yield during the first 3 wks postpartum, but increased milk

yield during the next 2 wks of the experiment. Piantoni et al. (2015b) observed that feeding a saturated FA supplement (C16:0 + C18:0) tended to decrease milk yield by 3.1 kg/d in cows in the immediate postpartum period. In early lactation cows, Weiss and Pinos-Rodriguez (2009) fed a FA supplement (C16:0 + C18:0) to early-lactation cows (21 to 126 d postpartum) and observed that when diets were supplemented with FA, energy intake was increased and directed mostly to milk yield in a lower forage diet, and to body reserves in a higher forage diet. These results indicated that milk yield usually is not affected when saturated FA supplements are fed in the immediate postpartum period (i.e. Piantoni et., 2015b; Beam and Butler, 1998), but milk yield may increase when these supplements are fed after 3-4 wks after calving (i.e. Hoffman et al., 1991; Weiss and Pinos-Rodriguez, 2009). Our results, therefore, agree with the previous literature feeding saturated fat and with our initial hypothesis in which we postulated that the response to FA supplementation may vary due to the timing when supplemental fat is fed.

We observed that PA increased milk fat yield during both FR (+280 g/d) and PK (+210 g/d) periods, but the magnitude of response was greater during FR than PK period. Most of our short-term studies involved feeding C16:0 supplements to post peak cows (fed at 1.5 to 2.0% diet DM) have indicated increases in milk fat yield (Piantoni et al., 2013; Lock et al., 2013; de Souza et al., 2017). In long-term feeding, de Souza and Lock (2018) observed that feeding a C16:0 supplement (1.5% diet DM) over a 10-wk period also increased milk fat yield by ~150 g/d. Although Rico et al. (2017) observed that maximum milk fat yield response occurred when C16:0 was fed at 1.5% of diet DM, the incorporation of C16:0 into milk fat increased linearly as C16:0 dose increased. Tzompa-Sosa et al. (2014) suggested that an increase in availability of C16:0 for lipid synthesis in mammary epithelial cells may increase the activity of GPAT in the mammary gland, increasing the proportion of C16:0 acylated at sn-1 that initiates TAG

synthesis. Overall, the increase in milk fat associated with our PA treatment during the FR period occurred due to an increase in yield of 16-carbon milk FA by 147g/d (derived from both de novo synthesis and extraction from plasma), and an increase in preformed milk FA by 96 g/d. The increase in 16-carbon milk FA by PA agrees with several previous studies that fed C16:0 supplements to post-peak cows (e.g. Piantoni et al., 2013; Lock et al 2013; de Souza et al., 2017), while the increase in preformed milk FA was likely associated with the greater BW loss in the PA treatment during FR period. Interestingly, during PK period we observed an interaction between diet fed in FR and PK periods for milk fat yield due to feeding PA during PK period increasing milk fat yield to a greater extent in cows that received the CON diet (+ 240 g/d) rather than PA (+ 180 g/d) diet during the FR period. This difference is associated with yield of preformed FA because we observed that feeding PA during the PK period increased the yield of preformed milk FA only in cows that received the CON diet during the FR period. Overall, the yield of de novo milk FA increased and the yield of preformed milk FA decreased for all treatments as the DMI increased over time. Although we did not observe treatment differences for de novo milk FA, the yield of C4:0 increased in both FR and PK periods when PA was fed; this is in line with our recent studies feeding C16:0 to post-peak cows (Piantoni et al., 2013; de Souza et al., 2016). It has been suggested that the increase yield of C4:0 might be part of the mechanism to maintain milk fat fluidity at body temperature, with an increase in C4:0 output due to the large diglyceride pool of high molecular weight FA that results from the incorporation of long-chain FA taken up from plasma (Barbano and Sherbon, 1980). Therefore, our results suggest that feeding PA during early lactation increased milk fat yield to a greater extent than previous studies with post-peak cows, but this is also partially related to an increase in the yield of preformed milk FA likely coming from adipose tissue.

Previous studies have observed that C16:0 supplementation increased 3.5% FCM and ECM in post-peak cows (Piantoni et al., 2013; Lock et al., 2013; de Souza et al., 2018). In our study, feeding PA during both the FR or PK periods increased both 3.5% FCM and ECM, and the increase in these variables over time after parturition was consistent. Also, the magnitude of increase in 3.5% FCM and ECM by PA was similar during the FR or PK periods. These results are associated with the increase in the yield of milk fat and protein. In contrast, Piantoni et al. (2015b) observed that feeding a saturated FA supplement (C16:0 + C18:0) did not affect the yield of 3.5% FCM, and ECM in cows in the immediately postpartum (1 to 29 DIM), but FA supplementation had a pronounced carryover effect (30 to 67 DIM) decreasing both 3.5% FCM and ECM in a low forage diet. Also, Moallem et al. (2007) fed a saturated FA and showed that the supplement did not affect 3.5% FCM or milk energy output. However, these diets were fed prepartum to 100 days postpartum, and the effects on performance were reported as least squares means for the whole 100 d in lactation. Thus, the effect of fat supplementation over time on production performance cannot be discerned. Therefore, in our study the pronounced increase in ECM due to PA supplementation is associated with the potential that C16:0 supplements have in increase yield of milk components, and the ECM response is not associated with timing of C16:0 supplementation.

Although we observed that feeding PA increased ECM in early lactation cows, it also resulted in increased BW and BCS loss. The increase in BW and BCS loss was more pronounced in the FR than PK period. In the FR period, PA induced a greater decrease in BW and BCS after the second week of treatments, and increased plasma levels of NEFA and reduced insulin (Chapter 6). Importantly, even though PA increased plasma NEFA concentration, NEFA levels were below the threshold considered critical for increased incidence of metabolic disorders

(Ospina et al., 2013). In the PK period, the magnitude of BW and BCS loss due to PA supplementation was much smaller, and cows start recovering BW and entered positive energy balance by wk 7 (Chapter 6). Similar to our results, Moallem et al. (2007) observed that feeding a saturated FA supplement increased milk yield, but also increased BCS loss compared with a control diet. In contrast, Piantoni et al. (2015b) feeding a saturated FA supplement observed that regardless of dietary forage content, FA supplementation decreased BW loss, and tended to decrease BCS loss in cows during the immediate postpartum period (1 to 29 DIM) at expense of milk production. With post peak cows, Mathews et al. (2016) observed a decrease in glucose-stimulated NEFA disappearance in cows fed C16:0, suggesting the possibility of localized adipose tissue insulin resistance with prolonged C16:0 supplementation. Since the development of insulin resistance in adipose and skeletal muscle tissues enables the dairy cow to partition nutrients toward the mammary gland during early lactation (Bell, 1995; Bell and Bauman, 1997), we postulate that the change in energy partitioning to milk at the expense of body reserves in the immediate postpartum period in the PA treatment may in part be related to changes in insulin resistance. The potential role of individual FA on nutrient partitioning to support lactation and its mechanisms requires further investigation.

To our knowledge, few studies were designed to evaluate the effects of timing of FA supplementation on production responses of dairy cows. Holter and Hayes (1994) evaluated the timing of feeding a Ca salts of palm FA supplement (3.75% diet DM) starting at 1, 29, and 57 DIM up to 112 DIM on production responses of dairy cows. The authors reported that most production responses including DMI, milk yield, and 4% FCM were not affected by timing of supplementation. In contrast, milk fat content decreased as the time of supplement introduction to the diet increased. In our study, although the increase with PA in milk energy output was

similar in FR and PK periods (Chapter 6), changes in body reserves were affected by the time of supplementation with cows reducing BW due to PA supplementation to a greater extent in the FR period. Increased rates of lipolysis and BW loss in the immediate postpartum period are expected since dairy cows exhibit this propensity to nurture the neonate from tissue reserves (Bauman and Currie, 1980). However, prolonged negative energy balance and increased BW loss may negatively affect reproduction (Roche et al., 2009). A negative association between BCS loss in early lactation and reproduction is associated with delayed ovarian activity, infrequent luteinizing hormone pulses, poor follicular response to gonadotropins, and reduced functional competence of the follicle (Chagas et al., 2007). Although there is general agreement regarding the importance of energy stores and energy balance on reproduction, some inconsistencies in this relationship also occur (Roche et al., 2009). For instance, feeding a Ca salts of palm FA supplement (2.6% diet DM) from parturition to 120 DIM increased milk yield and BW loss in dairy cows, but also increased plasma progesterone and pregnancy rate (Sklan et al., 1991). In contrast, Sklan et al. (1994) observed feeding a Ca salts of palm FA supplement (2.5% diet DM) from parturition to 120 DIM increased milk yield and BW loss in multiparous and primiparous cows, while reduced conception rate at first insemination only in primiparous cows. Further studies are needed to understand the mechanism by which C16:0 supplementation increases milk energy output at the expense of body reserves in the immediate postpartum period, and the possible effects of greater BW and BCS losses on health and reproduction of dairy cows.

Conclusion

In conclusion, feeding a C16:0 supplement to early lactation cows consistently increased the yield of ECM in both FR and PK periods compared with a non-fat supplemented control diet,

but also increased BW and BCS losses. Our results suggest that feeding C16:0 during early lactation increased milk fat yield to a greater extent than previous studies with post-peak cows, but this is also partially related to an increase in the yield of preformed milk FA likely coming from adipose tissue. For some variables, the effect of feeding C16:0 were affected by timing of supplementation since milk yield increased only during the PK period and BW reduced to a greater extent in the FR period when C16:0 supplement was fed. Regardless of diet fed during FR period, feeding a C16:0 supplement during PK period increased yield of milk and milk components.

APPENDIX

APPENDIX

Table 5.1. Ingredient and nutrient composition of close up and treatment diets.

	Diet[1]				
	Close up	FR		PK	
		CON	PA	CON	PA
Ingredient, % DM					
Corn Silage	34.2	27.5	27.5	26.4	26.4
Alfafa Silage	-	12.9	12.9	16.4	16.4
Alfafa Hay	-	11.2	11.2	-	-
Grass Hay	35.3	-	-	-	-
Wheat Straw	-	-	-	2.76	2.76
Ground Corn	9.59	18.6	18.6	13.9	13.9
High Moisture Corn	-	6.49	6.49	13.5	13.5
Soybean Meal	13.2	12.8	12.8	12.4	12.4
Soyhulls	-	2.82	1.36	5.57	4.06
Whole Cottonseed	-	3.50	3.50	5.49	5.49
Palmitic Acid Supplement[2]	-	-	1.46	-	1.51
Mineral and Vitamin mix[3,4,5]	7.68	4.29	4.29	3.58	3.58
Nutrient Composition, % DM[6]					
NDF	45.2	31.9	30.9	30.8	29.7
Forage NDF	41.2	24.0	24.0	21.0	21.0
CP	14.2	17.5	17.4	16.8	16.7
Starch	15.5	23.5	23.5	27.4	27.4
Gross energy, Mcal/kg of DM	-	4.49	4.61	4.97	5.05
FA	1.82	2.99	4.48	3.55	5.07
16:0	0.23	0.47	1.72	0.61	1.84
18:0	0.06	0.08	0.18	0.13	0.23
cis-9 18:1	0.30	0.54	0.68	0.65	0.79
cis-9, *cis*-12 18:2	0.76	1.48	1.52	1.79	1.81
cis-9, *cis*-12, *cis*-15 18:3	0.05	0.17	0.18	0.21	0.21

[1] Close up diet was fed from d -21 of expected calving date until calving. Diets fed during the fresh period (**FR**; 1 to 24 DIM) were either **CON** (control diet) or **PA** (1.5% of C16:0-enriched FA supplement replacing soyhulls). Diets fed during the peak period (**PK**; 25 to 67 DIM) were either CON (control diet) or PA (1.5% of C16:0-enriched FA supplement replacing soyhulls).

[2] C16:0-enriched fatty acid supplement (Palmit 80, Global Agri Trade Corporation, CA, USA). The supplement contained (g/100 g of fatty acid) 1.0 of C14:0, 85.1 of C16:0, 2.7 of C18:0, and 8.9 of *cis*-9 C18:1.

[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% di- calcium phosphate, 4.2% magnesium sulfate, 1.8% salt, 1.8% yeast, 4.4% trace minerals and vitamins, 0.3% selenium yeast 600 (600 mg of Se/kg), and 0.09% Smartamine.

[4]Vitamin-mineral mix for the FR 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, 0.4% selenium yeast 600 (600 mg of Se/kg), and 0.09% Smartamine.

[5]Vitamin-mineral mix for the PK 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, 0.2% selenium yeast 600 (600 mg of Se/kg), and 0.09% of Smartamine.

[6] Expressed as percent of as fed.

Table 5.2. Health incidents during the experiment for cows fed treatment diets.

	Diet[1]	
	CON	PA
During treatment period		
Fever with no apparent cause (>39.5°C)	1	0
Ketosis	4	5
Lame	0	0
Mastitis	1	0
Metritis	2	3
Milk fever	0	0
Retained Placenta	5	4
Displaced abomasum	1	0
During peak period		
Displaced abomasum	0	1
Ketosis	0	0
Lame	0	1
Mastitis	1	2

[1] Diets fed during the fresh period (FR; 1 to 24 DIM) were either CON (control diet) or PA (1.5% of C16:0-enriched FA supplement replacing soyhulls). Diets fed during the peak period (PK; 25 to 67 DIM) were either CON (control diet) or PA (1.5% of C16:0-enriched FA supplement replacing soyhulls).

Table 5.3. Milk production, milk composition, BW, and BCS for cows fed treatment diets during the fresh period (d 1 to 24 postpartum).

	Treatment (Trt)[1]		SEM	*P value*[2]		
Variable	CON	PA		Trt	Time	Trt x Time
DMI, kg/d	22.3	22.1	0.62	0.92	<0.01	0.91
Milk Yield, kg/d						
Milk	47.2	48.6	1.05	0.39	<0.01	0.61
3.5% FCM[3]	52.2	57.5	1.65	0.01	<0.01	0.19
ECM[4]	51.9	56.6	1.46	0.02	<0.01	0.17
Milk Composition						
Fat, kg/d	2.01	2.29	0.08	<0.01	<0.01	0.59
Fat, %	4.48	4.89	0.13	0.01	<0.01	0.32
Protein, kg/d	1.50	1.60	0.04	0.03	<0.01	0.25
Protein, %	3.37	3.41	0.06	0.65	<0.01	0.21
Lactose, kg/d	2.16	2.23	0.05	0.43	<0.01	0.32
Lactose, %	4.75	4.72	0.02	0.46	<0.01	0.22
Cumulative, kg						
Milk yield	1111	1145	33.4	0.25	NA[5]	NA
Fat yield	49.8	56.0	0.94	<0.01	NA	NA
Protein yield	36.7	38.6	0.86	0.05	NA	NA
ECM/DMI	2.34	2.60	0.08	<0.01	0.58	0.54
BW, kg	709	688	11.8	0.05	<0.01	0.05
BW change, kg/d	-1.89	-2.65	0.34	0.07	NA	NA
BCS	3.34	3.25	0.06	0.04	<0.01	0.07

[1] Diets fed during the fresh period (FR; 1 to 24 DIM) were either CON (control diet) or PA (1.5% of C16:0-enriched FA supplement replacing soyhulls).
[2] P values refer to the ANOVA results for the main effects of treatment (Trt), time, and its interactions.
[3] Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].
[4] Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)].
[5] Not applicable.

Table 5.4. Milk production, milk composition, BW, and BCS for cows fed treatment diets during the peak period (d 25 to 67 postpartum).

	Treatment[1]				SEM	*P value*[2]						
	CON-CON	CON-PA	PA-CON	PA-PA		FR	PK	FR*PK	Time	FR* Time	PK* Time	FR*PK* Time
DMI, kg/d	30.4	30.8	29.1	29.6	0.82	0.38	0.68	0.75	<0.01	0.42	0.79	0.21
Milk Yield, kg/d												
Milk	54.2	57.8	55.0	58.3	1.25	0.75	0.01	0.93	<0.01	0.58	0.31	0.76
3.5% FCM[3]	58.0	62.9	57.6	61.7	1.84	0.70	<0.01	0.83	0.05	0.48	0.29	0.85
ECM[4]	57.0	61.6	56.8	61.4	1.88	0.92	<0.01	0.95	0.01	0.45	0.46	0.83
Milk Composition												
Fat, kg/d	2.07	2.31	2.05	2.23	0.09	0.66	<0.01	0.07	<0.01	0.51	0.19	0.92
Fat, %	3.66	3.94	3.67	3.82	0.11	0.58	<0.01	0.52	<0.01	0.74	0.69	0.94
Protein, kg/d	1.65	1.74	1.66	1.85	0.08	0.35	0.04	0.43	<0.01	0.29	0.85	0.92
Protein, %	2.93	2.96	2.99	3.07	0.08	0.15	0.22	0.39	0.66	0.17	0.83	0.96
Lactose, kg/d	2.74	2.85	2.81	2.87	0.07	0.76	0.04	0.65	<0.01	0.54	0.45	0.21
Lactose, %	4.98	4.84	4.91	4.87	0.04	0.44	0.41	0.42	0.77	0.42	0.51	0.38
Cumulative, kg												
Milk yield	2493	2658	2530	2681	75.9	0.28	<0.01	0.54	NA	NA	NA	NA
Fat yield	94.3	106	95.2	103	2.86	0.45	<0.01	0.56	NA	NA	NA	NA
Protein yield	75.9	80.0	76.4	85.1	2.29	0.28	0.05	0.62	NA	NA	NA	NA
FCM/DMI	1.91	2.08	1.93	2.10	0.09	0.45	<0.01	0.78	<0.01	0.65	0.32	0.84
BW, kg	698	691	682	669	7.32	0.01	0.06	0.25	0.04	0.39	0.88	0.98
BW Change, kg/d	0.29	0.27	0.20	0.16	0.06	0.07	0.93	0.31	NA	NA	NA	NA
BCS	3.10	2.93	3.03	2.98	0.06	0.75	0.05	0.17	<0.01	0.72	0.27	0.31

[1] 1) CON-CON: cows that received control diet for both fresh (FR) and peak (PK) periods; 2) CON-PA: cows that received control diet during FR and changed to PA diet (1.5% of C16:0-enriched FA supplement replacing soyhulls) during PK period; 3) PA-CON: cows that received PA diet during FR and changed to CON diet during PK period; and 4) PA-PA: cows that received PA diet for both fresh (FR) and peak (PK) periods.

[2] P values refer to the ANOVA results for the main effects of treatments cows received during FR period, PK period, time, and their interactions.

[3] Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].

[4] Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)].

[5] Not applicable.

Table 5.5. Milk FA concentration and yield by source for cows fed treatment diets during the fresh period (d 1to 24 postpartum).

Variable	Treatment (Trt)[1]		SEM	*P value*[2]		
	CON	PA		Trt	Time	Trt x Time
Summation by Source[3], g/100 g FA						
De Novo	17.1	15.9	0.72	0.23	<0.01	0.18
Mixed	31.2	34.7	0.31	<0.01	0.45	<0.01
Preformed	51.7	49.5	0.91	0.07	<0.01	0.45
Summation by Source[4], g/d						
De Novo	285	303	13.5	0.32	<0.01	0.98
Mixed	524	671	23.3	<0.01	0.71	0.12
Preformed	870	966	44.5	0.05	0.42	0.25

[1] Diets fed during the fresh period (FR; 1 to 24 DIM) were either CON (control diet) or PA (1.5% of C16:0-enriched FA supplement replacing soyhulls).

[2] P values refer to the ANOVA results for the main effects of treatment (Trt), time, and its interactions.

[3] De novo FA originate from mammary de novo synthesis (<16 carbons), preformed FA originated from extraction from plasma (>16 carbons), and mixed FA originate from both sources (C16:0 plus *cis*-9 C16:1). Concentrations and yields of individual fatty acids are reported in Tables 5.7 and 5.8, respectively.

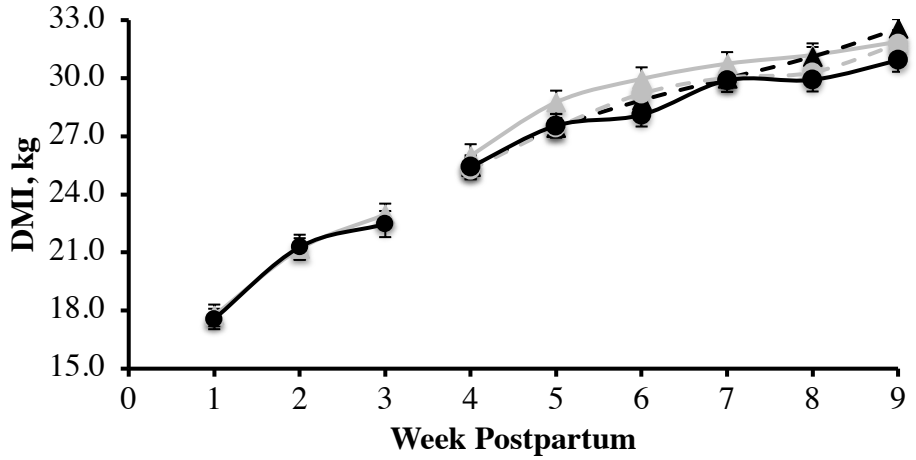
Table 5.6. Milk FA concentration and yield by source for cows fed treatment diets during the peak period (d 25 to 67 postpartum).

	Treatment[1]				SEM	*P value*[2]						
	CON-CON	CON-PA	PA-CON	PA-PA		FR	PK	FR* PK	Time	FR* Time	PK* Time	FR*PK* Time
Summation by Source[4], g/100 g FA												
de Novo	25.1	22.2	24.6	22.1	0.56	0.66	<0.01	0.82	<0.01	0.57	0.07	0.47
Mixed	31.8	35.3	30.9	36.4	0.48	0.75	<0.01	0.08	<0.01	0.76	0.71	0.59
Preformed	43.1	42.5	44.5	41.5	0.87	0.89	0.08	0.27	<0.01	0.72	0.19	0.82
Summation by Source[4], g/d												
de Novo	460	458	448	437	19.2	0.66	0.54	0.88	0.09	0.18	0.03	0.58
Mixed	587	720	572	730	28.6	0.95	<0.01	0.61	0.61	0.35	0.26	0.86
Preformed	803	864	836	829	41.8	0.85	0.72	0.06	<0.01	0.56	0.54	0.93

[1] 1) CON-CON: cows that received control diet for both fresh (FR) and peak (PK) periods; 2) CON-PA: cows that received control diet during FR and changed to PA diet (1.5% of C16:0-enriched FA supplement replacing soyhulls) during PK period; 3) PA-CON: cows that received PA diet during FR and changed to CON diet during PK period; and 4) PA-PA: cows that received PA diet for both fresh (FR) and peak (PK) periods.

[2] P values refer to the ANOVA results for the main effects of treatments cows received during FR period, PK period, time, and their interactions.

[3] De novo FA originate from mammary de novo synthesis (<16 carbons), preformed FA originated from extraction from plasma (>16 carbons), and mixed FA originate from both sources (C16:0 plus *cis*-9 C16:1). Concentrations and yields of individual fatty acids are reported in Tables 5.9 and 5.10, respectively.



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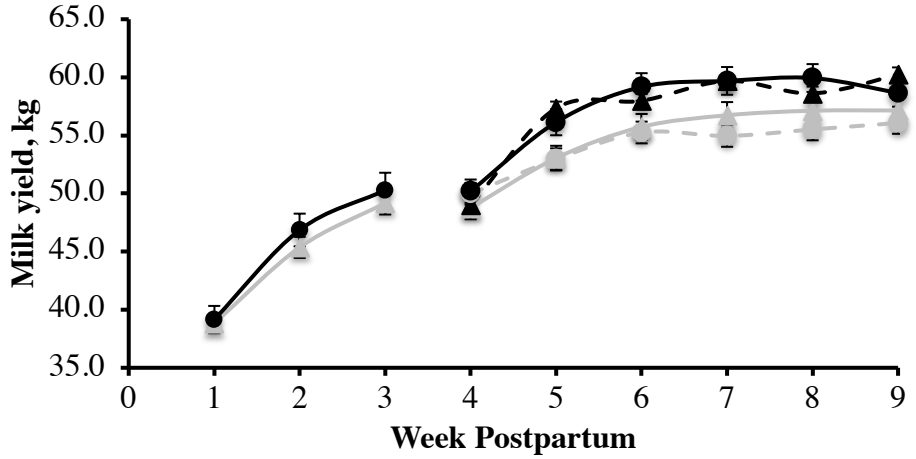

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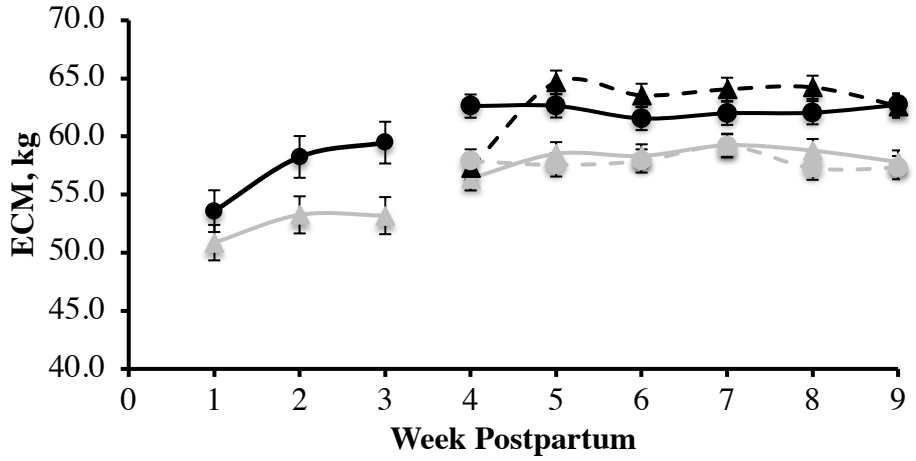
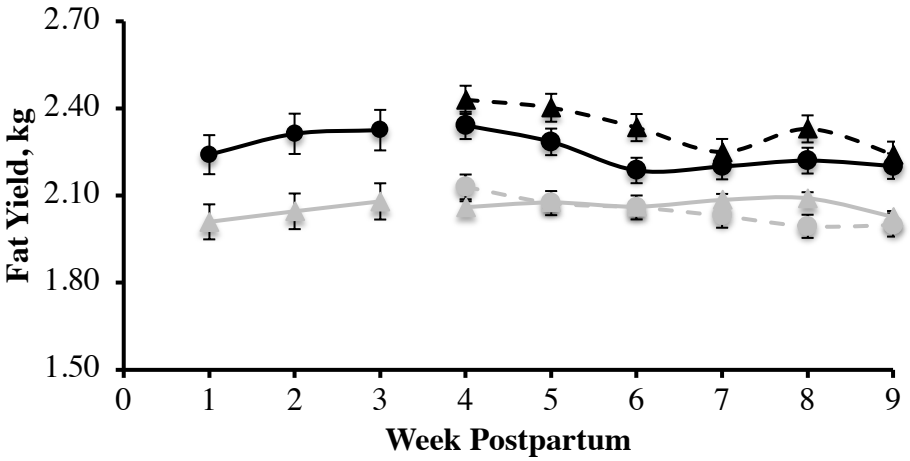


C

Figure 5.1. Effects of dietary treatments on DMI (A), milk yield (B) and ECM (C) over time during the fresh (weeks 1-3) and peak (weeks 4-9) periods.

Diets fed during the fresh period were either CON (control diet; black line) or PA (1.5% of C16:0-enriched supplement; grey line). During the peak period treatments were: CON-CON:

cows that received CON for both fresh (FR) and peak (PK) periods (grey line); 2) CON-PA: cows that received CON during FR and changed to PA diet (1.5% of C16:0-enriched supplement) during PK period (black broke line); 3) PA-CON: cows that received PA diet during FR and changed to CON diet during PK period (grey broke line); and 4) PA-PA: cows that received PA diet for FR and PK periods (black line). During FR, PA increased ECM ($P = 0.02$) and did not affect milk yield ($P=0.38$), and DMI ($P = 0.92$) compared with CON. DMI, milk yield and ECM increased over time in both treatments (all $P<0.01$) and we did not observed treatment by time interaction for DMI ($P = 0.91$), milk yield ($P = 0.61$) and ECM ($P = 0.63$). During PK, PA increased milk yield ($P = 0.01$) and ECM ($P < 0.01$) and did not affect DMI ($P = 0.68$) compared with CON. DMI, milk yield and ECM increased over time in all treatments (all $P < 0.01$), and we did not observed treatment by time interaction for DMI ($P = 0.79$), milk yield ($P = 0.31$) and ECM ($P = 0.46$). Error bars indicate SEM.



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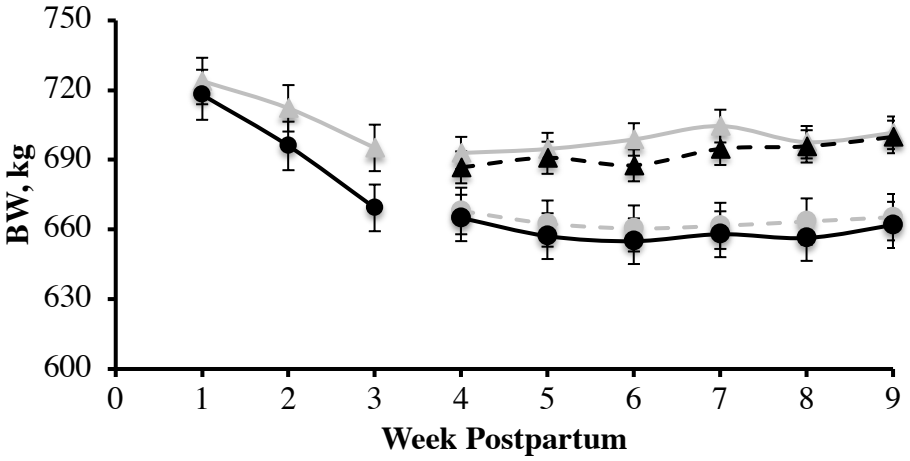

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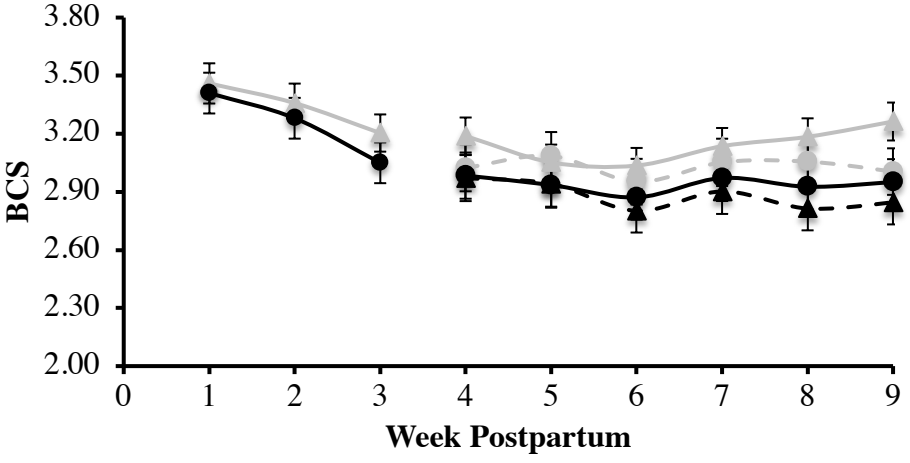
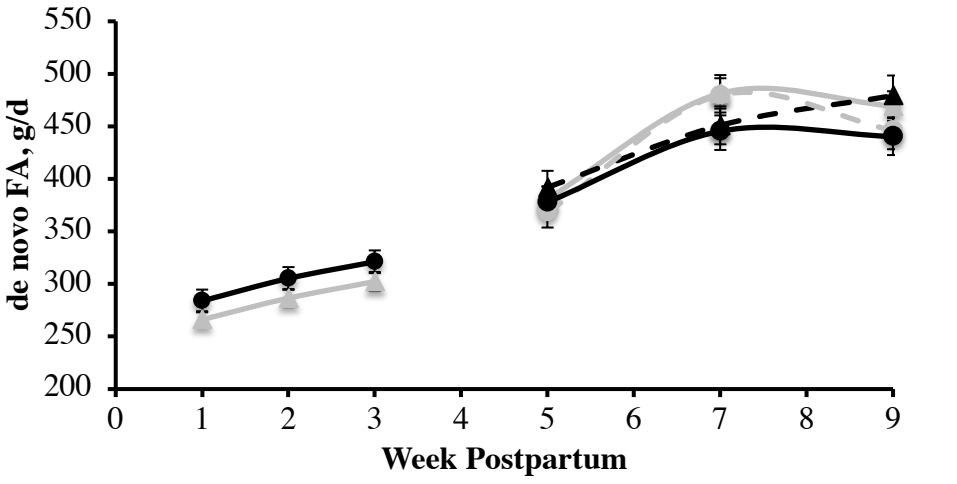


C

Figure 5.2. Effects of dietary treatments on milk fat yield (A), BW (B) and BCS (C) over time during the fresh (weeks 1-3) and peak (weeks 4-9) periods.
Diets fed during the fresh period were either CON (control diet; black line) or PA (1.5% of C16:0-enriched supplement; grey line). During the peak period treatments were: CON-CON: cows that received CON for both fresh (FR) and peak (PK) periods (grey line); 2) CON-PA:

cows that received CON during FR and changed to PA diet (1.5% of C16:0-enriched supplement) during PK period (black broke line); 3) PA-CON: cows that received PA diet during FR and changed to CON diet during PK period (grey broke line); and 4) PA-PA: cows that received PA diet for FR and PK periods (black line). During FR, PA increased milk fat yield ($P < 0.01$) and decreased BW ($P = 0.05$), and BCS ($P = 0.04$) compared with CON. Milk fat yield increased, and BW and BCS decreased over time in both treatments (all $P<0.01$) and we observed treatment by time interaction for BW ($P = 0.05$) and BCS ($P = 0.07$). During PK, PA increased milk fat yield ($P < 0.01$), decreased BCS ($P = 0.05$), and tended to decrease BW ($P = 0.06$), compared with CON. We did not treatment by time interaction for milk fat yield ($P = 0.99$), BW ($P = 0.88$) and BCS ($P = 0.27$). Error bars indicate SEM.



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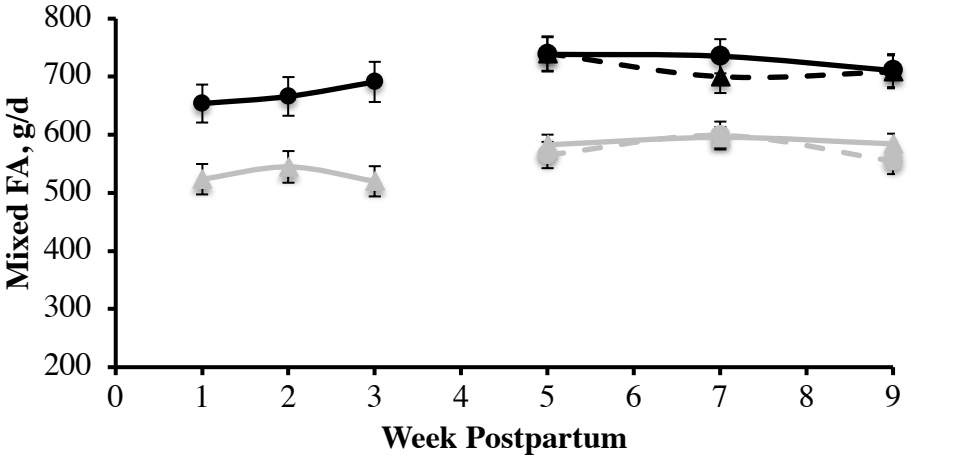

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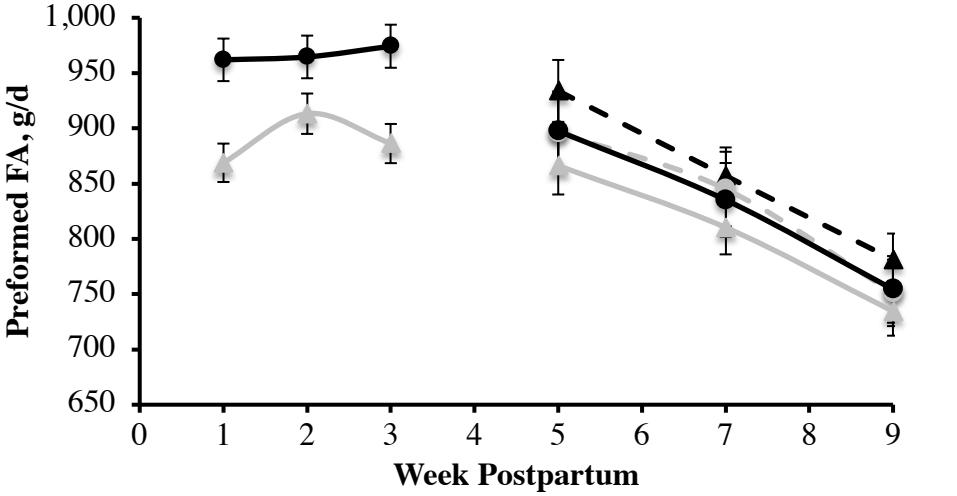

C

Figure 5.3. Effects of dietary treatments on the yield of de novo (A), mixed (B) and preformed (C) milk FA over time during the fresh (weeks 1-3) and peak (weeks 4-9) periods.

Diets fed during the fresh period were either CON (control diet; black line) or PA (1.5% of C16:0-

enriched supplement; grey line). During the peak period treatments were: CON-CON: cows that received CON for both fresh (FR) and peak (PK) periods (grey line); 2) CON-PA: cows that received CON during FR and changed to PA diet (1.5% of C16:0-enriched supplement) during PK period (black broke line); 3) PA-CON: cows that received PA diet during FR and changed to CON diet during PK period (grey broke line); and 4) PA-PA: cows that received PA diet for FR and PK periods (black line). During FR, PA increased mixed ($P < 0.01$) and preformed ($P = 0.05$) and did not affect de novo ($P = 0.32$) milk FA compared with CON. A tendency for a treatment by time interaction was observed for mixed ($P = 0.12$) but not for de novo ($P = 0.98$) and preformed ($P = 0.25$). During PK, PA increased mixed ($P < 0.01$) and did not affect de novo ($P = 0.54$) and preformed ($P = 0.72$) milk FA compared with CON. A treatment by time interaction was observed for de novo ($P = 0.03$) but not for mixed ($P = 0.26$) and preformed ($P = 0.54$). Error bars indicate SEM.

Table 5.7. Milk fatty acid concentrations for cows fed treatment diets during the fresh period (d 1 to 24 postpartum).

Variable	Treatment (Trt)[1]		SEM	*P value*[2]		
	CON	PA		Trt	Time	Trt x Time
Selected Individual FA, g/100 g FA						
C4:0	3.68	3.58	0.06	0.25	0.31	0.16
C6:0	1.76	1.62	0.06	0.16	<0.01	0.24
C8:0	0.84	0.75	0.04	0.21	<0.01	0.13
C10:0	1.63	1.46	0.12	0.35	<0.01	0.12
C12:0	1.69	1.55	0.13	0.42	<0.01	0.14
C14:0	7.01	6.47	0.33	0.25	<0.01	0.27
C16:0	28.6	32.4	0.35	<0.01	0.61	<0.01
cis-9 C16:1	2.52	2.27	0.09	0.07	0.19	0.39
C18:0	13.1	12.8	0.25	0.28	<0.01	0.12
cis-9 C18:1	29.3	27.9	0.82	0.27	0.12	0.74
cis-11 C18:1	1.04	0.94	0.04	0.08	<0.01	0.54
trans-6 to 8 C18:1	0.22	0.21	0.005	0.15	<0.01	0.58
trans-9 C18:1	0.13	0.14	0.002	0.71	<0.01	0.59
trans-10 C18:1	0.33	0.28	0.04	0.38	<0.01	0.63
trans-11 C18:1	0.83	0.79	0.03	0.37	<0.01	0.87
cis-9, *cis*-12 C18:2	2.30	2.17	0.05	0.08	0.12	0.24
cis-9, *trans*-11 C18:2	0.27	0.25	0.01	0.14	<0.01	0.33
cis-9, *cis*-12, *cis*-15 C18:3	0.37	0.34	0.009	0.01	<0.01	0.19

[1] Diets fed during the fresh period (FR; 1 to 24 DIM) were either CON (control diet) or PA (1.5% of C16:0-enriched fatty acid supplement replacing soyhulls).

[2] P values refer to the ANOVA results for the main effects of treatment (Trt), time, and its interactions.

Table 5.8. Milk fatty acid yields for cows fed treatment diets during the fresh period (d 1 to 24 postpartum).

	Treatment (Trt)[1]		SEM	*P value*[2]		
Variable	CON	PA		Trt	Time	Trt x Time
Selected Individual FA, g/d						
C4:0	61.6	69.6	2.79	0.02	0.85	0.36
C6:0	29.3	31.2	1.45	0.35	<0.01	0.91
C8:0	13.9	14.3	0.87	0.73	<0.01	0.88
C10:0	26.9	27.5	2.04	0.83	<0.01	0.72
C12:0	27.9	29.0	2.06	0.72	<0.01	0.71
C14:0	116	123	5.83	0.43	0.02	0.95
C16:0	481	625	21.2	<0.01	0.71	0.13
cis-9 C16:1	43.1	44.7	2.86	0.68	0.56	0.18
C18:0	219	248	10.1	0.03	0.05	0.32
cis-9 C18:1	494	550	30.1	0.05	0.56	0.20
cis-11 C18:1	17.7	18.5	1.12	0.62	0.11	0.18
trans-6 to 8 C18:1	3.73	4.13	0.15	0.03	<0.01	0.65
trans-9 C18:1	2.23	2.62	0.11	<0.01	<0.01	0.29
trans-10 C18:1	5.64	5.37	0.84	0.79	0.05	0.84
trans-11 C18:1	13.7	15.3	0.82	0.09	0.11	0.52
cis-9, *cis*-12 C18:2	38.5	41.8	1.66	0.11	0.46	0.47
cis-9, *trans*-11 C18:2	4.55	4.85	0.28	0.39	0.04	0.81
cis-9, *cis*-12, *cis*-15 C18:3	6.26	6.68	0.32	0.21	0.08	0.73

[1] Diets fed during the fresh period (FR; 1 to 24 DIM) were either CON (control diet) or PA (1.5% of C16:0-enriched fatty acid supplement replacing soyhulls).

[2] P values refer to the ANOVA results for the main effects of treatment (Trt), time, and its interactions.

Table 5.9. Milk fatty acid concentrations for cows fed treatment diets during the peak period (d 25 to 67 postpartum).

	Treatment[1]				SEM	*P value*[2]						
	CON-CON	CON-PA	PA-CON	PA-PA		FR	PK	FR* PK	Time	FR* Time	PK* Time	FR*PK* Time
Selected Individual FA, g/100 g FA												
C4:0	3.29	3.33	3.19	3.22	0.08	0.20	0.63	0.95	<0.01	0.53	0.12	0.55
C6:0	2.16	2.06	2.10	1.98	0.03	0.09	0.01	0.87	0.59	0.63	0.11	0.62
C8:0	1.28	1.16	1.25	1.11	0.02	0.28	<0.01	0.86	0.02	0.61	0.08	0.73
C10:0	3.16	2.71	3.12	2.62	0.16	0.68	<0.01	0.88	<0.01	0.51	0.07	0.72
C12:0	3.50	2.92	3.49	2.89	0.13	0.92	<0.01	0.96	<0.01	0.54	0.07	0.79
C14:0	11.1	9.43	10.8	9.59	0.29	0.95	<0.01	0.64	<0.01	0.66	0.14	0.25
C16:0	30.1	33.5	29.4	34.7	0.51	0.76	<0.01	0.09	<0.01	0.75	0.79	0.74
cis-9 C16:1	1.57	1.73	1.56	1.71	0.06	0.93	0.10	0.93	<0.01	0.61	0.05	0.25
C18:0	11.9	11.1	12.2	10.2	0.26	0.44	<0.01	0.08	0.03	0.72	0.68	0.87
cis-9 C18:1	20.4	20.9	21.3	20.6	0.73	0.76	0.90	0.55	<0.01	0.74	0.15	0.63
cis-11 C18:1	0.79	0.82	0.81	0.82	0.04	0.89	0.73	0.77	<0.01	0.77	0.03	0.91
trans-6 to 8 C18:1	0.27	0.27	0.27	0.28	0.01	0.79	0.81	0.75	<0.01	0.79	0.52	0.32
trans-9 C18:1	0.18	0.17	0.19	0.18	0.004	0.26	0.26	0.41	<0.01	0.75	0.22	0.89
trans-10 C18:1	0.44	0.68	0.53	0.71	0.11	0.63	0.07	0.76	0.09	0.48	0.63	0.41
trans-11 C18:1	0.94	0.86	0.94	0.95	0.03	0.39	0.39	0.43	<0.01	0.9	0.77	0.93
cis-9, *cis*-12 C18:2	2.57	2.42	2.58	2.35	0.05	0.62	0.01	0.60	0.04	0.75	0.71	0.98
cis-9, *trans*-11 C18:2	0.33	0.31	0.32	0.37	0.01	0.36	0.68	0.19	<0.01	0.99	0.87	0.83
cis-9, *cis*-12, *cis*-15 C18:3	0.42	0.38	0.41	0.37	0.007	0.52	<0.01	0.82	<0.01	0.95	0.55	0.32

[1] 1) CON-CON: cows that received control diet for both fresh (FR) and peak (PK) periods; 2) CON-PA: cows that received control diet during FR and changed to PA diet (1.5% of C16:0-enriched fatty acid supplement replacing soyhulls) during PK period; 3) PA-CON: cows that received PA diet during FR and changed to CON diet during PK period; and 4) PA-PA: cows that received PA diet for both fresh (FR) and peak (PK) periods.

[2] P values refer to the ANOVA results for the main effects of treatments cows received during FR period, PK period, time, and their interactions.

Table 5.10. Milk fatty acid yields for cows fed treatment diets during the peak period (d 25 to 67 postpartum).

	Treatment[1]				SEM	*P value*[2]						
	CON-CON	CON-PA	PA-CON	PA-PA		FR	PK	FR* PK	Time	FR* Time	PK* Time	FR*PK* Time
Selected Individual FA, g/d												
C4:0	61.1	67.2	59.8	64.4	3.18	0.56	0.10	0.85	<0.01	0.16	0.03	0.65
C6:0	40.1	41.5	39.9	39.5	1.92	0.45	0.61	0.85	0.48	0.17	0.02	0.62
C8:0	23.7	23.3	23.0	22.2	1.12	0.46	0.61	0.87	0.52	0.21	0.02	0.67
C10:0	57.9	54.1	56.5	52.2	2.88	0.63	0.23	0.93	0.01	0.19	0.02	0.68
C12:0	63.9	58.1	62.6	57.4	3.22	0.79	0.16	0.94	<0.01	0.21	0.02	0.74
C14:0	202	189	196	190	9.13	0.8	0.32	0.72	<0.01	0.21	0.05	0.44
C16:0	558	675	543	693	28.8	0.95	0.01	0.58	0.85	0.33	0.25	0.85
cis-9 C16:1	29.5	34.9	29.7	34.6	2.28	0.99	0.07	0.93	<0.01	0.98	0.61	0.75
C18:0	221	221	231	203	11.8	0.76	0.31	0.32	<0.01	0.64	0.29	0.83
cis-9 C18:1	382	416	405	404	16.1	0.73	0.46	0.07	<0.01	0.69	0.68	0.96
cis-11 C18:1	14.9	16.5	15.3	16.4	1.01	0.91	0.29	0.83	<0.01	0.92	0.99	0.91
trans-6 to 8 C18:1	4.96	5.29	4.91	5.45	0.17	0.81	0.02	0.56	0.01	0.21	0.48	0.52
trans-9 C18:1	3.34	3.45	3.38	3.61	0.12	0.53	0.31	0.75	0.23	0.42	0.02	0.67
trans-10 C18:1	8.11	12.0	8.73	13.1	1.46	0.57	0.01	0.87	0.13	0.72	0.81	0.65
trans-11 C18:1	17.2	17.3	17.5	18.6	0.79	0.48	0.58	0.65	<0.01	0.60	0.69	0.95
cis-9, *cis*-12 C18:2	47.1	48.6	47.5	46.9	2.09	0.76	0.81	0.62	0.07	0.29	0.26	0.86
cis-9, *trans*-11 C18:2	6.11	6.33	5.93	7.1	0.31	0.48	0.09	0.26	0.02	0.47	0.74	0.75
cis-9, *cis*-12, *cis*-15 C18:3	7.66	7.47	7.61	7.3	0.36	0.78	0.49	0.89	0.42	0.38	0.22	0.62

[1] 1) CON-CON: cows that received control diet for both fresh (FR) and peak (PK) periods; 2) CON-PA: cows that received control diet during FR and changed to PA diet (1.5% of C16:0-enriched fatty acid supplement replacing soyhulls) during PK period; 3) PA-CON: cows that received PA diet during FR and changed to CON diet during PK period; and 4) PA-PA: cows that received PA diet for both fresh (FR) and peak (PK) periods.

[2] P values refer to the ANOVA results for the main effects of treatments cows received during FR period, PK period, time, and their interactions.

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CHAPTER 6

EFFECTS OF TIMING OF PALMITIC ACID SUPPLEMENTATION ON NUTRIENT DIGESTIBILITY, ENERGY BALANCE AND METABOLISM OF EARLY LACTATION DAIRY COWS

Abstract

The objective of our study was to evaluate the effects of timing of palmitic acid (C16:0) supplementation on nutrient digestibility, energy intake and balance, and metabolic responses of early lactation dairy cows. Fifty-two multiparous cows were used in a randomized complete block design experiment and assigned to either a control diet containing no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving to 24 DIM (fresh period-FR) or from 25 to 67 DIM (peak period-PK). During the FR, PA compared with CON increased DM digestibility by 3.0% units and NDF digestibility by 4.4% units, and the increase of these variables was consistent over time. Although PA did not affect 18-carbon FA digestibility, it decreased 16-carbon FA digestibility by 10.8% units and total FA digestibility by 4.7% units compared with CON. We observed a tendency for an interaction between treatment and time for total FA digestibility, and 16-carbon FA digestibility, due to the difference in FA digestibility between PA and CON reducing over time. PA compared with CON increased digestible energy intake by 3.90 Mcal/d, ME intake by 3.50 Mcal/d, and NE_L intake by 2.70 Mcal/d. Compared with CON, PA increased milk energy output, the negative energy balance, and plasma NEFA concentration, while reduced plasma insulin. We also observed a tendency for an interaction between treatment and time for energy balance, due to cows receiving PA treatment were in a greater negative energy balance over time. During PK, PA compared with CON increased DM digestibility by 2.9% units and NDF digestibility by 3.5% units. Although PA decreased 16-carbon FA digestibility by 7.0% units compared with CON, PA did not affect

18-carbon FA digestibility, and total FA digestibility. A tendency for interaction between diets fed during FR and PK periods was observed for DM digestibility because feeding PA during PK tended to increase DM digestibility to a greater extent in cows that received the PA diet during FR. Interesting, feeding PA during PK only reduced total FA digestibility and 16-carbon FA digestibility in cows that received the CON diet during FR. Feeding PA during PK increased energy intake and milk energy output, but did not impact energy balance. In conclusion, feeding a C16:0 supplement to early lactation cows consistently increased DM and NDF digestibilities and energy intake compared with a non-fat control diet. Our results suggest that feeding C16:0 during early lactation increased milk energy output and reduced plasma insulin concentration, but also increased negative energy balance and plasma NEFA when fed in the FR period.

Introduction

The onset of lactation is a critical phase for health, fertility, and productivity of dairy cows (Zebeli et al., 2015). Lactogenesis, uterine involution, and pronounced changes in endocrine function and energy balance create a unique set of challenges, that trigger major adaptive changes in the metabolic function of dairy cows (Bradford et al., 2015). The major nutritional challenge is to meet the increasing requirements for energy and key nutrients while feed intake is limited. As part of the metabolic adaptive mechanisms, body fat reserves are mobilized around parturition because reduced plasma insulin concentration (Zachut et al., 2013), low insulin sensitivity of extra-hepatic tissues (Bell, 1995), and increased catecholamine-stimulated lipolysis (Contreras et al., 2017). Increased mobilization of body reserves results in increased plasma NEFA concentration (González et al., 2011). Since the control of feed intake is likely dominated by hepatic oxidation of NEFA during the immediate postpartum period (Allen et al., 2009), the increased NEFA supply to the liver can further decrease energy intake, thereby

increasing negative energy balance (Allen and Piantoni, 2013). Additionally, elevated plasma NEFA concentrations can also alter immune function and may increase the risk and severity of both metabolic and infectious diseases (Sordillo et al., 2009; Sordillo, 2016). Since nutrient status plays a critical role during early-lactation, nutrition-based management strategies to increase energy intake and minimize negative energy balance are required.

Several nutritional strategies have been suggested to improve the adaptation of the dairy cow around parturition. To support energy demands at parturition and decrease mobilization of body reserves, diets with high energy density could be used (Piantoni et al., 2015a,b). Grummer (1993) hypothesized that dietary fat could contribute to reducing fatty acid (FA) mobilization and spare glucose by decreasing the NADPH needed for mammary FA synthesis. Limited research is available and results are inconsistent regarding the effects of FA supplementation to early lactation cows on energy balance and metabolism. Beam and Butler (1998) reported that a saturated free FA supplement (C16:0 + C18:0) at 2.6% of diet DM during the first 6 wks postpartum had no effect on predicted NE_L intake, energy balance, and plasma concentration of insulin and NEFA when compared with a control diet with no supplemental fat. In contrast, Piantoni et al. (2015b) observed that feeding a saturated FA supplement (C16:0 + C18:0) at 2.0% of diet DM during the first 4 wks postpartum interacted with forage NDF level on the response of dairy cows; when the FA supplement was fed in a low forage diet (20% of forage NDF) it increased energy intake and energy balance and reduced body fat mobilization at the expense of milk yield.

Recently, considerable research has focused on palmitic acid (**C16:0**) and results have indicated that feeding C16:0 supplements in post-peak cows increased energy intake, NDF digestibility, and energy partitioning to milk, with no effect on BW when compared with non-fat

control diets (Piantoni et al., 2013; de Souza et al., 2017; de Souza et al., 2018). However, to our knowledge our research, and work by others, has been conducted only evaluating production and metabolic responses of C16:0 to post-peak cows (> 75 DIM). This raises a question regarding the response of early lactation cows to C16:0 supplements and when these supplements should be fed. Therefore, the objective of our study was to evaluate the effects of timing of C16:0 supplementation on nutrient digestibility, energy intake and balance, and metabolic responses of early lactation dairy cows. We hypothesized that feeding a C16:0-enriched supplement will increase energy intake and milk energy output in early lactation cows, and we postulated that the production and metabolic responses to supplemental fat would be greater if the supplementation starts after three weeks of parturition.

Materials And Methods

This article is the second article from an experiment that evaluated the effects of timing of C16:0 supplementation on performance and metabolism of early lactation cows. This chapter elaborates on the effect of these diets on nutrient digestibility, energy intake, energy balance, and plasma metabolites and hormones. The companion article (chapter 5) describe treatments effects on DMI, yield of milk and milk components, and milk FA profile.

Design and treatment diets

Fifty-two multiparous Holstein cows from the Michigan State University Dairy Field Laboratory were used in a randomized complete block design experiment with a 2x2 factorial arrangement of treatments. Cows were blocked by BCS (up to 0.50 unit difference using the 1=thin, 5=fat scale in 0.25 increments), previous lactation 305-ME (within 1,500 kg), and parity (up to 1 lactation difference). 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. Cows were assigned to either a control diet containing no supplemental fat (CON) or a 1.5% diet DM C16:0-supplemented diet (PA; Palmit 80, 85.1% of C16:0, 2.7 of C18:0, 8.9 of cis-9 C18:1, and 99.0% of total FA; Global Agri Trade Corporation, CA, USA) that was fed either from calving [1 to 24 DIM; fresh period (FR)] or after 3 weeks from calving [25 to 67 d; peak period (PK)]. Treatment diets were mixed daily in a tumble-mixer and were fed from the morning following parturition. FR diets contained 24% forage NDF, 23.5% starch, and 17.5% CP. PK diets contained 21% forage NDF, 27.4% starch, and 16.8% CP. 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 chapter (Chapter 5).

Data and Sample Collection

All samples and body measurements were collected or recorded on the same day of the week during the entire experiment, so all collection days are ±3 d. All data (milk yield, feed offered and refused, BW, and BCS) were recorded and samples (milk, feces, feed ingredients, and orts) collected and stored as described in our companion chapter (chapter 5).

On d 5, 12, 19, 33, 47 and 61 postpartum, 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. Blood samples were collected weekly by venipuncture of coccygeal vessels within 1 h before feeding on d 5, 12, 19, 33, 47 and 61 postpartum. 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 × *g* for 15 min immediately after sample collection, and plasma was harvested and stored at −20°C until analysis.

Sample Analysis

Diet ingredients, orts, and fecal samples were dried at 55°C in a forced-air oven for 72 h for DM determination. Dried samples were ground with a Wiley mill (1 mm-screen; Arthur H. Thomas, Philadelphia, PA). Feed ingredients, orts, and feces were analyzed for NDF, CP, starch and FA concentration as described by Boerman et al. (2017). Gross energy was assayed by bomb calorimeter (Parr Instrument Inc., Moline, IL). Indigestible NDF was used as an internal marker to estimate fecal output to determine the apparent total-tract digestibility of nutrients (Cochran et al., 1986). Indigestible NDF was estimated as NDF after a 240-h in vitro fermentation (Goering and Van Soest, 1970). All plasma samples were determined using an Olympus AU640e chemistry analyzer (Olympus America, Center Valley, PA) at the Diagnostic Center for Population and Animal Health of Michigan State University (Lansing, MI).

Energy intakes and balance were calculated using equations (NRC, 2001) according to Harvatine and Allen (2006). Digestible energy (DE) intake = gross energy (GE) intake × GE digestibility; NE_L intake was calculated from DE according to NRC (2001). Milk energy output (Mcal/d) was calculated according to NRC (2001) as: milk energy output (Mcal/d) = [9.29 × fat (kg) + 5.63 × true protein (kg) + 3.95 × lactose (kg)], where each component was based on the average output of a cow during the week. Energy for maintenance (Mcal/d) as 0.08 Mcal/kg × BW (kg) $^{0.75}$ (NRC, 2001). Energy balance (Mcal/d) = NE_L intake (Mcal/d) − milk NE_L (Mcal/d) − NE_L maintenance requirement (Mcal/d).

Statistical Analysis

Data were analyzed separately for FR (from 1 to 24 d postpartum) and for PK (from 25 to 67 d postpartum). All weekly data were analyzed using the MIXED procedure of SAS v.9.2 (SAS Institute, Inc. Cary, NC) with repeated measures.

For FR period, the model used included:

$Y_{ijklm} = \mu + B_i + C(B_iF_k)_j + F_k + T_l + J_m + F_kT_l + e_{ijklm}$

Where μ = overall mean B_i = random effect of block, $C(B_jK_kS_l)_j$ = random effect of cow within block and treatment diet, F_k = fixed effect of treatment during the fresh period, T_m = fixed effect of time, J_n = random effect of Julian date, e_{ijklm} = residual error.

For PK period, the model used included:

$Y_{ijklmn} = \mu + B_i + C(B_iF_kL_l)_j + F_k + L_l + F_kL_l + T_m + J_n + F_kT_m + L_lT_m + F_kL_lT_m + e_{ijklmn}$

Where μ = overall mean B_i = random effect of block, $C(B_jK_kS_l)_j$ = random effect of cow within block and treatment diet, F_k = fixed effect of treatment during the fresh period, L_l = fixed effect of treatment during the peak period, T_m = fixed effect of time, J_n = random effect of Julian date, e_{ijklmn} = residual error.

Unless otherwise specified, first-order autoregressive was the covariate structure used for analysis because it resulted in the lowest BIC for most of the variables measured. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals vs. 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-ME ($P = 0.79$), BW ($P = 0.84$), or BCS ($P = 0.43$) pre-calving. Two cows (one CON and one PA diets) had a displaced abomasum and underwent surgery and were excluded from the statistical analyses.

Results

Nutrient Digestibility During FR

During the FR period, we did not observe treatment differences for NDF intake (P = 0.25; Table 6.1). Compared with CON, PA increased the intakes of total FA by 317 g/d (P < 0.01), 16-carbon FA by 271 g/d (P < 0.01), and 18-carbon FA by 55 g/d (P < 0.01). PA increased DM digestibility by 3.0% units (P < 0.01) and NDF digestibility by 4.4% units (P < 0.01), and the increase of these variables was consistent over time (Figure 6.1). Although PA did not affect 18-carbon FA digestibility (P = 0.35), PA decreased 16-carbon FA digestibility by 10.8% units (P < 0.01) and total FA digestibility by 4.7% units (P < 0.01). PA increased absorbed total FA by 215 g/d (P < 0.01), absorbed 16-carbon FA by 157g/d (P < 0.01), and absorbed 18-carbon FA by 53/d (P < 0.01) compared with CON.

We observed a tendency for an interaction between treatment and time for total FA digestibility (P = 0.15), and 16-carbon FA digestibility (P = 0.14), due to the difference in FA digestibility between PA and CON reducing over time (Figure 6.2). Also, we observed a tendency for an interaction between treatment and time for absorbed total FA (P = 0.15), and an interaction between treatment and time for absorbed 16-carbon (P < 0.01), due to PA increasing absorbed FA to a greater extent over time (Figure 6.3).

Nutrient Digestibility During PK

During the PK period, the effect of diet fed during FR period was not significant for the variables related to nutrient digestibility and FA absorption (P > 0.10; Table 6.2).

We observed a tendency for PA decrease NDF intake by 0.45 kg/d (P = 0.07; Table 6.2) and increased DM digestibility by 2.9% units (P < 0.01) and NDF digestibility by 3.5% units (P = 0.01) compared with CON. Feeding PA during PK increased the intakes of total FA by 440 g/d

($P < 0.01$), 16-carbon FA by 359 g/d ($P < 0.01$), and 18-carbon FA by 66 g/d ($P < 0.01$) compared with CON. Although PA decreased 16-carbon FA digestibility by 7.0% units ($P < 0.01$), PA did not affect 18-carbon FA digestibility ($P = 0.31$), and total FA digestibility ($P = 0.22$) compared with CON. PA increased absorbed total FA by 288 g/d ($P < 0.01$), absorbed 16-carbon FA by 200 g/d ($P < 0.01$), and tended to increase absorbed 18-carbon FA by 68/d ($P = 0.10$).

The interaction between diet fed during FR and PK was also not significant for most of variables evaluated ($P > 0.15$). In contrast, a tendency for FR by PK diet interaction was observed for DM digestibility ($P = 0.13$) because feeding PA during PK tended to increase DM digestibility to a greater extent in cows that received the PA diet during FR (Figure 6.1). Interestingly, feeding PA during PK only reduced total FA digestibility (interaction; $P = 0.03$) and 16-carbon FA digestibility (interaction; $P = 0.05$) in cows that received CON diet during FR (Figure 6.2).

Energy intake and Energy Balance During FR

During the FR period, PA increased digestible energy (DE) intake by 3.90 Mcal/d (Table 6.3; $P = 0.05$), ME intake by 3.50 Mcal/d ($P = 0.05$), and NE_L intake by 2.50 Mcal/d ($P = 0.02$) compared with CON. The greater DE intake for PA compared with CON was consistent over time (Figure 6.4). Although PA did not affect energy for maintenance ($P = 0.33$), PA increased milk energy output by 3.70 Mcal/d ($P < 0.01$) and increased negative energy balance -1.30 Mcal/d ($P = 0.05$) compared with CON. The greater milk energy output for PA was consistent over time (Figure 6.4). There were no treatments differences for the efficiency of energy utilization for milk (NE_L milk/DE; $P = 0.46$) or for the efficiency of energy utilization for production [(NE_L milk+ NE_L maintenance)/DE; $P = 0.75$]. There were no interactions between

treatment and time for most of the variables evaluated ($P > 0.15$). However, we observed a tendency for an interaction between treatment and time for energy balance ($P = 0.15$), due to cows receiving PA treatment being in a greater negative energy balance over time (Figure 6.4).

Energy intake and Energy Balance During PK

During the PK period, the effect of diet fed during FR period was not significant for the variables related to energy intake and balance ($P > 0.10$; Table 6.4).

During the PK period, PA increased DE intake by 4.90 Mcal/d ($P = 0.05$; Table 6.4), ME intake by 4.70 Mcal/d ($P = 0.03$), and NE_L intake by 3.40 Mcal/d ($P = 0.02$) compared with CON. The greater DE intake for PA was consistent over time (Figure 6.4). Although PA did not affect energy for maintenance ($P = 0.56$) and energy balance ($P = 0.19$), PA increased milk energy output by 3.50 Mcal/d ($P < 0.01$). The greater milk energy output for PA was consistent over time (Figure 6.4). Additionally, both PA and CON treatments increased energy balance over time and entered in a positive balance by wk 7 (Figure 6.4). Compared with CON, PA increased the efficiency of energy utilization for milk (NE_L milk/DE; $P = 0.04$). There was not treatment differences for the efficiency of energy utilization for production [(NE_L milk+ NE_L maintenance)/DE; $P = 0.17$].

The interaction between diet fed during FR and PK was not significant for the variables related to energy intake and balance ($P > 0.15$).

Plasma Insulin and Metabolites During FR

During the FR period, we did not observe treatment differences for plasma glucose ($P = 0.18$; Table 6.5) or BHB concentration ($P = 0.15$). Compared with CON, PA increased plasma NEFA ($P = 0.03$) and cholesterol ($P = 0.03$), and reduced plasma insulin ($P = 0.05$). There were no treatment differences for albumin ($P = 0.87$), and Ca ($P = 0.94$). We observed an interaction

between treatment and time for BHB ($P = 0.10$) due to the difference in BHB between PA and CON reducing over time (Figure 6.5).

Plasma Insulin and Metabolites During PK

During the PK period, we only evaluated plasma NEFA, insulin, and BHB and the effect of diet fed during FR was not significant for these variables ($P > 0.10$; Table 6.6). Feeding PA during PK decreased plasma insulin ($P = 0.01$) and tended to decrease BHB ($P = 0.10$) compared with CON. There was no effect of treatment on NEFA ($P = 0.41$). We observed a tendency for an interaction between diet fed during FR and PK for BHB ($P = 0.15$) due to feeding PA during PK reducing plasma BHB only in cows that received PA in FR (Figure 6.5). Additionally, we observed a tendency for an interaction between diet fed at FR and PK for NEFA ($P = 0.13$) due to feeding PA during PK increasing plasma NEFA in cows that received CON in FR and this was more pronounced at wk 5 ($P = 0.10$; interaction FR×PK×time; Figure 6.5).

Discussion

During the weeks following parturition, the increased nutrient demands for milk production require homeorhetic adaptations to support both the increased energy demands of the mammary gland and peripheral tissue metabolism (Bauman and Currie, 1980). Several postpartum metabolic disorders are the result of insufficient energy intake in the period immediately surrounding parturition (MacCarthy et al., 2015). Therefore, to support energy demands at parturition and decrease mobilization of body reserves, feeding supplemental fat may be a strategy to increase energy intake and reduce negative energy balance, but inconsistency in responses to supplemental have been observed (e.g. Sklan et al., 1994; Moallem et al., 2007; Piantoni et al., 2015a,b). This may be associated with the FA profile of supplemental fat and timing when supplementation starts. To our knowledge there is scarce information about the

effects of individual FA on performance and metabolism of early lactation cows. In our study, we evaluated the effects of feeding a C16:0 supplement on nutrient digestibility and metabolic responses of early lactation cows, while production responses are presented elsewhere (chapter 5).

To our knowledge, few studies have evaluated FA digestibility in early lactation cows. During the FR period, we observed that PA decreased the digestibility of 16-carbon and total FA; however, the difference between treatment on these variables reduced over time. Due to these differences in FA digestibility, we observed that absorbed 16-carbon and total FA increased over time with PA supplementation. Bines et al. (1978) fed increasing levels of tallow in the first 13-wks of lactation and observed a quadratic response in total FA digestibility measured at wks 10-12 of lactation. With grazing cows, Batistel et al. (2017) reported that FA digestibility increased when cows received Ca-salts of palm FA from 3 to 16 wks of lactation compared with a non-fat control diet, but no differences over time were reported. With post-peak cows, although Rico et al. (2014) reported that feeding a C16:0 supplement had positive effects on 16-carbon and total FA digestibilities of low-producing cows, other studies with high-producing cows have observed reductions in FA digestibility when feeding similar supplements (de Souza et al., 2017; Rico et al., 2017a). In a recent meta-analysis, Boerman et al. (2015a) observed no reduction in FA digestibility when the duodenal flow of C16:0 increased up to 500 g/d, whereas increasing the duodenal flow of C18:0 to the same level reduced FA digestibility. In our study, the intake of 16-carbon FA was lower than 500 g/d for the PA treatments and PA treatments reduced FA digestibility. While the exact mechanisms for the reduction in FA digestibility as FA intake increases are unknown, potential causes have been suggested and include competition for absorption sites, and limitation in emulsification (Drackley, 2000). Additionally, in our study,

reasons for the treatment differences over time may include increased intake and flow of other FA to the duodenum since feed intake increased over time. While total flow of FA at the duodenum impacts FA digestibility (Boerman et al., 2015a), the profile of FA entering the duodenum is a critical factor affecting FA digestibility (Doreau and Chilliard, 1997; de Souza et al,. 2018). Along these lines, unsaturated FA not only have higher digestibility compared with SFA (Boerman et al., 2015a), but also they can increase the solubility of SFA facilitating transfer to micelles (Freeman, 1969), and uptake and re-esterification in enterocytes (Ockner et al., 1972). It is important to point out that the first measurement of digestibility during FR period was done for both treatments when cows were with 5 DIM, so that it is possible that cows were not totally adapted to diets and in a steady-state. Importantly, we also observed that feeding PA during PK period only reduced total FA digestibility and 16-carbon FA digestibility in cows that received the CON diet during FR period. This may suggest some adaptive mechanism in the digestion and absorption of FA in the intestine when FA supplements are fed long-term. Since most studies measuring digestibility in dairy cows have been conducted in short-term studies, further studies are needed to confirm these results and to determine the potential mechanisms associated with changes in FA absorption over time.

When fed at typical inclusion rates (≤3% of diet DM), fat supplements minimally influence the digestibility of large aggregated fractions, such as DM digestibility, even when the digestibility of total FA differs markedly (Grummer, 1988; Weiss and Wyatt, 2004). However, if the supplement has effects on fiber digestion, DM digestibility can be affected (Simas et al., 1998). In our study, the PA treatments consistently increased both NDF and DM digestibility compared with CON in both FR and PK periods. Piantoni et al. (2015a) observed that feeding a saturated FA supplement (C16:0 + C18:0) increased NDF digestibility by 4.0% units in a low

forage NDF diet, but had no effect in a high forage NDF diet during the immediate postpartum period (1-29 DIM). However, a saturated FA supplement had no effect on NDF digestibility when fed to cows across different forage NDF contents in the postpartum period and in early lactation (Jerred et al., 1990). With post-peak cows, previous studies have reported that feeding C16:0 supplement increased NDF digestibility compared with non-fat control diets (Piantoni et al., 2013; de Souza et al., 2017) and to other FA supplements (de Souza et al., 2018). This increase in NDF digestibility may be associated with an increase in retention time driven by an increase in CCK secretion (Piantoni et al., 2013). Alternatively, bacteria typically synthesize C16:0 de novo in order to produce phosphatidic acid, the precursor for FA components in membranes of *Butyrivibrio* bacteria (Hackmann and Firkins, 2015). However, if dietary C16:0 could be incorporated into rumen bacterial membranes, considerable ATP would be spared which may favor bacterial growth (Vlaeminck et al., 2006), potentially increasing NDF digestibility. Therefore, the results of our study with early-lactation cows agree with previous findings in post-peak cows indicating a positive effect of feeding C16:0 on fiber digestibility, while the exact mechanism still needs to be determined.

PA treatments increased energy intake including DE, ME and NE_L during both FR and PK periods when compared with CON. Additionally, we did not observe interactions with timing of supplementation indicating a consistent increase in energy intake when supplementing C16:0 over time. Previous studies feeding FA supplements around parturition have reported inconsistent results regarding energy intake. Piantoni et al. (2015b) reported that cows in the immediate postpartum period (1-29 DIM) had increased energy intake (+ 4.2 Mcal of NE_L/d) when fed with a saturated FA supplement (C16:0 + C18:0) regardless of dietary forage NDF level partially due to the high DMI in FA-supplemented diets (+1.4 Kg DMI/d). Similarly, Weiss

and Pinos-Rodríguez (2009) reported an interaction among FA supplementation, dietary forage NDF content, and time in early lactation cows (21 to 126 DIM): diets supplemented with a saturated FA supplement (C16:0 + C18:0) increased predicted NE_L intake in both high and low forage NDF diets before cows reached peak milk, but lower forage NDF diets increased predicted NE_L intake after peak lactation regardless of fat supplementation. In contrast, Moallem et al. (2007) fed a saturated FA supplement during the pre- and postpartum periods and reported that the supplement did not affect predicted energy intake compared with a diet with no supplemental fat. Consistent with these results, Beam and Butler (1998) showed that feeding a saturated FA supplement (C16:0 + C18:0) did not affect predicted energy intake during the first 6 wk of lactation. Reasons for these inconsistent results may include different methods to calculate or predict energy intake, duration of the supplementation period and the FA profile of supplemental fat. In the studies discussed above, energy intake was predicted from the diet and not actually measured as in Piantoni et al. (2015b) and in our study. In our study, predicting NE_L from dietary composition during the FR period using the NRC model suggested an increase in NE_L of 1 Mcal/d for PA treatments, while the actual calculated NE_L was 2.5 Mcal/d greater for the PA treatments. Considering the high variability in nutrient digestibility among cows (Piantoni et al., 2013) and the potential effect that individual FA may have on the digestibility of other fractions, using energy concentrations predicted from dietary composition is inadequate to calculate energy intake and energy balance (Piantoni et al., 2015b).

We observed that PA increased milk energy output consistently during FR and PK periods. This is associated with PA increasing ECM in both FR and PK periods (chapter 5). Similarly, previous studies with post-peak cows have observed that C16:0 supplementation increased ECM and milk energy output (Lock et al., 2013; de Souza et al., 2018). Additionally,

although PA increased energy intake (+2.5 Mcal/d of NE_L), due to the large increase in milk energy output (+3.7 Mcal/d) we observed that PA increased negative energy balance during FR period compared with CON (average of -1.3 Mcal/d). Previous studies feeding FA supplements to early lactation cows have observed inconsistent response regarding energy balance. Piantoni et al. (2015b) reported an increase in energy balance (+5.2 Mcal/d) mainly due to a higher energy intake (+4.2 Mcal/d of NE_L) when feeding a saturated FA supplement during the immediate postpartum period regardless of dietary forage NDF content, while milk energy output was not affected by treatments. Conversely, Beam and Butler (1998) reported that FA supplementation did not affect predicted energy balance compared with a control diet in the first 4 wks after parturition. In our study, we did not observe treatment effects on energy balance during PK period even though we observed similar increases in milk energy output (+3.50 Mcal/d) and energy intake (+3.40 Mcal/d of NE_L) due to PA supplementation. Also, we did observe an increase in efficiency of utilization of DE to NE_L in milk with PA compared with CON during PK. These results support our hypothesis that the timing of supplementation is important when feeding a FA supplement to early lactation cows.

In general, dairy cows are expected to have enhanced lipolysis during the immediate postpartum period as part of the normal homeorhetic regulation of their metabolism. In our study, the effect of PA on energy balance was consistent with a tendency for higher plasma NEFA concentrations and lower insulin levels during FR period, which suggests increased lipolysis and fat mobilization. Consistent with our blood markers results, we also observed that PA induced greater BW loss when fed during the FR period (chapter 5). In contrast, most of our short-term studies feeding C16:0 supplements to post-peak cows (fed at 1.5 to 2.0% diet DM) have indicated no changes in BW and BCS compared with non-fat control diets (Piantoni et al.,

2013; Lock et al., 2013; de Souza et al., 2017). Also, previous studies with saturated FA supplements have indicated that supplementation had no effect (Beam and Butler, 1998), increased (Moallem et al., 2007), or decreased (Piantoni et al., 2015b) plasma NEFA concentrations during early lactation. Although lipolysis ensures an adequate supply of energy around parturition, when lipolysis is intense and protracted, it predisposes cows to inflammatory and metabolic diseases by limiting the capacity of adipose tissue for energy buffering and contributing to chronically increased plasma NEFA (Bradford et al., 2015; Contreras et al., 2017). NEFA concentrations in serum greater than 0.7 mmol/L in the immediate postpartum period have been described as a risk factor for the development of clinical diseases postpartum (i.e., clinical ketosis, metritis, displaced abomasum), impaired reproduction in the subsequent breeding period, and early culling from the herd (Ospina et al., 2013; Ribeiro et al., 2013). Therefore, even though PA increased plasma NEFA concentration in our study, NEFA levels were below the threshold considered critical for increased incidence of metabolic disorders.

Additionally, we observed that PA only increased BHB levels during the first wk after calving compared with CON. Plasma BHB levels of 10 to 14 mg/dL have been suggested as cut off points for increased risk of metabolic disorders (Ospina et al., 2013; Overton et al., 2017). Furthermore, Ospina et al. (2010) reported that cows with BHB concentrations >10 mg/dL between 3 and 14 d postpartum had >4 times higher risk of postpartum diseases (e.g., displaced abomasum, metritis, clinical ketosis) and were >15% less likely to be pregnant after the voluntary waiting period (by 70 d). During the PK period, we did not observe treatment differences for NEFA, while PA decreased insulin compared with CON. With post peak cows, Mathews et al. (2016) observed a decrease in glucose-stimulated NEFA disappearance in cows fed C16:0, suggesting the possibility of localized adipose tissue insulin resistance with prolonged

C16:0 supplementation. Feeding C16:0 in early lactation rapidly increased circulating ceramide, especially C24:0-ceramide (Davis et al., 2017), which is inversely associated with glucose clearance rates following an insulin challenge postpartum (Rico et al., 2017b). Since we observe decreased plasma insulin concentration at both FR and PK periods due to C16:0 supplementation, it is possible to postulate that the effects of C16:0 driving energy partitioning towards milk at the expense of body reserves in early lactation are at least in part associated with its effect on insulin production or sensitivity. Altogether, our blood metabolites results agree with our energy calculations and performance outcomes. While we observed that PA supplementation increased negative energy balance and markers of lipolysis, further studies are needed to evaluate the potential effects of greater BW and BCS losses on health and reproduction of dairy cows.

Conclusion

In conclusion, feeding a C16:0 supplement to early lactation cows consistently increased DM and NDF digestibilities and energy intake compared with a non-fat control diet. For FA digestibility, the effect of feeding C16:0 were affected by the timing of supplementation since feeding PA during PK only reduced total FA digestibility and 16-carbon FA digestibility in cows that received the CON diet during FR. Our results suggest that feeding C16:0 during early lactation increased milk energy output, reduced insulin concentration, and only increased negative energy balance and plasma NEFA when fed at the immediate postpartum period.

APPENDIX

APPENDIX

Table 6.1. Nutrient intake and total-tract nutrient digestibility for cows fed treatment diets during the fresh period (d 1 to 24 postpartum).

	Treatment (Trt)[1]		SEM	*P value*[2]		
Variable	CON	PA		Trt	Time	Trt x Time
Intake, kg/d						
DM	22.3	22.1	0.62	0.92	<0.01	0.91
NDF	6.89	6.57	0.21	0.25	<0.01	0.59
Intake, g/d						
Total FA	675	992	24.2	<0.01	<0.01	0.24
16-carbon	126	396	8.52	<0.01	<0.01	0.18
18-carbon	515	570	15.5	0.01	<0.01	0.49
Digestibility, %						
DM	63.5	66.5	0.28	<0.01	<0.01	0.59
NDF	35.7	40.1	0.58	<0.01	0.16	0.58
Total FA	83.4	78.7	0.83	<0.01	0.82	0.15
16-carbon	78.2	67.4	1.19	<0.01	0.31	0.14
18-carbon	86.6	87.5	0.68	0.35	0.12	0.39
Absorbed FA, g/d						
Total FA	563	778	19.5	<0.01	<0.01	0.15
16-carbon	98.6	266	6.65	<0.01	<0.01	0.01
18-carbon	445	498	13.1	<0.01	<0.01	0.29

[1] Diets fed during the fresh period (FR; 1 to 24 DIM) were either CON (control diet) or PA (1.5% of C16:0-enriched fatty acid supplement replacing soyhulls).
[2] P values refer to the ANOVA results for the main effects of treatment (Trt), time, and its interactions.

Table 6.2. Nutrient intake and total-tract nutrient digestibility for cows fed treatment diets during the peak period (d 25 to 67 postpartum).

	Treatment[1]				SEM	*P value*[2]						
	CON-CON	CON-PA	PA-CON	PA-PA		FR	PK	FR x PK	Time	FR x Time	PK x Time	FR x PK x Time
Intake, kg/d												
DM	30.4	30.8	29.1	29.6	0.82	0.38	0.68	0.75	<0.01	0.42	0.79	0.21
NDF	9.38	9.09	9.55	8.95	0.25	0.98	0.07	0.53	<0.01	0.49	0.73	0.51
Intake, g/d												
Total FA	1113	1576	1138	1554	39.5	0.98	<0.01	0.54	<0.01	0.74	0.29	0.18
16-carbon	213	578	220	572	15.7	0.97	<0.01	0.64	<0.01	0.82	0.03	0.31
18-carbon	868	950	884	934	25.5	0.99	<0.01	0.51	<0.01	0.63	0.64	0.15
Digestibility, %												
DM	60.4	62.7	59.9	63.4	0.51	0.85	<0.01	0.13	0.14	0.58	0.21	0.88
NDF	41.4	44.2	39.5	43.4	1.15	0.49	0.01	0.37	0.57	0.77	0.31	0.85
Total FA	77.3	72.7	72.8	72.3	1.91	0.21	0.02	0.03	<0.01	0.18	0.22	0.93
16-carbon	73.4	62.4	69.8	66.6	2.44	0.15	<0.01	0.05	0.42	0.29	0.27	0.85
18-carbon	79.9	80.0	75.9	79.8	1.92	0.27	0.31	0.32	<0.01	0.18	0.81	0.82
Absorbed FA, g/d												
Total FA	813	1130	878	1137	39.5	0.32	<0.01	0.42	0.05	0.03	0.31	0.45
16-carbon	150	355	167	363	15.1	0.35	<0.01	0.74	0.04	0.65	0.21	0.85
18-carbon	660	751	706	750	27.3	0.39	0.10	0.35	0.02	0.44	0.59	0.19

[1] 1) CON-CON: cows that received control diet for both fresh (FR) and peak (PK) periods; 2) CON-PA: cows that received control diet during FR and changed to PA diet (1.5% of C16:0-enriched fatty acid supplement replacing soyhulls) during PK period; 3) PA-CON: cows that received PA diet during FR and changed to CON diet during PK period; and 4) PA-PA: cows that received PA diet for both FR and PK periods.

[2] P values refer to the ANOVA results for the main effects of treatments cows received during FR period, PK period, time, and their interactions.

Table 6.3. Energy intake, energy output and balance, and efficiency of energy utilization for cows fed treatment diets during the fresh period (FR, d 1 to 24 postpartum).

Variable	Treatment (Trt)[1]		SEM	*P value*[2]		
	CON	PA		Trt	Time	Trt x Time
Energy intake, Mcal/d						
DE[3]	62.9	66.8	1.55	0.05	<0.01	0.66
ME[4]	53.8	57.2	1.59	0.05	<0.01	0.71
NE_L[5]	33.7	36.2	0.85	0.02	<0.01	0.72
Energy output, Mcal/d						
Milk[6]	35.6	39.3	1.26	<0.01	0.01	0.41
Maintenance[7]	11.0	10.9	0.18	0.33	<0.01	0.38
Energy Balance, Mcal/d[8]	-12.9	-14.2	0.55	0.03	<0.01	0.15
Efficiency						
NE_L milk/ DE	0.565	0.580	0.03	0.46	<0.01	0.25
NE_L production[9]/ DE	0.742	0.744	0.04	0.75	<0.01	0.46

[1] Diets fed during the fresh period (FR; 1 to 24 DIM) were either CON (control diet) or PA (1.5% of C16:0-enriched fatty acid supplement replacing soyhulls).
[2] P values refer to the ANOVA results for the main effects of treatment (Trt), time, and its interactions.
[3]Digestible energy intake = gross energy intake (Mcal/d) × gross energy digestibility.
[4]Metabolizable energy intake = was calculated from DE according to NRC (2001).
[5]Net energy of lactation intake was calculated from DE through ME according to NRC (2001).
[6]Milk NE_L (Mcal/d) = milk yield (kg/d) × [(fat % × 0.0929) + (true protein % × 0.0563) + (lactose % × 0.0395)] (NRC, 2001).
[7]NE_L maintenance (Mcal/d) = 0.08 Mcal/kg × BW (kg) 0.75 (NRC, 2001).
[8]Energy balance (Mcal/d) = NE_L intake (Mcal/d) − milk NE_L (Mcal/d) − NE_L maintenance requirement (Mcal/d).
[9]NE_L production = milk NE_L + NE_L required for maintenance.

Table 6.4. Energy intake, energy output and balance, and efficiency of energy utilization for cows fed treatment diets during the peak period (PK, d 25 to 67 postpartum).

	Treatment[1]				SEM	*P value*[2]						
	CON-CON	CON-PA	PA-CON	PA-PA		FR	PK	FR x PK	Time	FR x Time	PK x Time	FR x PK x Time
Energy intake, Mcal/d												
DE[3]	87.2	92.4	87.2	92.8	2.69	0.91	0.05	0.92	<0.01	0.35	0.36	0.28
ME[4]	74.4	79.6	74.2	79.8	2.56	0.89	0.03	0.98	<0.01	0.36	0.33	0.34
NE_L[5]	46.5	50.0	46.4	50.6	1.63	0.88	0.01	0.97	<0.01	0.35	0.31	0.38
Energy output, Mcal/d												
Milk[6]	36.5	39.8	36.4	40.1	1.73	0.97	0.03	0.94	0.12	0.19	0.81	0.82
Maintenance[7]	11.0	10.8	10.7	10.9	0.21	0.41	0.56	0.73	0.41	0.77	0.63	0.18
Energy Balance, Mcal/d[8]	-1.00	-0.80	-0.70	-1.10	0.48	0.84	0.19	0.81	<0.01	0.64	0.77	0.33
Efficiency												
NE_L milk/ DE	0.419	0.429	0.417	0.437	0.01	0.65	0.04	0.93	<0.01	0.75	0.63	0.32
NE_L production[9]/ DE	0.545	0.545	0.540	0.552	0.02	0.91	0.17	0.92	<0.01	0.79	0.58	0.27

[1] 1) CON-CON: cows that received control diet for both fresh (FR) and peak (PK) periods; 2) CON-PA: cows that received control diet during FR and changed to PA diet (1.5% of C16:0-enriched fatty acid supplement replacing soyhulls) during PK period; 3) PA-CON: cows that received PA diet during FR and changed to CON diet during PK period; and 4) PA-PA: cows that received PA diet for both FR and PK periods.

[2] P values refer to the ANOVA results for the main effects of treatments cows received during FR period, PK period, time, and their interactions.

[3]Digestible energy intake = gross energy intake (Mcal/d) × gross energy digestibility.

[4]Metabolizable energy intake = was calculated from DE according to NRC (2001).

[5]Net energy of lactation intake was calculated from DE through ME according to NRC (2001).

[6]Milk NE_L (Mcal/d) = milk yield (kg/d) × [(fat % × 0.0929) + (true protein % × 0.0563) + (lactose % × 0.0395)] (NRC, 2001).

[7]NE_L maintenance (Mcal/d) = 0.08 Mcal/kg × BW (kg) 0.75 (NRC, 2001).

[8]Energy balance (Mcal/d) = NE_L intake (Mcal/d) − milk NE_L (Mcal/d) − NE_L maintenance requirement (Mcal/d).

[9]NE_L production = milk NE_L + NE_L required for maintenance.

Table 6.5. Blood metabolites for cows fed treatment diets during the fresh period (FR, d 1 to 24 postpartum).

	Treatment (Trt)[1]		SEM	*P value*[2]		
Variable	CON	PA		Trt	Time	Trt xTime
Glucose, mg/dL	50.4	45.3	3.27	0.18	<0.01	0.24
NEFA, mEq/L	0.59	0.65	0.02	0.03	<0.01	0.33
BHB, mg/dL	12.4	13.6	1.75	0.15	<0.01	0.10
Insulin, ug/L	0.24	0.21	0.01	0.05	<0.01	0.67
Albumin, g/dL	3.04	3.02	0.15	0.87	<0.01	0.45
Cholesterol, mg/dL	79.5	89.0	4.29	0.03	<0.01	0.43
Ca, mg/dL	8.9	9.0	0.32	0.94	<0.01	0.80

[1] Diets fed during the fresh period (FR; 1 to 24 DIM) were either CON (control diet) or PA (1.5% of C16:0-enriched fatty acid supplement replacing soyhulls).
[2] P values refer to the ANOVA results for the main effects of treatment (Trt), time, and its interactions.

Table 6.6. Blood metabolites for cows fed treatment diets during the peak period (PK, d 25 to 67 postpartum).

	Treatment[1]				SEM	*P value*[2]						
	CON-CON	CON-PA	PA-CON	PA-PA		FR	PK	FR x PK	Time	FR x Time	PK x Time	FR x PK x Time
NEFA, mEq/L	0.30	0.39	0.33	0.31	0.03	0.46	0.41	0.13	<0.01	0.94	0.82	0.10
BHB, mg/dL	6.14	6.05	6.39	5.18	1.05	0.53	0.10	0.15	<0.01	0.63	0.76	0.25
Insulin, ug/L	0.33	0.23	0.31	0.27	0.02	0.73	0.01	0.22	<0.01	0.37	0.78	0.16

[1] 1) CON-CON: cows that received control diet for both fresh (FR) and peak (PK) periods; 2) CON-PA: cows that received control diet during FR and changed to PA diet (1.5% of C16:0-enriched fatty acid supplement replacing soyhulls) during PK period; 3) PA-CON: cows that received PA diet during FR and changed to CON diet during PK period; and 4) PA-PA: cows that received PA diet for both FR and PK periods.

[2] P values refer to the ANOVA results for the main effects of treatments cows received during FR period, PK period, time, and their interactions.

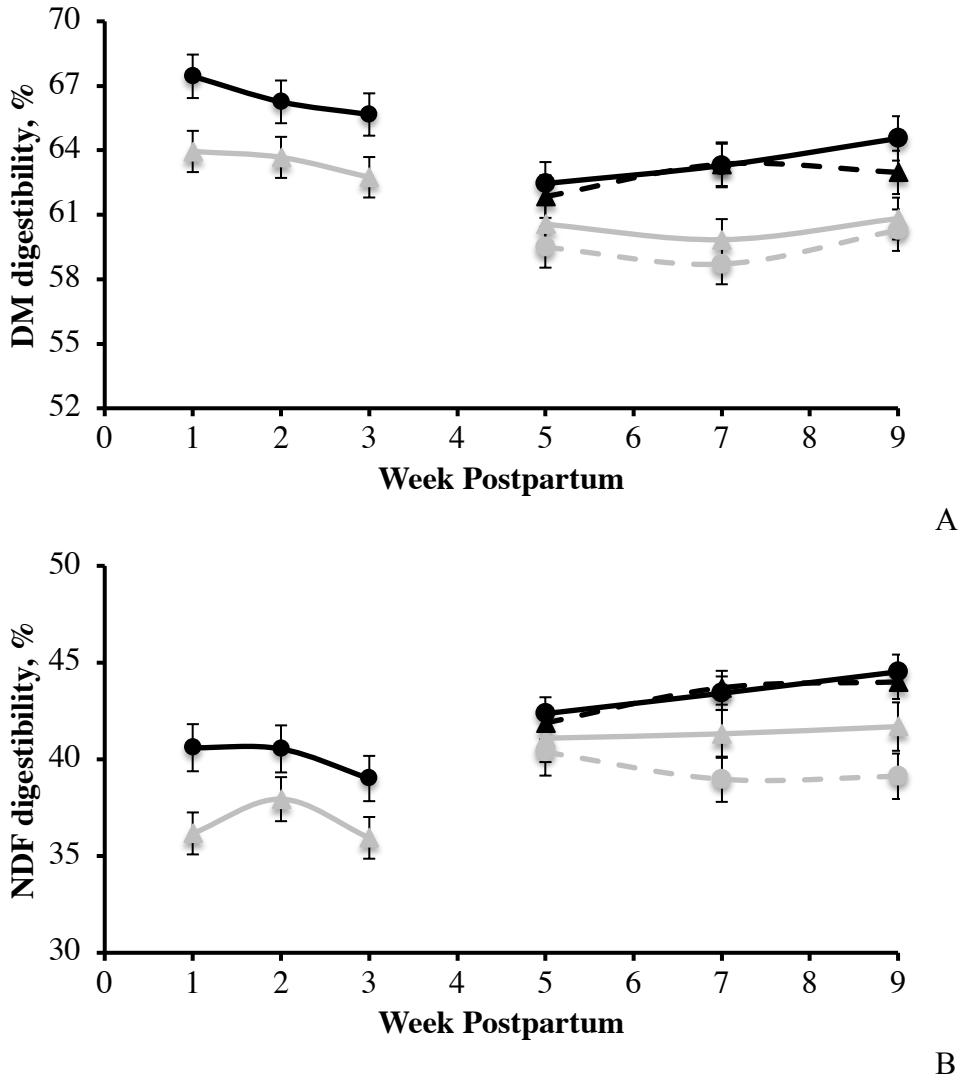


Figure 6.1. Effects of dietary treatments on DM digestibility (A), and NDF digestibility (B) over time during the fresh (1-3 weeks) and peak (4-9 weeks) periods.
Diets fed during the fresh period were either CON (control diet; black line) or PA (1.5% of C16:0-enriched supplement; grey line). During the peak period treatments were: CON-CON: cows that received CON for both fresh (FR) and peak (PK) periods (grey line); 2) CON-PA: cows that received CON during FR and changed to PA diet (1.5% of C16:0-enriched supplement) during PK period (black broke line); 3) PA-CON: cows that received PA diet during FR and changed to CON diet during PK period (grey broke line); and 4) PA-PA: cows that received PA diet for FR and PK periods (black line). During FR and PK, PA increased digestibilities of DM ($P < 0.01$) and NDF ($P < 0.01$) compared with CON. DM digestibility decreased over time in both treatments (all $P < 0.01$) and we did not observe treatment by time interaction for both variables during FR period. During PK, a tendency for FR by PK diet interaction was observed for DM digestibility because feeding PA during PK tended to increase DM digestibility to a greater extent in cows that received the PA diet during FR. Error bars indicate SEM.

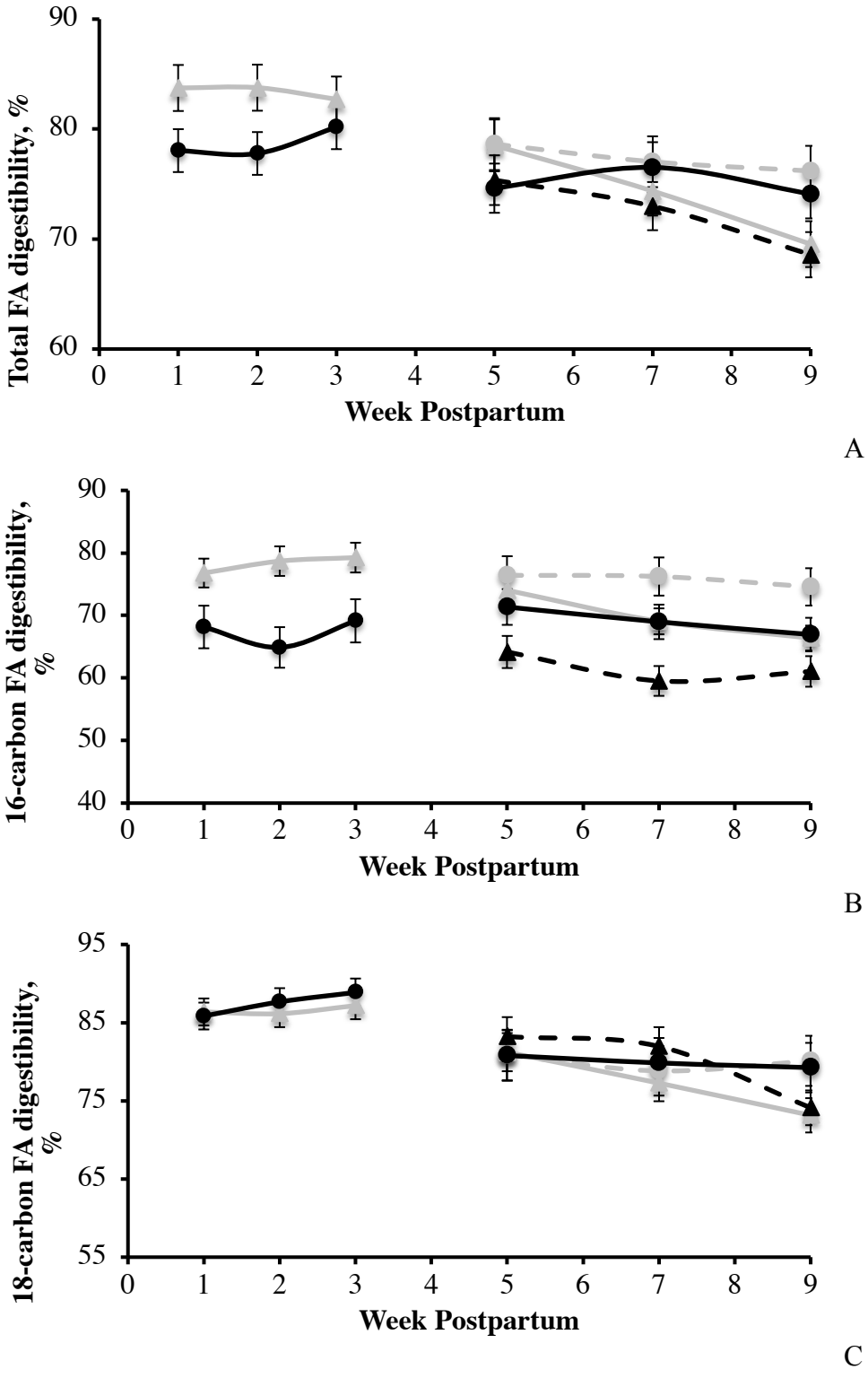
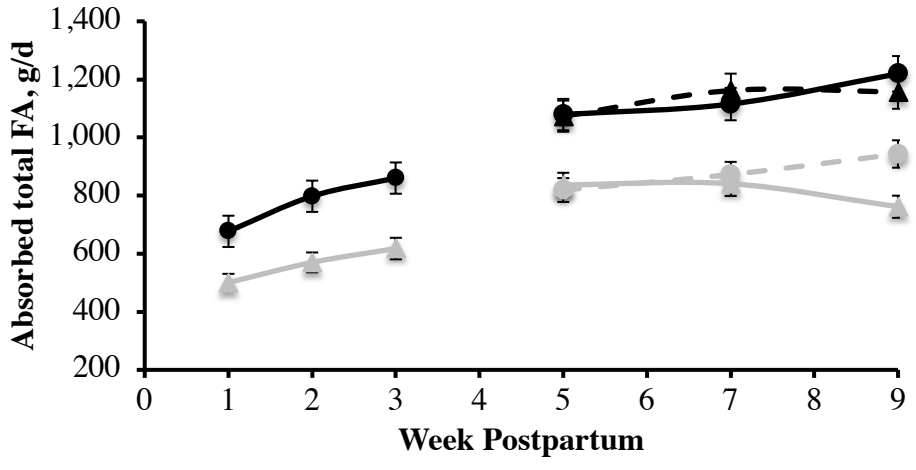


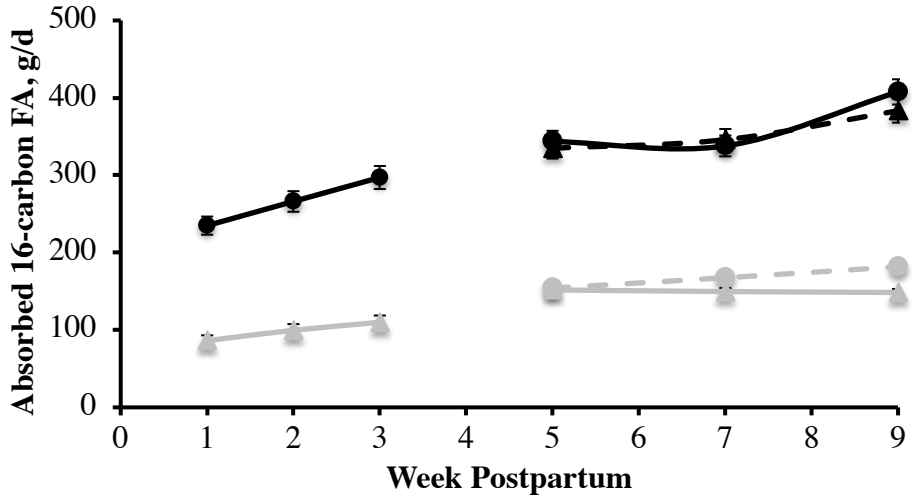
Figure 6.2. Effects of dietary treatments on total FA digestibility (A), 16-carbon FA digestibility (B), and 18-carbon FA digestibility (C) over time during the fresh (1-3 weeks) and peak (4-9 weeks) periods.

Diets fed during the fresh period were either CON (control diet; black line) or PA (1.5% of

C16:0-enriched supplement; grey line). During the peak period treatments were: CON-CON: cows that received CON for both fresh (FR) and peak (PK) periods (grey line); 2) CON-PA: cows that received CON during FR and changed to PA diet (1.5% of C16:0-enriched supplement) during PK period (black broke line); 3) PA-CON: cows that received PA diet during FR and changed to CON diet during PK period (grey broke line); and 4) PA-PA: cows that received PA diet for FR and PK periods (black line). During FR, PA decreased digestibilities of total FA (P <0.01) and 16-carbon FA (P < 0.01) compared with CON. We observed a tendency for an interaction between treatment and time for total FA digestibility (P = 0.15), and 16-carbon FA digestibility (P = 0.14), due to the difference in FA digestibility between PA and CON reduced over time. During PK, PA decreased 16-carbon FA digestibility (P < 0.01) compared with CON. Also, we observed FR by PK diet interactions for 16-carbon (P = 0.05) and total FA digestibility (P = 0.03) because feeding PA during PK only reduced total FA digestibility and 16-carbon FA digestibility in cows that received the CON diet during FR. Error bars indicate SEM.



A



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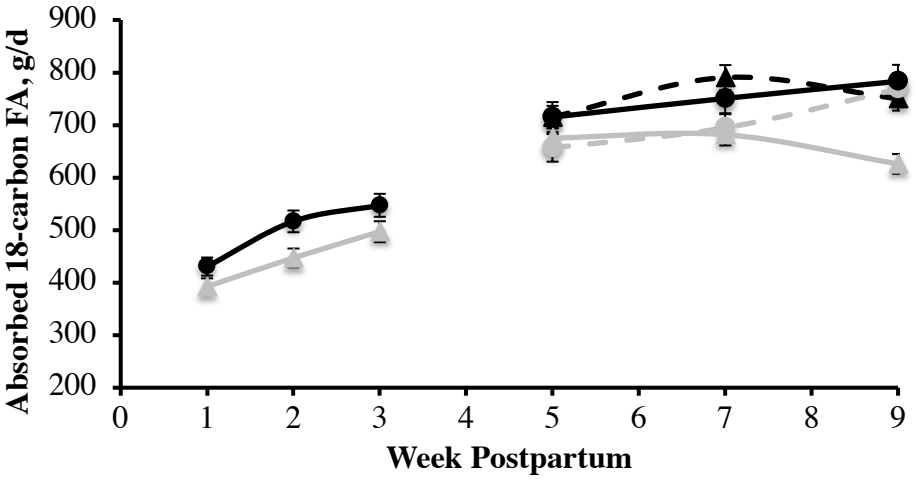
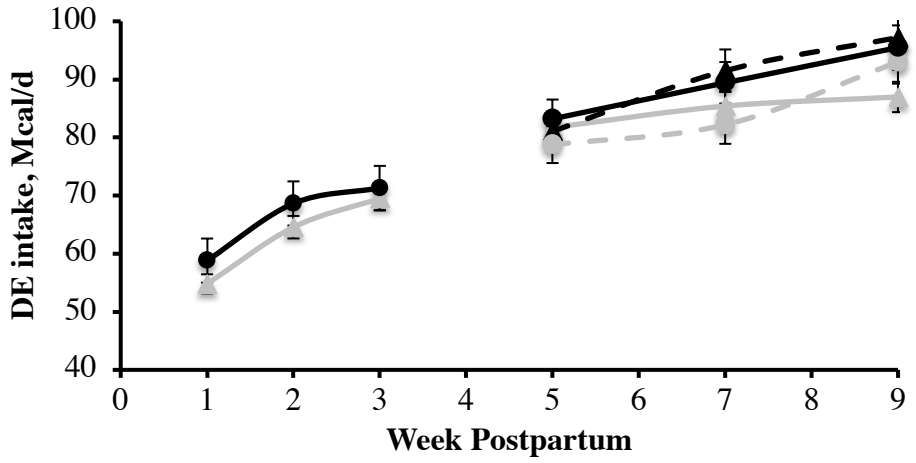


C

Figure 6.3. Effects of dietary treatments on absorbed total FA (A), 16-carbon FA (B), and 18-carbon FA (C) over time during the fresh (1-3 weeks) and peak (4-9 weeks) periods. Diets fed during the fresh period were either CON (control diet; black line) or PA (1.5% of C16:0-enriched supplement; grey line). During the peak period treatments were: CON-CON:

cows that received CON for both fresh (FR) and peak (PK) periods (grey line); 2) CON-PA: cows that received CON during FR and changed to PA diet (1.5% of C16:0-enriched supplement) during PK period (black broke line); 3) PA-CON: cows that received PA diet during FR and changed to CON diet during PK period (grey broke line); and 4) PA-PA: cows that received PA diet for FR and PK periods (black line). During FR, PA increased (all $P < 0.01$) absorbed total FA, 16-carbon FA, and 18-carbon FA compared with CON. We observed a tendency for an interaction between treatment and time for absorbed total FA ($P = 0.15$), and an interaction between treatment and time for absorbed 16-carbon ($P < 0.01$), due to PA increasing absorbed FA to a greater extent over time. During PK, PA increased absorbed 16-carbon FA and total FA (both $P < 0.01$) and tended to increase absorbed 18-carbon FA ($P = 0.10$) compared with CON. Error bars indicate SEM.



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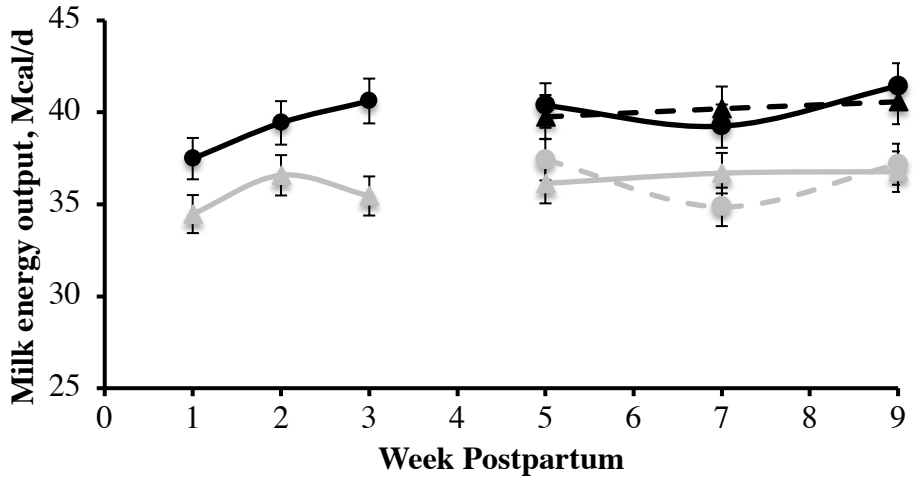

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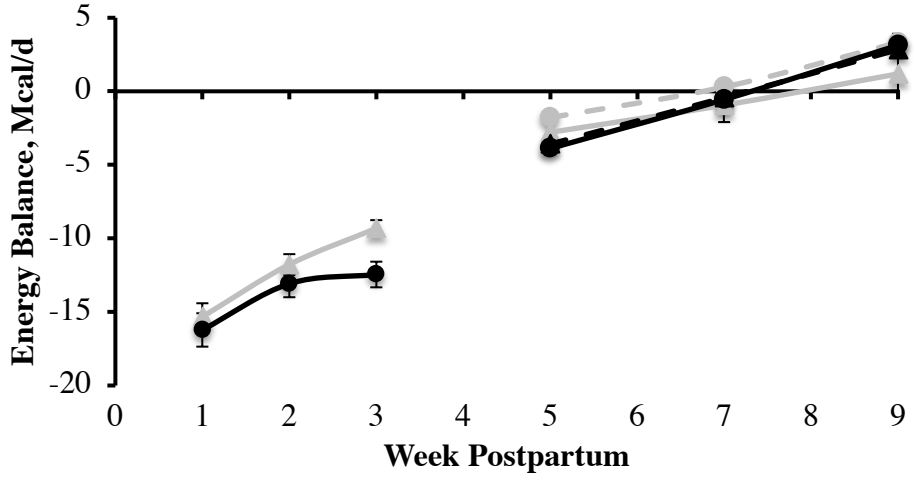
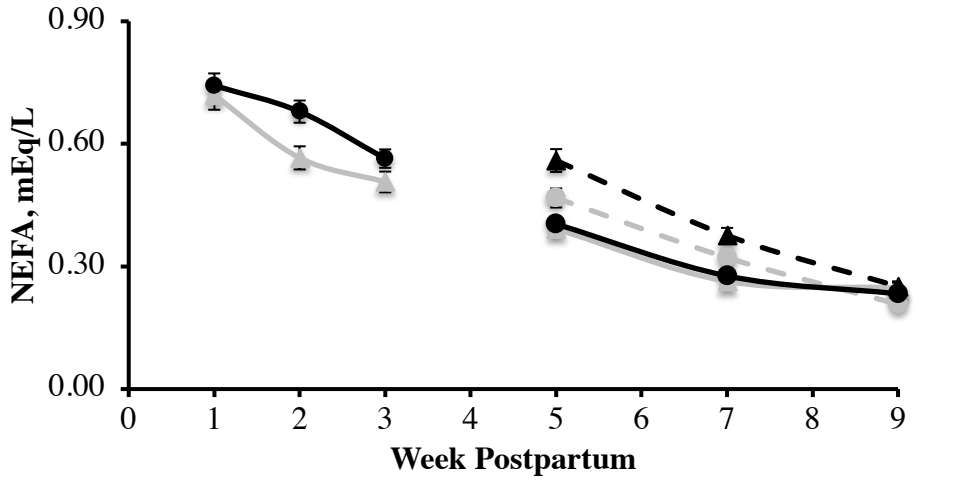


C

Figure 6.4. Effects of dietary treatments on digestible energy (DE) intake (A), milk energy output (B), and energy balance (C) over time during the fresh (1-3 weeks) and peak (4-9 weeks) periods.

Diets fed during the fresh period were either CON (control diet; black line) or PA (1.5% of

C16:0-enriched supplement; grey line). During the peak period treatments were: CON-CON: cows that received CON for both fresh (FR) and peak (PK) periods (grey line); 2) CON-PA: cows that received CON during FR and changed to PA diet (1.5% of C16:0-enriched supplement) during PK period (black broke line); 3) PA-CON: cows that received PA diet during FR and changed to CON diet during PK period (grey broke line); and 4) PA-PA: cows that received PA diet for FR and PK periods (black line). During FR, PA increased DE intake (P = 0.05), milk energy output ($P < 0.01$) and reduced energy balance (P = 0.05) compared with CON. We observed a tendency for an interaction between treatment and time for energy balance (P = 0.15), due to cows receiving PA treatment were in a greater negative energy balance over time. During PK, PA increased DE intake (P = 0.05) and milk energy output (P = 0.03) compared with CON. DE intake, milk energy output, and energy balance increased over time for all treatments. Error bars indicate SEM.



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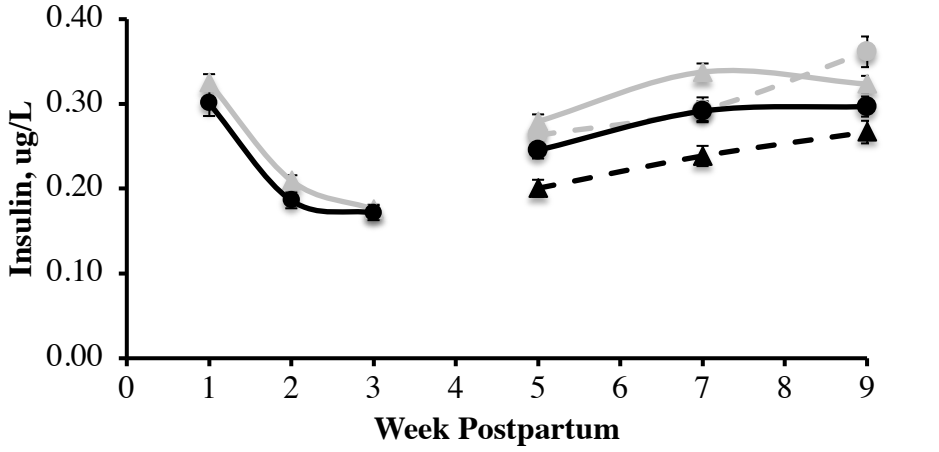

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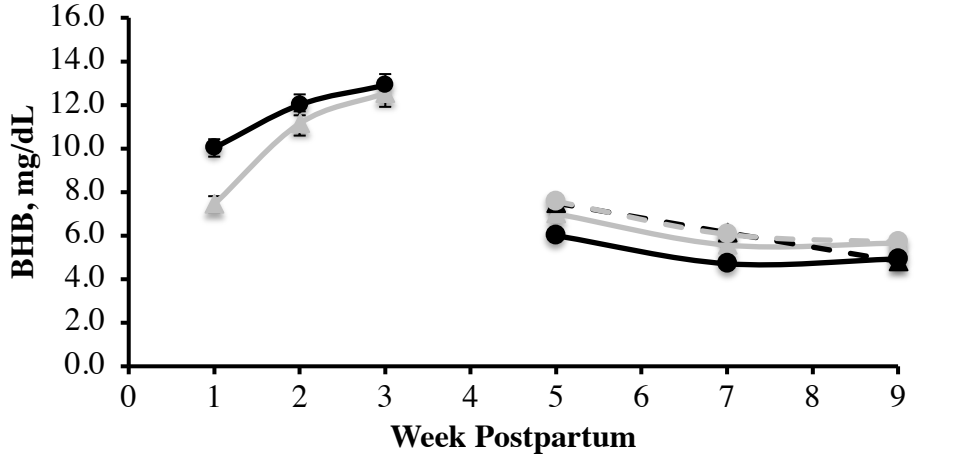

C

Figure 6.5. Effects of dietary treatments on plasma NEFA (A), BHB (B), and insulin (C) over time during the fresh (1-3 weeks) and peak (4-9 weeks) periods.
Diets fed during the fresh period were either CON (control diet; black line) or PA (1.5% of

C16:0-enriched supplement; grey line). During the peak period treatments were: CON-CON: cows that received CON for both fresh (FR) and peak (PK) periods (grey line); 2) CON-PA: cows that received CON during FR and changed to PA diet (1.5% of C16:0-enriched supplement) during PK period (black broke line); 3) PA-CON: cows that received PA diet during FR and changed to CON diet during PK period (grey broke line); and 4) PA-PA: cows that received PA diet for FR and PK periods (black line). During FR, PA increased plasma NEFA ($P = 0.03$) and reduced plasma insulin ($P = 0.05$) compared with CON. We observed an interaction between treatment and time for BHB ($P = 0.10$) due to the difference in BHB between PA and CON reduced over time. During PK, feeding PA during PK decreased plasma insulin ($P = 0.01$) and tended to decrease BHB ($P = 0.10$) compared with CON. We observed a tendency for an interaction between diet fed at FR and PK and time for NEFA ($P = 0.13$) due to feeding PA during PK increased plasma NEFA on cows that received CON in FR and this was more pronounced at wk 5. Error bars indicate SEM.

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CHAPTER 7

ALTERING THE RATIO OF DIETARY PALMITIC AND OLEIC ACIDS INTERACTS WITH PRODUCTION LEVEL IN DAIRY COWS: EFFECTS ON PRODUCTION RESPONSES AND ENERGY PARTITIONING

Abstract

The objective of this study was to evaluate the effects the effects of altering the dietary ratio of palmitic (C16:0) and oleic (*cis*-9 C18:1) acids associated with production level on nutrient digestibility, energy partitioning, and production response of lactating dairy cows. Cows were blocked by milk yield and assigned to three groups (12 cows per group) in a main plot. Production groups were: a) low (45.2±1.7 kg/d); b) medium (53.0±1.6 kg/d); and c) high (60.0±1.9 kg/d). Within each production group, a truncated Latin square arrangement of fatty acids (FA) treatments was used in two consecutive 35-d periods. The FA treatments supplemented at 1.5% diet DM were: 1) 80:10 (80% C16:0 + 10% *cis*-9 C18:1); 2) 73:17 (73% C16:0 + 17% *cis*-9 C18:1); 3) 66:24 (66% C16:0 + 24% *cis*-9 C18:1); and 4) 60:30 (60% C16:0 + 30% *cis*-9 C18:1). Treatment by production group interactions were observed for milk yield, FCM, ECM, milk fat yield, milk protein yield, milk lactose yield, and energy partitioned to milk. Increasing *cis*-9 C18:1 in FA treatments reduced FCM, ECM, and milk energy output in low producing cows, but increased these in high producing cows. Increasing *cis*-9 C18:1 in FA treatments did not impact milk yield, milk protein yield, and milk lactose yield in low and medium producing cows, but increased these in high producing cows. Regardless of production level, there was no effect of treatments on DMI; however, increasing *cis*-9 C18:1 in FA treatments increased BW change, BCS change, and energy partitioned to body reserves. Increasing *cis*-9 C18:1 in FA treatments increased total FA digestibility due to a linear increase in 16 and 18-carbon FA digestibilities. Overall, increasing *cis*-9 C18:1 in supplemental fat

linearly decreased mixed FA, while increased preformed milk FA and did not change milk fat yield. However, interactions between FA treatments and production level was observed for the yield of milk fat and source of milk FA. In low producing cows, increasing *cis*-9 C18:1 in supplemental fat decreased milk fat yield due to a decrease in de novo and mixed milk FA without changes in preformed milk FA. In contrast, in high producing cows, increasing *cis*-9 C18:1 in supplemental fat increased milk fat yield due to an increase in de novo and preformed milk FA. Our results indicated that high producing dairy cows (averaging 60 kg/d) responded better to fat supplements containing more *cis*-9 C18:1, while low producing cows (averaging 45 kg/d) responded better to supplements containing more C16:0.

Introduction

The addition of supplemental fat sources to diets is a common practice in dairy nutrition to increase dietary energy density and to support milk production and recovery of body condition. Although in general fat supplementation has been shown to increase milk yield, the efficiency of milk production, and reproductive performance, great variation has been reported for different fat types, and indeed the same supplement across different diets and studies (Rabiee et al., 2012; Rodney et al., 2015). Understanding the effects of different fatty acid (FA) sources on milk production and energy partitioning is crucial, and attention has lately been given to determining the effects of specific individual FA. However, to our knowledge, few studies were designed to evaluate the effects of different FA ratios on the production responses of dairy cows. Recently, we observed that feeding a FA blend with high content of C16:0 (80% C16:0) increased milk energy output and energy partitioning towards milk, while feeding a FA blend with a combination of C16:0 and *cis*-9 C18:1 (45% C16:0 and 35% *cis*-9 C18:1) increased energy allocated to BW and the partitioning of energy to BW compared with non-fat control

diets (de Souza et al., 2018). These results suggest that C16:0 and *cis*-9 C18:1 are able to alter nutrient partitioning between the mammary gland and adipose tissue, which may allow for different FA supplements to be used in different situations according to the metabolic priority of dairy cows and management needs. Unfortunately, in the study of de Souza et al. (2018) only one combination of C16:0 and *cis*-9 C18:1 was evaluated and therefore, determining the impact of other dietary ratios among these FA is of particular importance.

Additionally, reasons for variability across experiments to different FA supplements could be associated with physiological state of cows. Production level is well established as a potential factor that interacts with nutrition impacting production responses of dairy cows (e.g. Harvatine and Allen, 2005; Piantoni et al., 2015). Palmquist and Jenkins (1980) reported that low producing cows did not respond to fat supplementation compared with high producing cows in their feeding trials. Interestingly, supplementation with C16:0 has been shown to increase milk fat yield regardless of level of milk production (Piantoni et al., 2013, Rico et al., 2014). In contrast, DMI and milk production increased with C18:0 supplementation for higher-producing cows than for lower-producing cows (Piantoni et al., 2015). Although the reasons for this interaction between C18:0 and production level were not determined by Piantoni et al. (2015), these results suggested that high producing cows may respond better to supplements containing 18-carbon FA. Although it is well established that high producing cows have greater energy demands resulting in changes in DMI and microbial fermentation, other differences in partitioning of absorbed FA between milk and body reserves are also possible.

The objective of our study was to evaluate the effects of altering the dietary ratio of C16:0 and *cis*-9 C18:1 in cows with different production level on nutrient digestibility, energy partitioning, and production responses of lactating dairy cows. We hypothesized that increasing

the amount of *cis*-9 C18:1 in supplemental fat would increase energy partitioning to body reserves, and that feeding *cis*-9 C18:1 would increase milk yield in high producing cows but not in low-producing cows.

Materials And Methods

Design and Treatments

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University. This trial was designed to test the interaction between production level and feeding fat supplements varying in the ratios of C16:0 and *cis*-9 C18:1. Third-six mid-lactation multiparous Holstein cows, from the Michigan State University Dairy Field Laboratory, were used in a split-plot truncated Latin square design. All animals received a common diet with no fat supplementation during a 14-d preliminary period to obtain baseline values. Cows were blocked by production level based on the data collected during the preliminary period (Table 7.1) and assigned to three groups in a main plot. Production groups were: a) low producing cows (milk yield = 45.2 ± 1.7 kg/d; 115 ± 42 DIM); b) medium producing cows (milk yield = 53.0 ± 1.6 kg/d; 105 ± 41 DIM); and c) high producing cows (milk yield = 60.0 ± 1.9 kg/d; 104 ± 43 DIM). Within each production level group, a 4 × 2 truncated Latin square arrangement of treatments was used in two consecutive 35-d periods. The four treatments were combinations of two commercially available FA supplements that differed in FA profile that were blended to achieve different ratios of C16:0 and *cis*-9 C18:1 in the FA supplement blends (Table 7.2). The FA treatments were: 1) 80:10 (80% C16:0 + 10% *cis*-9 C18:1); 2) 73:17 (73% C16:0 + 17% *cis*-9 C18:1); 3) 66:24 (66% C16:0 + 24% *cis*-9 C18:1); and 4) 60:30 (60% C16:0 + 30% *cis*-9 C18:1). The FA supplement blends provided 1.5% FA (% diet DM) and diets were balanced for Ca concentration (Table 7.3).

Dry matter concentration of forages was determined twice weekly and diets were adjusted when necessary. Throughout the experiment cows were housed in individual tie stalls. Access to feed was blocked daily from 0830 to 1000 h to allow for the collection of orts and offering feed. Cows were fed 115% of expected daily intake, and feed intake was recorded. Water was available ad libitum in each stall and stalls were bedded with sawdust and cleaned twice per day.

Data and Sample Collection

Samples and data for production variables, digestibility, and plasma metabolites and hormones were collected during the last 5 d of each treatment period (d 30 to 35). Samples (0.5 kg) of all diet ingredients and orts (12.5%) from each cow were collected daily and composited by period for analysis. Fecal (~400 g) and blood (~15 mL) samples were collected every 15 h during the last 5 d of each period totaling eight samples per cow per period. The 15 h interval over 5 d simulates sampling every 3 h over a 24-h period. Feces were stored in a sealed plastic cup at -20°C. Blood was stored on ice until centrifugation at 2,000 × g for 15 min at 4°C (within 30 min of sample collection). Plasma was transferred into microcentrifuge tubes and stored at −20°C until composited by cow period. Milk yield was recorded and two milk samples collected at each milking. One aliquot was collected in a sealed tube with preservative (Bronopol tablet; D&F Control Systems, San Ramon, CA) and stored at 4°C for milk component analysis. The second aliquot was stored without preservative at -20°C until analyzed for FA composition.

BW measurements were taken three times per week following the afternoon milking, and BW change was calculated according to Boerman et al. (2015b). On the last d of the preliminary period and last d of each treatment period, three trained investigators determined BCS on a 5-point scale in 0.25-point increments (Wildman et al., 1982).

Sample Analysis

Diet ingredients, orts, and fecal samples were dried at 55°C in a forced-air oven for 72 h for DM determination. Dried samples were ground with a Wiley mill (1 mm-screen; Arthur H. Thomas, Philadelphia, PA). Feed ingredients, orts, and feces were analyzed for NDF, CP, starch and FA concentration as described by Boerman et al. (2017). Indigestible NDF was determined after 240 h in vitro fermentation (Goering and Van Soest, 1970).

Milk samples were analyzed for fat, true protein, and lactose concentrations by mid-infrared spectroscopy (AOAC, 1990; method 972.160) (Universal Lab Services, Lansing, MI). Yields of 3.5% FCM, ECM, milk energy, and milk components were calculated using milk yield and component concentrations from each milking, summed for a daily total, and averaged for each collection period. Milk samples used for analysis of FA composition were composited based on milk fat yield (d 30-35 of each period). Milk lipids were extracted, and FA-methyl esters prepared and quantified using GLC according to Lock et al. (2013). Yield of individual FA (g/d) in milk fat were calculated by using milk fat yield and FA concentration to determine yield on a mass basis using the molecular weight of each FA while correcting for glycerol content and other milk lipid classes (Piantoni et al., 2013).

Energy Partitioning Calculations

We determined energy partitioning using the procedures described by Boerman et al. (2015b). Energy partitioning was determined during treatment periods using weekly milk samples taken twice a week and analyzed for fat, protein, and lactose concentrations, BW measurements 3 times per week following the afternoon milking, and BCS determined by 3 trained investigators on the last day of each period. Data were used to calculate milk energy output, metabolic BW, and body tissue gain throughout treatment periods.

Milk energy output (Mcal/d) was calculated according to NRC (2001) as: milk energy output (Mcal/d) = [9.29 × fat (kg) + 5.63 × true protein (kg) + 3.95 × lactose (kg)], where each component was based on the average output of a cow during the 35-d period. Metabolic BW (MBW) was estimated as $BW^{0.75}$, where BW was the mean BW for a cow during the 35-d period. Mean daily BW change (kg/d) was calculated for each cow within period by linear regression after 2 iterations of removing outliers. Energy partitioned to body tissue gain (Mcal/d) was estimated according to NRC (2001) as: body tissue gain (Mcal/d) = [(2.88+1.036 × BCS) × ΔBW], where BCS was the average BCS for each cow during a 35-d period. NE_L intake was calculated based on the sum of milk energy output, maintenance energy calculated from MBW, and body energy gain for each cow on each diet according to Boerman et al. (2015b).

Statistical Analysis

All data were analyzed using the mixed model procedure of SAS (Version 9.4, SAS Institute, Cary, NC) according to the following model:

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

Where Y_{ijkl} = dependent variable, μ = overall mean, F_i = fixed effect of production level ($i = 3$), $C_j (F_i)$ = random effect of cow within the main plot ($j = 1$ to 12), P_k = fixed effect of period ($k = 1$ to 2), T_l = fixed effect of FA treatment ($l = 1$ to 4), and e_{ijkl} = residual error. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals vs. predicted values. Main effects were declared significant at $P \leq 0.05$, and tendencies were declared at $0.05 < P \leq 0.10$. Interactions were declared significant at $P \leq 0.10$, and tendencies were declared at $0.10 < P \leq 0.15$. Interactions between treatment and period, and production level and period were evaluated, but removed from the statistical model when not significant ($P > 0.20$). Orthogonal contrasts were used to test the linear, quadratic and

cubic effects of *cis*-9 C18:1 inclusion for the main effect of FA treatment and the interaction between production level × FA treatments.

Results

Nutrient Intake and Total-tract Digestibility

FA treatments did not affect intakes of DM ($P = 0.68$; Table 7.4), NDF ($P = 0.66$), and total FA ($P = 0.70$). Increasing *cis*-9 C18:1 in FA treatments decreased intake of 16-carbon FA (linear, $P < 0.01$), and increased the intake of 18-carbon FA (linear, $P < 0.01$). Increasing *cis*-9 C18:1 in FA treatments increased total FA digestibility (linear, $P = 0.02$), 16-carbon FA digestibility (linear, $P < 0.01$), and 18-carbon FA digestibility (quadratic, $P = 0.05$). Increasing *cis*-9 C18:1 in FA treatments decreased absorbed 16-carbon FA (quadratic, $P = 0.03$) and increased linearly absorbed 18-carbon FA ($P < 0.01$).

The interaction between FA treatments and production level was not significant for variables related with nutrient intake and digestibility (Table 7.4).

Production Responses

Increasing *cis*-9 C18:1 in FA treatments tended to increase milk yield (linear, $P = 0.08$; Table 7.5). Although increasing *cis*-9 C18:1 in FA treatments decreased milk fat content (linear, $P < 0.01$), we did not observe treatment differences for 3.5% FCM ($P = 0.97$), ECM ($P = 0.87$), or milk fat yield ($P = 0.92$). Increasing *cis*-9 C18:1 in FA treatments increased milk lactose content (linear, $P < 0.01$) and yield (linear, $P = 0.03$) and tended to increase milk protein yield (linear, $P = 0.06$). Increasing dietary *cis*-9 C18:1 in FA treatments also increased BW change (quadratic, $P = 0.02$) and BCS change (linear, $P < 0.01$).

We observed an interaction between FA treatments and production level for milk yield ($P = 0.09$; Table 7.5) due to increasing dietary *cis*-9 C18:1 in FA treatments increasing milk yield in

high producing cows but not in low and medium producing cows (linear, $P < 0.05$; Figure 7.1A). We also observed an interaction between FA treatments and production level for 3.5% FCM ($P = 0.05$), ECM ($P = 0.05$), and fat yield ($P = 0.08$) due to increasing *cis*-9 C18:1 in FA treatments decreasing these variables in low producing cows, but increasing in high producing cows (linear, $P < 0.05$; Figure 7.1B-1D). Also, increasing *cis*-9 C18:1 in FA treatments increasing yields of milk protein and lactose in high producing cows but not in low and medium producing cows (linear interaction; $P < 0.05$; Figure 7.1E-1F).

Milk Fatty Acid Concentration and Yield

Milk FA are derived from 2 sources: < 16 carbon FA from de novo synthesis in the mammary gland and > 16 carbon FA originating from extraction from plasma. Mixed source FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis in the mammary gland and extraction from plasma. Increasing *cis*-9 C18:1 in FA treatments decreased the concentration of mixed (linear, $P < 0.01$; Table 7.6), while linearly increased the concentration of preformed (linear, $P < 0.01$) milk FA. These results were due to increasing *cis*-9 C18:1 in FA treatments decreasing the concentration of C16:0 (linear, $P < 0.01$; Table 7.9), while increasing several *trans* milk FA (linear, all $P < 0.05$) and *cis*-9 C18:1 (quadratic, $P = 0.02$). On a yield basis, increasing *cis*-9 C18:1 in FA treatments increased the yield of preformed FA (linear, $P < 0.01$) due to an increase in *cis*-9 C18:1 and several *trans* milk FA (linear, all $P < 0.05$; Table 7.10). Although we did not observe treatment differences on the yield of de novo FA, increasing *cis*-9 C18:1 in FA treatments tended to decrease mixed milk FA (linear, $P = 0.06$).

We observed an interaction between FA treatments and production level for the yield of de novo milk FA ($P = 0.10$; Table 7.6) due to increasing dietary *cis*-9 C18:1 in FA treatments decreasing de novo FA in low producing cows, while increasing it in high producing cows

(linear, $P < 0.05$; Figure 7.2A). Increasing dietary *cis*-9 C18:1 in FA treatments decreased mixed FA in low and medium producing cows, but did not affect it in high producing cows (quadratic interaction; $P = 0.10$; Figure 7.2B). Also, increasing dietary *cis*-9 C18:1 in FA treatments increased preformed FA in medium producing cows (quadratic interaction; $P = 0.01$; Figure 7.2C), while increased in high producing cows (linear; $P = 0.01$; Figure 7.2C).

Blood Metabolites

Although FA treatments did not affect plasma NEFA concentration ($P = 0.48$; Table 7.7), increasing *cis*-9 C18:1 in FA treatments increased BHB (linear, $P = 0.05$), and tended to increase insulin (linear, $P = 0.08$) concentrations. We also observed an interaction between FA treatments and production level for plasma insulin ($P = 0.06$) due to increasing dietary *cis*-9 C18:1 in FA treatments increasing insulin in low producing cows (linear, $P < 0.05$; Figure 7.3A), while increasing it in high producing cows (quadratic, $P < 0.05$; Figure 7.3A).

Calculated Energy and Energy Partitioning

Increasing *cis*-9 C18:1 in FA treatments increased NE_L intake (linear, $P = 0.01$; Table 7.8), and NE_L per kg of DM (quadratic, $P = 0.01$). Also, increasing *cis*-9 C18:1 in FA treatments increased energy output in body reserves (quadratic, $P = 0.05$) and tended to increase energy partitioned to body reserves (quadratic, $P = 0.06$). We also observed an interaction between FA treatments and production level for milk energy output ($P = 0.04$) and energy partitioned to milk ($P = 0.05$) due to increasing dietary *cis*-9 C18:1 in FA treatments reduced these variables in low producing cows, while increased them in high producing cows (linear, $P < 0.05$; Figure 7.3B and 3C).

Discussion

Post-peak cows are usually in positive energy balance and the goals are to maximize milk

and component yields to maximize milk price and income. C16:0 and *cis*-9 C18:1 are typically the most abundant FA found in commercially available FA supplements fed to dairy cows. Also, these FA normally comprise the majority of FA present in milk fat, ranging from 20 to 40, and 20 to 30 g/100 g FA for C16:0 and *cis*-9 C18:1, respectively (Jensen, 2002). Although we recently observed that altering the dietary ratio of C16:0 and *cis*-9 C18:1 may alter nutrient partitioning between the mammary gland and adipose tissue, only one combination of these FA was evaluated (de Souza et al., 2018). Therefore, our aim in the current study was to evaluate the effects of altering the dietary ratio of C16:0 and *cis*-9 C18:1 in supplemental fat on performance of dairy cows. Additionally, cow milk production level was used as a blocking factor to investigate possible treatment interactions with metabolic demand. Although production levels were similar in DIM, they differed in average milk production and milk fat concentration. Evaluating potential interaction between dietary strategies and production level is important, and this information is readily available to the dairy producer and can be easily used for grouping and feeding cows.

The effect of fat supplements on DMI is variable and usually depends on the profile of the FA supplement being fed (Allen, 2000; Rabiee et al., 2012). Harvatine and Allen (2006b) observed that DMI decreased linearly as the degree of unsaturation of supplemental fat increased. The more pronounced decrease in DMI for unsaturated FA is likely mediated in part by increased secretion of cholecystokinin (Bradford et al., 2008). In contrast, in our study we did not observe changes in DMI as we increased *cis*-9 C18:1 in FA treatments, and no interaction between FA treatments and production level was detected. de Souza et al (2018) reported that feeding a FA blend with 45% C16:0 and 35% *cis*-9 C18:1 decreased DMI compared with non-fat control diet in cottonseed basal diets, but not in the soyhulls basal diets, which is likely related to

the greater intake and duodenal flow of unsaturated FA on the cottonseed diet. He et al. (2012) did not observe effects on DMI of varying levels of C18:1 *cis*-9 (from 0.98 to 3.32% of diet DM), but DMI was linearly reduced as C18:2 *cis*-9, *cis*-12 concentration increased in the diet (from 1.51 to 3.86% of diet DM). These results may indicate that the depression in feed intake caused by supplemental fat may be related with the level of supplemental fat, potential interaction with other dietary components, and specific FA and/or degree of unsaturation. In our study, although increasing dietary *cis*-9 C18:1 in supplemental fat mainly increased *cis*-9 C18:1, C18:2 *cis*-9, *cis*-12 intake also increased. Further research is needed to determine whether a higher amount of unsaturated FA or a higher amount from a specific FA is related to the depression in feed intake.

In our study, there was no treatment differences for NDF digestibility. Similarly, de Souza et al. (2018) observed no differences in NDF digestibility when feeding a FA blend with high content of C16:0 (80% C16:0) and a FA blend with a combination of C16:0 and *cis*-9 C18:1 (45% C16:0 and 35% *cis*-9 C18:1), whereas both treatments increased NDF digestibility compared with a non-fat control diet and a diet supplemented with a FA blend with 40% C16:0 and 40% C18:0. Previous studies feeding C16:0 supplements have indicated increases in NDF digestibility compared with a non-fat control diet (de Souza et al., 2017; Rico et al., 2017). In a recent meta-analysis, Weld and Armentano (2017) observed that calcium salts of palm FA and saturated fat containing a mixture of C16:0 and C18:0 did not affect NDF digestibility.

Additionally, we also observed that increasing *cis*-9 C18:1 in FA treatments increased digestibilities of total FA, 16- and 18-carbon FA. Likewise, Harvatine and Allen (2006b) observed that total FA, 16- and 18-carbon FA digestibilities increased linearly as the degree of unsaturation of supplemental fat increased. Usually unsaturated FA have higher digestibility than

saturated FA (Boerman et al., 2015a), which may be due to the greater solubility of unsaturated FA facilitating transfer of FA to micelles (Freeman, 1969), and rapid uptake and re-esterification in enterocytes compared with saturated FA (Ockner et al., 1972). Recently, Rico et al. (2017) observed that 16-carbon FA digestibility was reduced when feeding a C16:0 supplement (85% C16:0) to a greater extent in a soyhulls basal diet compared with a whole cottonseed basal diet. While total flow of FA at the duodenum impacts FA digestibility (Boerman et al., 2015a) these findings support the hypothesis that the profile of FA entering the duodenum is a critical factor affecting FA digestibility. Furthermore, previous results have indicated that *cis*-9 C18:1 has greater digestibility than C16:0 and C18:0 (Boerman et al., 2015a) and *cis*-9 C18:1 has been suggested as having amphiphilic properties (Moate et al., 2004). This is supported by Freeman (1969) that examined the amphiphilic properties of polar lipid solutes and found that *cis*-9 C18:1 had a positive effect on the micellar solubility of C18:0. Additional research to understand the mechanisms by which *cis*-9 C18:1 may increase digestibility of other FA is required.

To our knowledge, responses to altering the dietary ratio of FA have not been previously reported using cows at different levels of production. We observed a tendency for increasing *cis*-9 C18:1 in FA treatments increasing milk yield, which was driven by the effect of FA treatments on high producing cows. In our study, higher-yielding cows responded more favorably to a FA blend containing higher content of *cis*-9 C18:1 for yields of milk and milk components compared with lower-yielding cows. Previous research has shown that the production and metabolic responses to FA supplements can differ in cows at different levels of milk production (Harvatine and Allen, 2005; Warntjes et al., 2008). A previous study has reported that C16:0 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). In contrast,

DMI and milk production increased with C18:0 supplementation for higher-producing cows than for lower-producing cows (Piantoni et al., 2015). These results suggest that high producing cows may potentially respond better to supplements containing 18-carbon FA. Although we increased dietary *cis*-9 C18:1, it is likely that this treatment increased rumen outflow of other 18-carbon FA so that it is unclear if these results are associated with an overall effect of 18-carbon FA or a specific FA. Also, Bionaz et al. (2013) indicated that FA can affect gene expression of several metabolic pathways. Gluconeogenesis in perfused liver of rats was stimulated by *cis*-9 C18:1 (Teufel et al., 1967), possibly indicating a role of this FA in mediating liver glucose metabolism. Conversely, 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 *cis*-9 C18:1 did not affect the expression of gluconeogenic enzymes, while C18:0 decreased expression of pyruvate carboxylase, a key gluconeogenic enzyme. Although results from the later experiment suggest that both C18:0 and *cis*-9 C18:1 would either not affect or decrease milk yield through a decrease in gluconeogenesis, which is contrary to our results, we cannot rule out a potential effect of these FA at liver and other tissues.

We observed an interaction between FA treatments and production level for milk energy output and energy partitioning to milk. This was due to increasing *cis*-9 C18:1 in FA treatments decreasing milk fat yield and ECM in low producing cows, but increasing these in high producing cows. de Souza et al. (2018) observed that, compared with a non-fat control diet, ECM and milk energy output increased when feeding a FA blend with high content of C16:0 (80% C16:0), but not with a FA blend with a combination of C16:0 and *cis*-9 C18:1 (45% C16:0 and 35% *cis*-9 C18:1). Previous studies have observed that C16:0 supplementation increased ECM (Piantoni et al., 2013; Lock et al., 2013; de Souza et al., 2017), and this increase was

consistent across a wide range of production level (Piantoni et al., 2013). Similar to our results, 3.5% FCM and ECM increased with C18:0 supplementation for higher-producing cows than for lower-producing cows (Piantoni et al., 2015). In contrast, Rico et al. (2014) did not observe treatment differences for ECM when evaluating the effects of a C16:0 supplement and a Ca-salts of palm FA supplement in low (average 34 kg/d) and high producing cows (average 48 kg/d). It is important to point out that in our trial, low producing cows averaged 45 kg/d, so that further research is needed to assess the effects of dietary ratio of FA in cows with a lower level of milk production. Based on our results and the ones from Piantoni et al. (2015), further studies are needed to determine whether high producing cows may respond better to supplements containing 18-carbon FA, or to a specific 18-carbon FA.

Overall, increasing *cis*-9 C18:1 in supplemental fat linearly decreased mixed FA, while increased preformed milk FA and did not change milk fat yield. However, interactions between FA treatments and production level were observed for the yield of milk fat and sources of milk FA. In low producing cows, increasing *cis*-9 C18:1 in supplemental fat decreased milk fat yield due to a decrease in de novo and mixed milk FA without changes in preformed milk FA. In contrast, in high producing cows, increasing *cis*-9 C18:1 in supplemental fat increased milk fat yield due to an increase in de novo and preformed milk FA. Hansen and Knudsen (1987) reported that C16:0 stimulated de novo FA synthesis and incorporation into triglycerides, whereas other FA (C18:0, C18:1, and C18:2) had no effect in dispersed goat mammary epithelial cells. In our study, the effect of FA treatments on milk FA profile was influenced by production level. Dorea and Armentano (2017) observed in a meta-analysis a negative relationship between dietary *cis*-9 C18:1 content and de novo milk FA yield. This substitution effect of preformed for de novo milk FA has been reported previously (He and Armentano, 2011; He et al., 2012), in

which the reduction in yield of de novo milk FA was compensated for by an increase in the yield of preformed milk FA when fat supplements were fed. In contrast, Glasser et al. (2008) suggested that a positive relationship between de novo synthesized and long-chain FA can be expected in low fat diets; however, when FA are supplemented to the diet, a simultaneous decrease in de novo FA and an increase in long-chain FA occurs, corresponding to an inverse relationship between these two FA sources (Enjalbert et al., 1998; Glasser et al., 2008). Our results indicate an interdependence between de novo and preformed in high producing cows driving milk fat yield, while in low producing cows a substitution effect seems to occur. Similar to our results, Piantoni et al. (2015) also observed that C18:0 supplementation increased milk fat yield in higher producing cows compared with lower producing cows due to an increase in both de novo and preformed milk FA.

Regardless of production level, increasing *cis*-9 C18:1 in supplemental fat increased BW and BCS change and tended to increase energy partitioning to body reserves. Although increasing dietary *cis*-9 C18:1 did not cause milk fat depression (MFD) since milk fat yield was unchanged, we observed a linear reduction in milk fat content and an increase in concentration and yield of several *trans* FA in milk fat indicating a mild MFD condition. Possibly, increasing dietary *cis*-9 C18:1 provided a higher load of unsaturated FA in the diet, which likely overcome normal rumen biohydrogenation capacity and changed biohydrogenation pathways. This is likely associated with repartitioning of energy towards body fat reserves. Recently, Urrutia and Harvatine (2017) observed reduced lipogenic capacity of adipose tissue explants without changes in gene expression of key lipogenic enzymes during 4-d of *trans*-10, *cis*-12 C18:2 infusion. In our study, we did not detect levels of *trans*-10, *cis*-12 C18:2 in milk fat for most of our samples, but it is important to consider that other FA produced as intermediates in rumen

biohydrogenation have been shown to reduce milk fat (Bauman et al., 2011) and potentially may be involved with energy partitioning. Additionally, we observed that increasing dietary *cis*-9 C18:1 increased plasma insulin. Similarly, we observed higher plasma insulin levels in post peak cows when feeding a FA blend containing 45% C16:0 and 35% *cis*-9 C18:1 compared with control and other FA-supplemented diets containing C16:0 and C18:0 (de Souza et al., 2018). Previous studies using rats as animal model have observed that free FA including *cis*-9 C18:1 may stimulate insulin secretion from pancreatic β-cells (Itoh et al., 2003; Fujiwara et al., 2005). Insulin is an antilipolytic hormone and elevated insulin concentrations may reduce lipolysis or increase lipogenesis in adipose tissue (Vernon, 2005). Therefore, the effect of *cis*-9 C18:1 on energy partitioning to body reserves could be linked to increased insulin concentrations and/or production of biohydrogenation intermediates.

Conclusion

Regardless of production level, increasing *cis*-9 C18:1 in the FA supplement did not affect DMI and increased FA digestibility, BW and BCS change. Our results indicate that higher producing dairy cows (averaging 60 kg/d) respond better to fat supplements containing more *cis*-9 C18:1, while lower producing cows (averaging 45 kg/d) respond better to supplements containing more C16:0. Increasing *cis*-9 C18:1 did not impact milk yield and milk protein yield in low and medium producing cows, but linearly increased them in high producing cows. Increasing *cis*-9 C18:1 in a FA supplement linearly reduced FCM and ECM in low producing cows, but linearly increased in high producing cows.

APPENDIX

APPENDIX

Table 7.1. Baseline data for cows used in this study, obtained during the preliminary period when cows were fed a common diet (mean ± SE).

	Production level		
Parameter	Low	Medium	High
Milk yield, kg	45.2 ± 1.7	53.0 ± 1.6	60.0 ± 1.9
Fat content, %	3.53 ± 0.18	3.43 ± 0.19	3.31 ± 0.20
Protein content, %	3.15 ± 0.15	3.12 ± 0.13	3.08 ± 0.16
Lactose content, %	4.84± 0.10	4.82± 0.09	4.80 ± 0.11
Fat yield, kg	1.59 ± 0.12	1.77 ± 0.13	1.98 ± 0.15
Protein yield, kg	1.42 ± 0.12	1.65 ± 0.18	1.84 ± 0.20
Lactose yield, kg	2.17 ± 0.23	2.55 ± 0.25	2.88 ± 0.28
BW, kg	688 ± 73	651 ± 51	700 ± 63
BCS	3.18 ± 0.38	3.17 ± 0.30	3.13 ± 0.35
DIM	115 ± 42	105 ± 41	104 ± 43

Table 7.2. Proportion of each FA supplement used in the treatment blends and FA profile of FA blends.

	Treatment[1]			
	80:10	73:17	66:24	60:30
% of each FA supplement in treatment blends				
Nutracor[2]	90.0	66.5	45.5	29.0
Nutracal[3]	10.0	33.5	54.5	71.0
FA profile of each FA blend, g/100g FA				
C14:0	0.67	0.75	0.82	0.88
C16:0	80.7	73.6	66.3	59.7
C18:0	1.83	1.79	1.75	1.70
cis-9 C18:1	10.2	17.5	23.9	29.7
cis-9, *cis*-12 C18:2	2.95	4.23	4.45	5.55
cis-9, *cis*-12, *cis*-15 C18:3	0.11	0.15	0.19	0.23

[1]Treatments were: 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 2) 73:17 (1.5% of FA supplement blend to provide ~ 73% C16:0 and 17% *cis*-9 C18:1); 3) 66:24 (1.5% of FA supplement blend to provide ~ 66% C16:0 and 24% *cis*-9 C18:1); and 4) 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1).

[2]Palmitic acid-enriched FA supplement (Nutracor; Wawasan Agrolipids, Johor, Malaysia). The supplement contained (g/100 g of fatty acid) 0.60 of C14:0, 84.5 of C16:0, 1.80 of C18:0, 7.90 of C18:1 *cis*-9, and 98.8% total fatty acids.

[3]Ca salts of palm FA supplement (Nutracal; Wawasan Agrolipids, Johor, Malaysia). The supplement contained (g/100 g of fatty acid) 1.0 of C14:0, 48.1 of C16:0, 1.12 of C18:0, 39.9 of C18:1 *cis*-9, and 83.4% total fatty acids.

Table 7.3. Ingredient and nutrient composition of treatment diets.

	Treatments[1]			
	80:10	73:17	66:24	60:30
Ingredient, % DM				
Corn Silage	25.5	25.5	25.5	25.5
Alfalfa Silage	16.3	16.3	16.3	16.3
Wheat Straw	5.32	5.32	5.32	5.32
Ground Corn	15.9	15.9	15.9	15.9
High Moisture Corn	14.2	14.2	14.2	14.2
Soybean Meal	12.1	12.1	12.1	12.1
Soyhulls	4.82	4.76	4.70	4.65
Protein supplement[2]	1.09	1.09	1.09	1.09
Nutracor[3]	1.37	1.06	0.76	0.48
Nutracal[4]	0.17	0.54	0.90	1.23
Mineral and Vitamin mix[5]	3.23	3.23	3.23	3.23
Nutrient Composition, % DM[6]				
NDF	29.0	29.0	29.0	29.0
CP	16.5	16.5	16.5	16.5
Starch	28.8	28.8	28.8	28.8
FA	4.00	3.98	4.00	3.98
16:0	1.58	1.44	1.33	1.26
18:0	0.05	0.04	0.04	0.04
cis-9 18:1	0.68	0.78	0.88	0.98
cis-9, *cis*-12 18:2	1.25	1.25	1.27	1.29
cis-9, *cis*-12, *cis*-15 18:3	0.20	0.20	0.20	0.20

[1]Treatments were: 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 2) 73:17 (1.5% of FA supplement blend to provide ~ 73% C16:0 and 17% *cis*-9 C18:1); 3) 66:24 (1.5% of FA supplement blend to provide ~ 66% C16:0 and 24% *cis*-9 C18:1); and 4) 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1).

[2] Spectrum Agriblue (Perdue Agribussiness, Salisbury, MD).

[3]Palmitic acid-enriched FA supplement (Nutracor; Wawasan Agrolipids, Johor, Malaysia). The supplement contained (g/100 g of fatty acid) 0.60 of C14:0, 84.5 of C16:0, 1.80 of C18:0, 7.90 of C18:1 *cis*-9, and 98.8% total fatty acids.

[4]Ca salts of palm FA supplement (Nutracal; Wawasan Agrolipids, Johor, Malaysia). The supplement contained (g/100 g of fatty acid) 1.0 of C14:0, 48.1 of C16:0, 1.12 of C18:0, 39.9 of C18:1 *cis*-9, and 83.4% total fatty acids.

[5]Vitamin and mineral mix contained 34.1% dry ground shelled corn, 25.6% white salt, 21.8% calcium carbonate, 9.1% Biofos (The Mosaic Co., Plymouth, MN), 3.9% magnesium oxide, 2% soybean oil, and < 1% of each of the following: manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, iodine, cobalt carbonate, vitamin E, vitamin A, vitamin D, and selenium.

[6] Expressed as percent of as fed.

Table 7.4. Nutrient intake and nutrient digestibility for cows fed treatment diets (n = 36).

Variable	Treatments[1]				SEM	P value[2]			*Contrast*[3]		
	80:10	73:17	66:24	60:30		Trt	Production	Trt x Production	Linear	Quadratic	Cubic
Intake, kg/d											
DMI	29.1	29.0	28.9	29.3	0.52	0.68	<0.01	0.89	0.82	0.88	0.89
NDF	8.43	8.42	8.40	8.35	0.16	0.66	0.01	0.92	0.64	0.85	0.98
Intake, g/d											
Total FA	1164	1148	1132	1142	23.0	0.70	0.01	0.81	0.36	0.50	0.78
16-carbon	464	425	379	370	8.92	<0.01	0.03	0.73	<0.01	0.06	0.19
18-carbon	644	661	693	721	13.4	<0.01	0.01	0.86	<0.01	0.62	0.71
Digestibility, %											
DM	66.2	66.7	67.0	67.3	0.66	0.17	0.21	0.18	0.10	0.71	0.97
NDF	40.2	39.0	40.0	40.9	0.99	0.58	0.60	0.24	0.90	0.27	0.39
Total FA	76.5	80.1	79.0	80.6	1.10	0.04	0.43	0.17	0.02	0.32	0.13
16-carbon	72.9	76.8	75.2	78.1	1.07	<0.01	0.22	0.16	<0.01	0.62	0.12
18-carbon	79.8	83.5	82.8	82.7	1.11	0.04	0.52	0.18	0.06	0.05	0.22
Absorbed, g/d											
Total FA	892	921	896	924	10.4	0.23	<0.01	0.50	0.09	0.50	0.13
16-carbon	337	326	285	287	6.98	<0.01	0.01	0.33	<0.01	0.03	0.12
18-carbon	518	551	573	595	9.63	<0.01	<0.01	0.47	<0.01	0.49	0.75

[1]Treatments were: 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 2) 73:17 (1.5% of FA supplement blend to provide ~ 73% C16:0 and 17% *cis*-9 C18:1); 3) 66:24 (1.5% of FA supplement blend to provide ~ 66% C16:0 and 24% *cis*-9 C18:1); and 4) 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1).

[2] P values associated with treatment, production level and interaction.

[3] Contrasts evaluated refers to the linear, quadratic and cubic effects of *cis*-9 C18:1 inclusion in supplemental fat.

Table 7.5. Milk yield, milk composition, BW, and BCS of cows fed treatment diets (n = 36).

Variable	Treatments (Trt)[1]				SEM	P value[2]			Contrast[3]		
	80:10	73:17	66:24	60:30		Trt	Production	Trt x Production	Linear	Quadratic	Cubic
Milk Yield, kg/d											
Milk	48.1	49.5	49.4	50.3	1.11	0.09	<0.01	0.09	0.08	0.14	0.52
3.5% FCM[4]	49.6	49.8	50.0	50.1	1.16	0.97	<0.01	0.05	0.65	0.98	0.99
ECM[5]	49.6	50.1	50.2	50.6	1.15	0.87	<0.01	0.05	0.42	0.97	0.88
Milk Composition											
Fat, kg/d	1.78	1.75	1.76	1.74	0.05	0.92	0.02	0.08	0.62	0.85	0.68
Fat, %	3.71	3.57	3.57	3.48	0.09	0.04	0.27	0.47	<0.01	0.59	0.31
Protein, kg/d	1.53	1.58	1.58	1.62	0.05	0.07	<0.01	0.03	0.06	0.91	0.51
Protein, %	3.18	3.21	3.21	3.24	0.05	0.47	0.02	0.42	0.16	0.87	0.51
Lactose, kg/d	2.15	2.24	2.25	2.30	0.06	0.05	<0.01	0.05	0.03	0.71	0.53
Lactose, %	4.47	4.54	4.55	4.57	0.03	0.05	0.05	0.08	<0.01	0.47	0.53
FCM/DMI	1.71	1.71	1.72	1.73	0.03	0.95	<0.01	0.04	0.58	0.95	0.98
BW, kg	710	705	704	709	10.2	0.25	0.06	0.66	0.74	0.05	0.65
BW change kg/d	0.50	0.84	0.96	0.84	0.09	0.01	0.74	0.61	0.01	0.02	0.97
BCS	3.31	3.36	3.38	3.35	0.05	0.63	0.14	0.13	0.46	0.29	0.95
BCS change	0.08	0.15	0.22	0.28	0.04	<0.01	0.25	0.76	<0.01	0.87	0.79

[1]Treatments were: 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 2) 73:17 (1.5% of FA supplement blend to provide ~ 73% C16:0 and 17% *cis*-9 C18:1); 3) 66:24 (1.5% of FA supplement blend to provide ~ 66% C16:0 and 24% *cis*-9 C18:1); and 4) 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1).

[2] P values associated with treatment, production level and interaction.

[3] Contrasts evaluated refers to the linear, quadratic and cubic effects of *cis*-9 C18:1 inclusion in supplemental fat.

[4] Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].

[5] Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)].

Table 7.6. Fatty acid concentration and yield by source of milk FA for cows fed treatment diets (n = 36).

Variable	Treatments[1]				SEM	P value[2]			*Contrast*[3]		
	80:10	73:17	66:24	60:30		Trt	Production	Trt x Production	Linear	Quadratic	Cubic
Summation by Source[4], g/100g FA											
De Novo	25.6	24.9	24.9	25.4	0.39	0.23	0.14	0.59	0.60	0.04	0.84
Mixed	40.9	39.6	39.1	37.9	0.38	<0.01	0.18	0.78	<0.01	0.87	0.21
Preformed	33.5	35.5	35.9	36.7	0.56	<0.01	0.93	0.54	<0.01	0.17	0.37
Summation by Source[4], g/d											
De Novo	414	410	412	418	16.1	0.96	0.29	0.10	0.81	0.64	0.98
Mixed	665	654	645	628	21.3	0.10	0.02	0.10	0.06	0.81	0.86
Preformed	540	576	591	596	14.5	<0.01	<0.01	0.01	<0.01	0.16	0.81

[1]Treatments were: 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 2) 73:17 (1.5% of FA supplement blend to provide ~ 73% C16:0 and 17% *cis*-9 C18:1); 3) 66:24 (1.5% of FA supplement blend to provide ~ 66% C16:0 and 24% *cis*-9 C18:1); and 4) 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1).

[2] P values associated with treatment, production level and interaction.

[3] Contrasts evaluated refers to the linear, quadratic and cubic effects of *cis*-9 C18:1 inclusion in supplemental fat.

[4] De novo FA originate from mammary de novo synthesis (<16 carbons), preformed FA originated from extraction from plasma (>16 carbons), and mixed FA originate from both sources (C16:0 plus *cis*-9 C16:1). Concentrations and yields of individual fatty acids are reported in Tables 7.9 and 7.10, respectively.

Table 7.7. Blood metabolites for cows fed treatment diets (n = 36).

Variable	Treatments[1]				SEM	P value[2]			*Contrast*[3]		
	80:10	73:17	66:24	60:30		Trt	Production	Trt x Production	Linear	Quadratic	Cubic
NEFA, mEq/L	0.10	0.11	0.11	0.11	0.005	0.48	0.26	0.20	0.51	0.23	0.46
BHB, mg/dL	7.76	8.43	8.81	9.35	0.31	0.10	0.01	0.88	0.05	0.21	0.42
Insulin, ug/L	0.58	0.62	0.63	0.65	0.03	0.10	0.15	0.06	0.08	0.72	0.78

[1]Treatments were: 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 2) 73:17 (1.5% of FA supplement blend to provide ~ 73% C16:0 and 17% *cis*-9 C18:1); 3) 66:24 (1.5% of FA supplement blend to provide ~ 66% C16:0 and 24% *cis*-9 C18:1); and 4) 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1).

[2] P values associated with treatment, production level and interaction.

[3] Contrasts evaluated refers to the linear, quadratic and cubic effects of *cis*-9 C18:1 inclusion in supplemental fat.

Table 7.8. Calculated energy, and energy partitioning toward milk, body tissues and maintenance for cows fed treatment diets (n = 36).

Variable	Treatments[1]				SEM	P value[2]			*Contrast*[3]		
	80:10	73:17	66:24	60:30		Trt	Production	Trt x Production	Linear	Quadratic	Cubic
Energy intake, Mcal/d											
NE_L[4]	48.2	49.8	50.1	50.7	0.65	0.02	0.01	0.29	0.01	0.79	0.80
NE_L Kg/DM[5]	1.66	1.72	1.73	1.73	0.02	0.04	0.01	0.22	0.01	0.01	0.82
Energy output, Mcal/d											
Milk[6]	33.2	34.0	34.1	34.4	0.83	0.52	<0.01	0.04	0.12	0.67	0.76
Body reserves[7]	4.60	5.10	5.40	5.60	0.44	0.08	0.33	0.48	0.05	0.01	0.18
Maintenance[8]	11.0	10.9	10.9	11.0	0.12	0.11	0.07	0.68	0.97	0.17	0.77
Partitioning, % energy intake											
Milk	67.8	68.1	68.1	67.7	0.84	0.67	<0.01	0.05	0.80	0.67	0.94
Body reserves	9.20	9.80	9.70	10.5	0.56	0.09	0.07	0.28	0.09	0.06	0.21
Maintenance	23.0	22.1	22.2	21.8	0.52	0.41	0.01	0.21	0.14	0.68	0.48

[1] Treatments were: 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 2) 73:17 (1.5% of FA supplement blend to provide ~ 73% C16:0 and 17% *cis*-9 C18:1); 3) 66:24 (1.5% of FA supplement blend to provide ~ 66% C16:0 and 24% *cis*-9 C18:1); and 4) 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1).

[2] P values associated with treatment, production level and interaction.

[3] Contrasts evaluated refers to the linear, quadratic and cubic effects of *cis*-9 C18:1 inclusion in supplemental fat.

[4] From the sum of milk energy output, maintenance energy calculated from metabolic BW, and body energy gain for each cow on each diet throughout the 35-d period.

[5]Net energy of lactation intake/DMI.

[6]Milk NE_L (Mcal/d) = milk yield (kg/d) × [(fat % × 0.0929) + (true protein % × 0.0563) + (lactose % × 0.0395)] (NRC, 2001).

[7]Body reserves (Mcal/d) = [(2.88+1.036 × BCS) × ΔBW], where BCS was the average BCS for study and ΔBW was BW change.

[8]NE_L maintenance (Mcal/d) = NE_L intake (Mcal/d) − milk NE_L (Mcal/d) − NE_L reserves (Mcal/d).

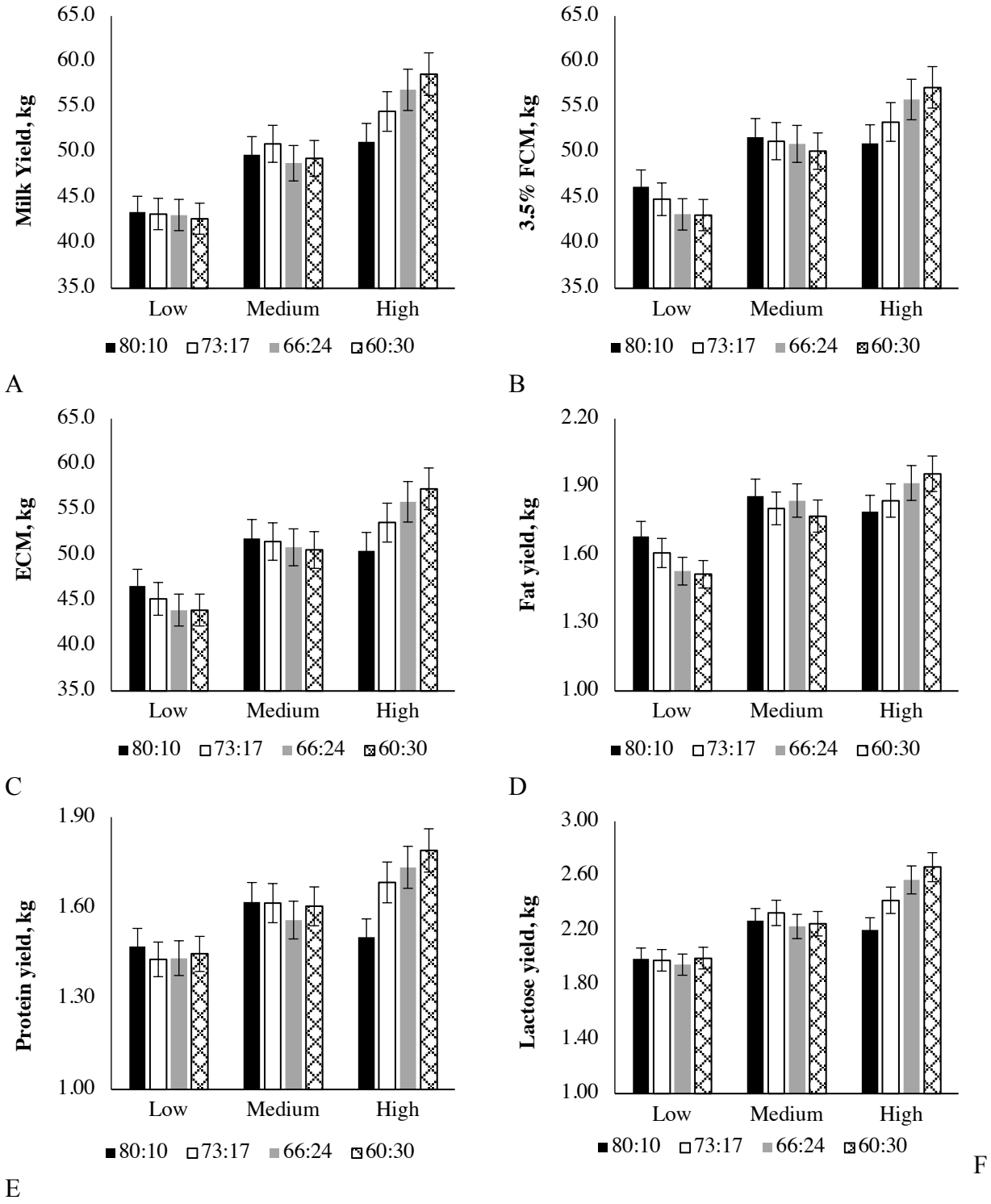
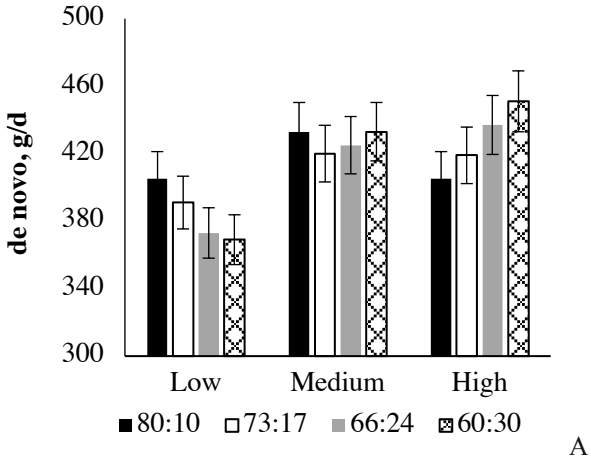


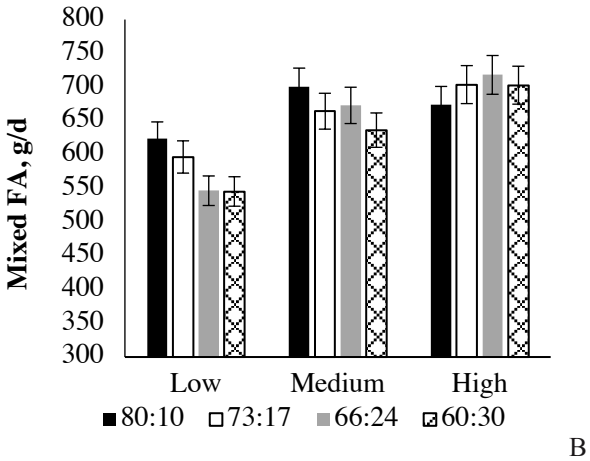
Figure 7.1. Effects of altering dietary ratio of palmitic and oleic acids on milk yield (A), 3.5% FCM (B), ECM (C), milk fat yield (D), milk protein yield (E) and milk lactose yield (F) of dairy cows across different production levels (low, medium and high producing groups).

Treatments were: 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 2) 73:17 (1.5% of FA supplement blend to provide ~ 73% C16:0 and 17% *cis*-9 C18:1);

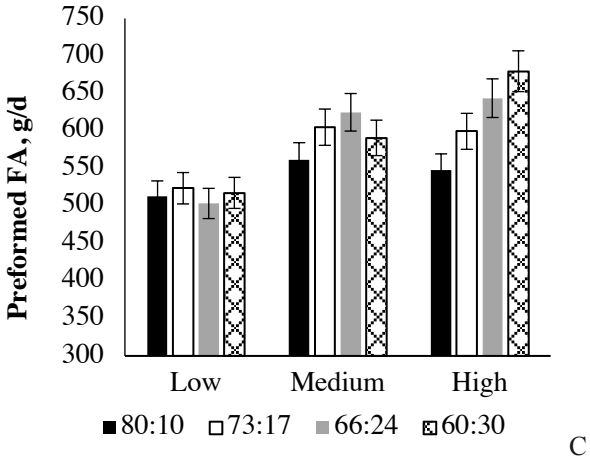
3) 66:24 (1.5% of FA supplement blend to provide ~ 66% C16:0 and 24% *cis*-9 C18:1); and 4) 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1). Significant interaction between FA treatments and production level were detected for milk yield ($P = 0.09$), 3.5% FCM ($P = 0.05$), ECM ($P = 0.05$), milk fat yield ($P = 0.08$), milk protein yield ($P = 0.03$) and milk lactose yield ($P = 0.05$). ** linear (L), quadratic (Q) and cubic (C) contrasts ($P<0.05$) of the effects of *cis*-9 C18:1 inclusion in supplemental fat. Error bars represent SEM.



A



B



C

Figure 7.2. Effects of altering dietary ratio of palmitic and oleic acids on yield of de novo milk FA (A), mixed (B), and preformed (C) of dairy cows across different production levels (low, medium and high producing groups).
Treatments were: 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 2) 73:17 (1.5% of FA supplement blend to provide ~ 73% C16:0 and 17% *cis*-9 C18:1); 3) 66:24 (1.5% of FA supplement blend to provide ~ 66% C16:0 and 24% *cis*-9 C18:1); and 4)

60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1). Significant interaction between FA treatments and production level were detected for de novo ($P = 0.10$), mixed ($P = 0.10$), and preformed milk FA ($P = 0.01$). ** linear (L), quadratic (Q) and cubic (C) contrasts ($P<0.05$) of the effects of *cis*-9 C18:1 inclusion in supplemental fat. Error bars represent SEM.

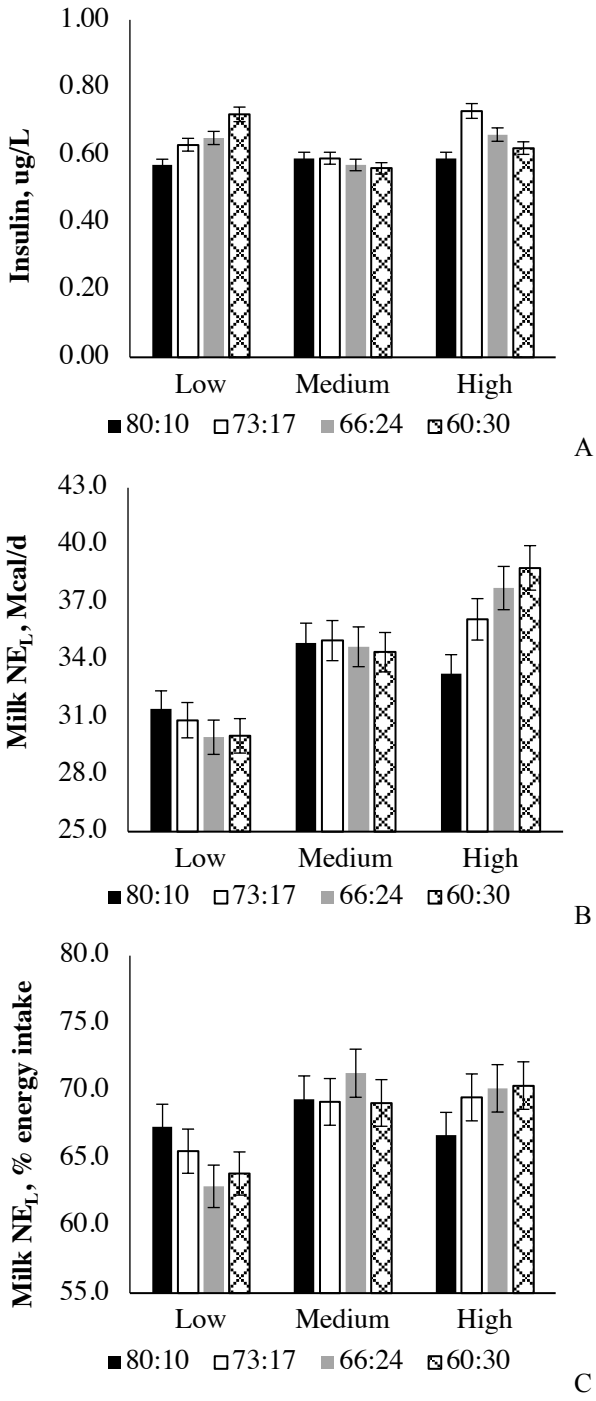


Figure 7.3. Effects of altering dietary ratio of palmitic and oleic acids on plasma insulin (A), milk energy output (B), and energy partitioned to milk (C) of dairy cows across different production levels (low, medium and high producing groups).
Treatments were: 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 2) 73:17 (1.5% of FA supplement blend to provide ~ 73% C16:0 and 17% *cis*-9 C18:1); 3) 66:24 (1.5% of FA supplement blend to provide ~ 66% C16:0 and 24% *cis*-9 C18:1); and 4) 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1). Significant

interaction between FA treatments and production level were detected for plasma insulin (P = 0.06), milk energy output (P = 0.04), and energy partitioned to milk (P = 0.05). ** linear (L), quadratic (Q) and cubic (C) contrasts (P<0.05) of the effects of *cis*-9 C18:1 inclusion in supplemental fat. Error bars represent SEM.

Table 7.9. Milk fatty acid concentrations for cows fed treatment diets (n = 36).

Variable	Treatments[1]				SEM	P value[2]			*Contrast*[3]		
	80:10	73:17	66:24	60:30		Trt	Prod.	Trt x Prod.	Linear	Quadratic	Cubic
Selected Individual FA[4], g/ 100g FA											
C4:0	3.11	3.17	3.14	3.15	0.07	0.91	0.14	0.78	0.77	0.65	0.61
C6:0	2.11	2.07	2.07	2.08	0.05	0.76	0.78	0.61	0.47	0.44	0.89
C8:0	1.22	1.19	1.21	1.21	0.03	0.65	0.62	0.49	0.62	0.29	0.64
C10:0	3.07	2.93	2.95	3.0	0.08	0.24	0.07	0.55	0.45	0.07	0.57
C12:0	3.59	3.41	3.43	3.49	0.10	0.19	0.02	0.47	0.34	0.06	0.62
C14:0	11.5	11.1	11.2	11.5	0.15	0.07	0.01	0.51	0.65	0.01	0.85
C16:0	39.4	37.9	37.5	35.4	0.36	<0.01	0.25	0.68	<0.01	0.62	0.15
cis-9 C16:1	1.59	1.67	1.63	1.56	0.08	0.67	0.31	0.59	0.62	0.27	0.78
C18:0	7.36	7.38	7.58	7.79	0.26	0.51	0.84	0.31	0.15	0.67	0.87
cis-9 C18:1	16.6	18.0	18.2	18.5	0.33	<0.01	0.59	0.39	<0.01	0.02	0.33
cis-11 C18:1	0.58	0.63	0.62	0.64	0.02	0.18	0.96	0.56	0.08	0.38	0.31
trans-6 to 8 C18:1	0.23	0.28	0.29	0.32	0.01	<0.01	0.63	0.55	<0.01	0.15	0.24
trans-9 C18:1	0.15	0.18	0.18	0.21	0.007	<0.01	0.76	0.78	<0.01	0.23	0.13
trans-10 C18:1	0.42	0.59	0.54	0.65	0.09	0.03	0.39	0.47	0.01	0.55	0.10
trans-11 C18:1	0.63	0.69	0.72	0.76	0.03	0.02	0.57	0.62	<0.01	0.54	0.68
cis-9, *cis*-12 C18:2	2.0	2.08	2.10	2.20	0.04	<0.01	0.75	0.95	<0.01	0.56	0.32
cis-9, *trans*-11 C18:2	0.33	0.38	0.39	0.41	0.02	<0.01	0.68	0.62	<0.01	0.28	0.66
cis-9, *cis*-12, *cis*-15 C18:3	0.33	0.34	0.33	0.34	0.007	0.19	0.69	0.91	0.12	0.34	0.24

[1]Treatments were: 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 2) 73:17 (1.5% of FA supplement blend to provide ~ 73% C16:0 and 17% *cis*-9 C18:1); 3) 66:24 (1.5% of FA supplement blend to provide ~ 66% C16:0 and 24% *cis*-9 C18:1); and 4) 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1).

[2]P values associated with treatment, production level and interaction.

[3] Contrasts evaluated refers to the linear, quadratic and cubic effects of *cis*-9 C18:1 inclusion in supplemental fat.

[4]A total of approximately 80 individual FA were quantified. Only select FA are reported in the table.

Table 7.10. Milk fatty acid yields for cows fed treatment diets (n = 36).

Variable	Treatments[1]				SEM	P value[2]			*Contrast*[3]		
	80:10	73:17	66:24	60:30		Trt	Prod.	Trt x Prod.	Linear	Quadratic	Cubic
Selected Individual FA[4], g/d											
C4:0	50.5	52.3	52.1	52.2	2.16	0.81	0.01	0.33	0.46	0.58	0.74
C6:0	34.2	34.4	34.4	34.5	1.52	0.99	0.10	0.51	0.84	0.97	0.91
C8:0	20.0	19.9	20.0	20.1	0.93	0.99	0.33	0.71	0.82	0.82	0.96
C10:0	49.9	48.5	48.8	48.8	2.43	0.89	0.69	0.81	0.97	0.45	0.90
C12:0	58.4	56.3	56.6	57.8	2.79	0.82	0.82	0.66	0.85	0.37	0.87
C14:0	186	183	185	188	6.66	0.89	0.29	0.29	0.75	0.49	0.92
C16:0	638	626	621	599	20.8	0.10	0.02	0.13	0.03	0.69	0.68
cis-9 C16:1	26.0	27.3	26.9	25.2	1.41	0.42	0.02	0.41	0.53	0.13	0.92
C18:0	119	121	125	127	5.61	0.57	0.10	0.09	0.17	0.91	0.91
cis-9 C18:1	269	292	299	307	7.42	<0.01	<0.01	<0.01	<0.01	0.19	0.65
cis-11 C18:1	9.44	10.1	10.1	10.1	0.41	0.15	0.12	0.03	0.11	0.15	0.39
trans-6 to 8 C18:1	3.77	4.49	4.83	5.12	0.16	<0.01	0.05	0.48	<0.01	0.11	0.57
trans-9 C18:1	2.39	2.85	3.03	3.24	0.09	<0.01	0.01	0.12	<0.01	0.07	0.32
trans-10 C18:1	6.54	8.66	8.37	9.36	1.07	0.01	0.76	0.48	<0.01	0.36	0.18
trans-11 C18:1	9.83	11.4	11.7	12.5	0.55	0.01	<0.01	0.21	<0.01	0.52	0.45
cis-9, *cis*-12 C18:2	32.2	33.9	34.3	35.0	0.97	0.09	<0.01	0.22	0.01	0.48	0.63
cis-9, *trans*-11 C18:2	5.27	6.14	6.44	6.58	0.33	0.01	0.04	0.24	<0.01	0.21	0.74
cis-9, *cis*-12, *cis*-15 C18:3	5.32	5.42	5.44	5.61	0.17	0.49	<0.01	0.28	0.15	0.75	0.66

[1]Treatments were: 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 2) 73:17 (1.5% of FA supplement blend to provide ~ 73% C16:0 and 17% *cis*-9 C18:1); 3) 66:24 (1.5% of FA supplement blend to provide ~ 66% C16:0 and 24% *cis*-9 C18:1); and 4) 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1).

[2]P values associated with treatment, production level and interaction.

[3] Contrasts evaluated refers to the linear, quadratic and cubic effects of *cis*-9 C18:1 inclusion in supplemental fat.

[4]A total of approximately 80 individual FA were quantified. Only select FA are reported in the table.

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CHAPTER 8

CHANGING THE RATIO OF DIETARY PALMITIC AND OLEIC ACIDS ALTERS PRODUCTION AND METABOLIC RESPONSES DURING THE IMMEDIATE POSTPARTUM PERIOD AND CARRYOVER PERIOD IN DAIRY COWS

Abstract

The objectives of our study were to determine the effects of altering the dietary ratio of C16:0 and *cis*-9 C18:1 on production and metabolic responses of early lactation dairy cows during the immediate postpartum period and to evaluate carryover effects of the treatment diets in early lactation. Fifty-six multiparous cows were used in a randomized complete block design and randomly assigned to one of four treatments fed from 1 to 24 DIM. The treatments were: 1) CON (control; non-FA supplemented diet); 2) 80:10 (80% C16:0 + 10% *cis*-9 C18:1); 3) 70:20 (70% C16:0 + 20% *cis*-9 C18:1); and 4) 60:30 (60% C16:0 + 30% *cis*-9 C18:1). The FA blends were fed at 1.5% diet DM replacing soyhulls. From d 25 to 60 postpartum (carryover period), all cows were offered a common diet to evaluate carryover effects. During the treatment period, FA-supplemented diets increased milk yield, 3.5% FCM, and ECM compared with CON. Compared with CON, FA-supplemented diets increased milk fat content, milk fat yield, and tended to increase protein yield and lactose yield. Also, compared with CON, FA-supplemented diets tended to increase BCS change and increased plasma insulin. A treatment by time interaction was observed for BW due to 80:10 inducing a greater decrease in BW over time compared with the other treatments. Also, we tended to observe an interaction between treatment and time for BHB due to FA-supplemented diets increasing BHB compared with CON at wk 3. Increasing *cis*-9 C18:1 in FA treatments tended to linearly increase DMI. In contrast, altering *cis*-9 C18:1 in FA treatments did not affect milk yield, 3.5% FCM, ECM, and the yields of milk fat, protein and lactose. Increasing *cis*-9 C18:1 in FA treatments linearly decreased milk fat content and milk

lactose content. Also, increasing *cis*-9 C18:1 in FA treatments linearly decreased BW and BCS losses and plasma NEFA, and tended to increase BW, plasma insulin and BHB. During the carryover period, compared with CON, FA-supplemented diets tended to increase milk yield and milk protein yield. Also, FA-supplemented diets increased 3.5% FCM, ECM, and milk fat yield. Our results indicate that feeding FA supplements containing C16:0 and *cis*-9 C18:1 during the immediate postpartum period increased milk yield and ECM compared with a non-fat control diet. Additionally, the diets fed during the immediate postpartum period had a tremendous carryover effect during early lactation, when cows were fed a common diet.

Introduction

During the immediate postpartum period (from calving to 3-4 wks following parturition), high-producing cows are challenged with large metabolic demands due to the sudden increase in energy requirements which cannot be met by feed intake alone (van Knegsel et al., 2007). During this stage, dairy cows enter a state of negative energy balance leading to an increased mobilization of adipose tissue and release of NEFA into circulation (Drackley, 1999). When body reserve mobilization is intense and plasma NEFA concentrations elevated, it can lead to alterations in immune function and increase the risk and severity of metabolic and infectious diseases (Sordillo et al., 2009, Sordillo, 2016). Increased energy intake during the immediate postpartum period results in lower circulating NEFA (Rabelo et al., 2005) and has been associated with improved health (Esposito et al., 2014) and performance (Rabelo et al., 2003). Approaches for increasing energy intake of postpartum cows include raising dietary starch content and supplementing fat to increase the energy density of the diet (McCarthy et al., 2015, Piantoni et al., 2015a). Inconsistent responses to supplemental fat on production and metabolic responses of early lactation cows have been reported, which are likely associated with the fatty

acid (FA) profile of the supplement fed, timing and level of supplementation, and interactions with other dietary and animal factors. Importantly, research has evolved from feeding traditional animal- and plant-based fats to the increased interest in the effects of feeding individual FA, extending beyond their energy contribution to include potentially structural, metabolic, and physiological effects (Palmquist and Jenkins, 2017). Therefore, determining dairy cow responses to specific FA or combination of FA is of particular importance.

To our knowledge, few studies were designed to evaluate the effects of different FA ratios on production responses of dairy cows. Greco et al. (2015) observed that decreasing the ratio of omega-6 to omega-3 FA in the diet of lactating dairy cows while maintaining similar dietary concentrations of total FA improved productive performance in early lactation (14 to 104 d postpartum). A dietary omega-6 to omega-3 ratio of approximately 4:1 increased DMI and the yield of milk and milk components compared with a 6:1 ratio. In post peak cows, we observed that feeding a FA blend with high content of C16:0 (80% C16:0) increased milk energy output and energy partitioning towards milk, while feeding a FA blend with a combination of C16:0 and *cis*-9 C18:1 (45% C16:0 and 35% *cis*-9 C18:1) increased plasma insulin, energy allocated to BW and the partitioning of energy to BW compared with non-fat control diets (de Souza et al., 2018). Similarly, we observed that decreasing the ratio of C16:0 to *cis*-9 C18:1 (from 80:10 to 60:30) in supplemental fat increased BW change and energy allocated to body reserves in post-peak cows (Chapter 7). Interestingly, we observed that feeding a C16:0 supplement to early lactation cows consistently increased ECM and milk energy output, but also increased BW and BCS losses and plasma NEFA when fed during the first 24 d after calving (Chapters 5 and 6). Thus, determining the impact of combinations between C16:0 and *cis*-9 C18:1 on production responses and body reserve mobilization during early lactation is warranted.

Therefore, our objectives were to determine the effects of altering the dietary ratio of C16:0 and *cis*-9 C18:1 on production and metabolic responses of early lactation dairy cows during the immediate postpartum period and to evaluate carryover effects of the treatment diets early in lactation. We hypothesized that increasing the amount of *cis*-9 C18:1 in supplemental fat would reduce milk energy output due to differences in milk fat yield responses, and that feeding *cis*-9 C18:1 would reduce body reserves mobilization in early lactation. We also postulated that feeding FA supplements in the immediate postpartum period would result in positive carryover effects on performance during 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 February 22th, 2017 and finished on September 15th, 2017. Cows were fed once daily (9000 h) at 120% of expected intake during the treatment and carryover periods, 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

Fifty-six multiparous Holstein cows at the Michigan State University Dairy Field Laboratory were used in a randomized complete block design. Cows were blocked by BCS (up to 0.50 unit difference using the 1=thin, 5=fat scale in 0.25 increments), previous lactation 305-ME (within 2,000 kg), and parity (up to 1 lactation difference). The BCS used to block cows was the last measurement before parturition. Cows within each block were randomly assigned to one of four treatments fed from 1 to 24 DIM. The treatments were combinations of two commercially

available FA supplements with different FA profiles that were blended to achieve the required ratios of C16:0 and *cis*-9 C18:1 in the FA supplement blends (Table 8.1). The treatments were: 1) CON (control; non-FA supplemented diet); 2) 80:10 (80% C16:0 + 10% *cis*-9 C18:1); 3) 70:20 (70% C16:0 + 20% *cis*-9 C18:1); and 4) 60:30 (60% C16:0 + 30% *cis*-9 C18:1). The FA supplement blends were added at 1.5% of diet DM replacing soyhulls in the CON diet. Treatment diets were mixed daily in a tumble-mixer and fed from the morning following parturition. From d 25 to 60 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 the close-up ration for reference, are described in Table 8.2. 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, so all collection days are ±3 d. Daily milk yield and feed offered and refused were recorded daily throughout the experiment. Samples of all diet ingredients (0.5 kg) and orts from each cow (~12.5%) were collected weekly during the experiment and stored in plastic bags at -20°C until processed. Milk samples were collected twice a week 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 35 d postpartum, and stored without preservative at -20°C for determination of FA profile. BW was recorded three times per week from d -21 of expected parturition and throughout the experiment. Body condition was scored weekly by 3 trained investigators on a 5-point scale, where 1 = thin and 5 = fat, as described by Wildman et al.

(1982). Blood samples were collected weekly by venipuncture of coccygeal vessels within 1 h before feeding on d 5, 12, 19, and 35 postpartum. 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 × *g* for 15 min immediately after sample collection, and plasma was harvested and stored at −20°C until analysis.

Sample Analysis

Feed and orts samples were dried in a 55°C forced-air oven for 72 h and analyzed for DM content. Before drying, ingredients were composited monthly. Orts were dried to calculate DMI weekly, but only orts collected on d 5, 12, and 19 postpartum were processed further and analyzed for nutrient composition. Once dried, samples of feed ingredients and orts were ground in a Wiley mill (1-mm screen; Arthur H Thomas Co., Philadelphia, PA) and analyzed for ash, NDF, indigestible NDF, CP, starch and FA concentration as described by Boerman et al. (2017).

Milk samples were analyzed for fat, true protein, and lactose concentrations by mid-infrared spectroscopy (AOAC, 1990; method 972.160) (Universal Lab Services, Lansing, MI). Yields of 3.5% FCM, ECM, milk energy, and milk components were calculated using milk yield and component concentrations from each milking, summed for a daily total, and averaged for each week. Milk samples stored without preservative were composited by milk fat yield and milk lipids were extracted, and FA-methyl esters prepared and quantified using GLC according to Lock et al. (2013). Yields of individual FA (g/d) in milk fat were calculated using milk fat yield and FA concentration to determine yield on a mass basis using the molecular weight of each FA while correcting for glycerol content and other milk lipid classes (Piantoni et al., 2013).

All plasma samples were determined using an Olympus AU640e chemistry analyzer (Olympus America, Center Valley, PA) at the Diagnostic Center for Population and Animal

Health of Michigan State University (Lansing, MI).

Statistical Analysis

Data were analyzed separately for the treatment (from 1 to 24 d postpartum) and for the carryover periods (from 25 to 60 d postpartum) periods. Data was average by week and all weekly data were analyzed using the MIXED procedure of SAS v.9.2 (SAS Institute, Inc. Cary, NC) with repeated measures.

The model used included:

$Y_{ijkl} = \mu + B_i + C(B_iF_k)_j + F_k + T_l + F_kT_l + e_{ijkl}$

Where μ = overall mean B_i = random effect of block, $C(B_iF_k)_j$ = random effect of cow within block and treatment diet, F_k = fixed effect of treatment during the treatment period, T_l = fixed effect of time, e_{ijkl} = residual error.

Unless otherwise specified, first-order autoregressive was the covariate structure used for analysis because it resulted in the lowest BIC for most of the variables measured. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals vs. predicted values. Pre calving BCS and BW were used as covariates for BW, BCS, BW change and BCS change. Significance was declared at $P \leq 0.05$ for main effects and $P \leq 0.10$ for interactions. Tendencies were declared at $P \leq 0.10$ for main effects and $P \leq 0.15$ for interactions. Three preplanned contrasts were used to compare treatment differences: 1) CON vs. FAT [control vs. FA-supplemented diets; (80:10 + 70:20 + 60:30)/3]; 2) the linear effect of *cis*-9 C18:1 inclusion in supplemental fat and 3) the quadratic effect of *cis*-9 C18:1 inclusion in supplemental fat. All cows were in apparent good health at the beginning of the study, and treatment groups were not different in terms of 305-ME ($P = 0.84$), BW ($P = 0.44$), or BCS ($P = 0.93$) pre-calving.

Results

Diets, Nutrient Composition, and Health Incidents

All cows received the same close-up diet before calving (Table 8.2). During the treatment period, the CON diet contained (DM basis) 30.7% NDF, 23.0% forage NDF, 24.6% starch, and 2.49% total FA. As expected, 80:10 treatment mainly increased dietary C16:0, while 70:20 and 60:30 increased C16:0 and *cis*-9 C18:1 compared with CON. During the carryover period, diets were adjusted to reduce forage and increase starch content. Therefore, the carryover diet contained (DM basis) 28.8% NDF, 20.3% forage NDF, 27.6% starch, and 2.94% total FA.

Our study was not designed to evaluate treatment effects on health incidents. Therefore, only a summary of health incidents is presented in Table 8.3. Ketosis was the major health incident with 2, 3, 2 and 2 for CON, 80:10, 70:20, and 60:30, respectively. Also, we observed 3, 1, 1, and 3 cases of retained placenta for CON, 80:10, 70:20, and 60:30, respectively. The major health incident during the carryover period was mastitis (3 cases).

Production Responses During the Treatment Period

FA-supplemented diets increased milk yield ($P = 0.05$; Table 8.4), 3.5% FCM ($P < 0.01$), and ECM ($P = 0.01$) compared with CON. The increase in DMI, milk yield, and ECM was consistent over time for all treatments. Compared with CON, FA-supplemented diets increased milk fat content ($P = 0.03$), milk fat yield ($P < 0.01$), and tended to increase protein yield ($P = 0.06$) and lactose yield ($P = 0.10$). We did not observe treatment differences for milk protein ($P = 0.21$) or milk lactose content ($P = 0.26$). Compared with CON, FA-supplemented diets tended to increase BCS change ($P = 0.09$). A treatment by time interaction was observed for BW ($P < 0.01$) due to 80:10 inducing a greater decrease in BW over time compared with other treatments.

Increasing *cis*-9 C18:1 in FA treatments tended to linearly increase DMI ($P = 0.09$; Table

8.4). In contrast, increasing *cis*-9 C18:1 in supplemental fat did not affect milk yield, 3.5% FCM, ECM, and the yields of milk fat, protein and lactose (all $P > 0.10$; Table 8.4). Increasing *cis*-9 C18:1 in FA treatments linearly decreased milk fat content ($P = 0.01$) and milk lactose content ($P = 0.02$). However, increasing *cis*-9 C18:1 in FA treatments linearly decreased BW ($P = 0.02$) and BCS ($P = 0.04$) losses, and tended to increase BW ($P = 0.10$).

Production Responses During the Carryover Period

During the carryover period, FA-supplemented diets increased 3.5% FCM ($P = 0.02$; Table 8.5), ECM ($P = 0.02$), and milk fat yield ($P = 0.02$) compared with CON. Additionally, FA-supplemented diets tended to increase milk yield compared with CON ($P = 0.08$) and milk protein yield ($P = 0.10$). Although we did not observe treatment differences for DMI, DMI increased consistently over time peaking at wk 6 for all treatments. For milk yield and ECM, FA-supplemented diets compared with CON consistently increased these variables over time peaking at wk 5. Although FA-supplemented diets increased milk lactose content ($P < 0.01$), we did not observe treatment differences for milk fat ($P = 0.19$) or milk protein content ($P = 0.65$). Compared with CON, FA-supplemented diets decreased BCS ($P = 0.02$). A treatment by time interaction was observed for BW ($P = 0.10$) due 80:10 increasing BW over time compared with CON.

Altering *cis*-9 C18:1 in FA treatments did not affect production variables during the carryover period (all $P > 0.10$; Table 8.5).

Plasma Insulin and Metabolites During the Treatment Period

Compared with CON, FA-supplemented diets increased plasma insulin ($P = 0.02$; Table 8.6). Also, we tended to observe an interaction between treatment and time for BHB ($P = 0.15$)

due to FA-supplemented diets increasing BHB compared with CON at wk 3 (Figure 8.1). We did not observe differences between CON and FA-supplemented diets for NEFA ($P = 0.57$) or albumin ($P = 0.11$).

Increasing *cis*-9 C18:1 in FA treatments linearly decreased plasma NEFA ($P = 0.03$; Table 8.6), and tended to linearly increase plasma insulin ($P = 0.07$) and BHB ($P = 0.10$).

Plasma Insulin and Metabolites During the Carryover Period

During the carryover period, blood metabolites were only evaluated at one time point (d 35). We did not observe difference between CON and FA-supplemented diets or among the FA treatments for the metabolites evaluated ($P > 0.10$; Table 8.7).

Discussion

The potential response of different FA during the immediate postpartum period (3 to 4 wks after parturition) and when supplemental fat should be fed is not well described and previous results are inconsistent. Grummer (1992) suggested based on studies conducted in the early 1990's that supplemental fat had little benefits on cow performance when fed in the first 5 to 7 wks of lactation. However, our recent advances in understanding the role of individual FA and their impact on digestion and metabolism requires a reevaluation of fat supplementation during early lactation. Previously, we observed that feeding a C16:0 supplement from 1-24 d postpartum consistently increased the yield of ECM compared with a non-fat supplemented control diet, but also increased BW and BCS losses (Chapter 5). Additionally, our research has indicated that altering the dietary ratio of C16:0 and *cis*-9 C18:1 may alter nutrient partitioning between the mammary gland and adipose tissue in post-peak cows (de Souza et al., 2018), and increasing the level of *cis*-9 C18:1 increased BW change and energy partitioning to body reserves in post-peak cows (Chapter 7). Since metabolic state plays a critical role in energy

partitioning, our aim in the current study was to evaluate the effects of altering the dietary ratio of C16:0 and *cis*-9 C18:1 in a fat supplement on production and metabolic responses of early lactation dairy cows.

Some authors suggest that feeding FA supplements to cows in the immediate postpartum period may depress feed intake (Kuhla et al., 2016), because DMI is likely controlled primarily by mechanisms related to oxidation of fuels in the liver in early postpartum (Allen and Piantoni, 2013). Previous studies reported that feeding a saturated FA supplement (C16:0 + C18:0) increased DMI (Moallem et al., 2007; Piantoni et al., 2015a), and feeding a C16:0 supplement did not affect DMI (Chapter 5) in cows in the immediate postpartum period and early lactation. Importantly, the effect of FA on feed intake is associated with the FA profile of the supplement fed (Allen, 2000; Rabiee et al., 2012) with DMI decreasing linearly as the degree of unsaturation of supplemental fat increases (Drackley et al., 1992; Harvatine and Allen, 2006). In our study, unexpectedly we observed a tendency for DMI increasing as we increased *cis*-9 C18:1 in the FA treatments. Interestingly, we also observed that increasing *cis*-9 C18:1 in the FA treatments increased plasma insulin and decreased NEFA in the immediate postpartum period. Piantoni et al. (2015b) reported that greater reductions in plasma NEFA concentrations after feeding were positively related to greater intakes in early postpartum cows, suggesting that decreased β-oxidation in the liver might allow for higher DMI. Plasma insulin concentration increases during and after meals, decreasing lipolysis and plasma NEFA concentrations (Allen et al., 2005). Therefore, the increase in DMI observed as we increased *cis*-9 C18:1 in the FA treatments may be related to a decreased flux of fuels to the liver that could have potentially decreased satiety and improved DMI (Allen et al., 2009).

We observed that milk yield increased with FA supplementation in the immediate

postpartum period, but there were no differences among the FA treatments. Milk yield responses to FA supplementation in the immediate postpartum period have been inconsistent. Beam and Butler (1998) supplemented a saturated FA supplement (C16:0 + C18:0) and reported an interaction between diet and time for milk yield due to supplemental fat decreasing milk yield during the first 3 wks postpartum, but increasing milk yield during the next 2 wks of the experiment. Piantoni et al. (2015a) observed that feeding a saturated FA supplement (C16:0 + C18:0) tended to decrease milk yield by 3.1 kg/d in cows in the immediate postpartum period (1-29 DIM). Feeding a Ca-salts of palm FA supplement increased milk yield by 2.2 kg/d without changes in DMI during the first 150 d of lactation compared with a nonfat control diet (Moallem et al., 2000). However, the effects on milk yield and DMI were reported as least squares means for the whole 150 d in lactation so that the effect of FA supplementation during the immediate postpartum on production performance cannot be discerned. In our study, the increase in milk yield during the treatment period for 70:20 and 60:30 treatments are associated with the tendency for higher DMI. For the 80:10 treatment these results are likely related at least in part to the greater body reserves mobilization during the immediate postpartum period compared with other treatments similar to what we previously reported when a C16:0 supplement was fed to cows in the immediate postpartum period (Chapter 5).

Although increasing *cis*-9 C18:1 in FA treatments linearly decreased milk fat content, no differences in milk fat yield was observed among the FA-supplemented treatments. Also, compared with CON, the FA-supplemented treatments increased milk fat yield, milk protein yield, 3.5% FCM and ECM and the increase in these variables over time after parturition was consistent. Previous studies have observed that C16:0 supplementation increased 3.5% FCM and ECM in post-peak cows (Piantoni et al., 2013; Lock et al., 2013) and when fed from calving to

67 DIM (Chapter 5). Also, de Souza et al. (2018) observed that, compared with a nonfat control diet, ECM and milk energy output increased when feeding a FA blend containing 80% of C16:0, but not with a FA blend containing 45% C16:0 and 35% *cis*-9 C18:1. Also, feeding a Ca salts of palm FA supplement (2.6% diet DM) from parturition to 120 DIM increased 3.5% FCM in dairy cows (Sklan et al., 1991; Sklan et al., 1994), but no interaction with time was reported. Therefore, the effects of feeding FA supplements varying in the ratio of C16:0 and *cis*-9 C18:1 on 3.5% FCM and ECM was consistent with previous studies supplementing Ca-salts and C16:0 supplements to dairy cows.

Interestingly, we observed that increasing *cis*-9 C18:1 in FA treatments linearly decreased BW and BCS losses, and tended to increase BW. This difference in nutrient partitioning is probably driven by insulin and/or greater DMI, as we observed that increasing *cis*-9 C18:1 in FA treatments increased plasma insulin concentration and tended to increase DMI. Previous studies using rats as a model have observed that *cis*-9 C18:1 stimulated insulin secretion from pancreatic β-cells (Itoh et al., 2003; Fujiwara et al., 2005). Similarly, we observed higher plasma insulin levels in post peak cows when feeding a FA blend containing 45% C16:0 and 35% *cis*-9 C18:1 compared with control (de Souza et al., 2018), and that increasing *cis*-9 C18:1 in supplemental fat increased plasma insulin (Chapter 7). Elevated insulin concentrations would reduce plasma NEFA through reducing lipolysis or increasing lipogenesis (Vernon, 2005). In addition, increased concentrations of plasma triglycerides (TAG) could result from higher intake of dietary FA increasing the supply of TAG-rich lipoproteins available in circulation. As a result, increases in insulin could partition circulating TAG into other tissues and reduce lipolysis from adipose tissues. Furthermore, Yanting et al. (2018) reported that *cis*-9 C18:1 increased adipocyte number and size through enhancing adipogenic commitment and lipogenesis compared

with saturated FA (C14:0, C16:0, and C18:0). Also, they reported that in mature adipocytes treated with FA, the lipid content in adipocytes increased with *cis*-9 C18:1 compared to C14:0, C16:0, C18:0, and *cis*-9 *cis*-12 C18:2 (Yanting et al., 2018). Piantoni et al. (2015a) observed that regardless of forage NDF level of the diets evaluated, feeding a saturated FA supplement (C16:0 + C18:0) decreased BCS loss, but tended to decrease milk yield in the immediate postpartum period (1 to 29 DIM). In contrast, we observed that feeding a C16:0 supplement increased plasma NEFA and body reserves mobilization compared with a nonfat control diet mainly when fed to cows in the immediate postpartum period (Chapter 5 and 6). Interesting in our study, increasing *cis*-9 C18:1 in FA treatments tended to reduce body reserve mobilization despite the increase in milk energy output. It is important to point out that although we increased dietary *cis*-9 C18:1, it is likely that this treatment increased rumen outflow of other 18-carbon FA. While it is unclear if these results are associated with an overall effect of 18-carbon FA or a specific FA, in post-peak cows we observed that feeding a FA blend containing 45% C16:0 and 35% *cis*-9 C18:1 increased BW change and plasma insulin compared with a FA blend containing 40% C16:0 and 40% C18:0 (de Souza et al., 2018). Further research is needed to determine whether a higher amount of 18-carbon FA or a higher amount of a specific FA is related to energy partitioning toward body reserves, and to determine the mechanism associated with it.

Changes in production responses during the supplementation period not only have an immediate effect, but can have an effect on subsequent lactation performance (Jorgensen et al., 2016). One of our goals was to evaluate the potential carryover effects of FA supplementation during the immediate postpartum on production responses during early lactation. 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 yield of milk and milk components,

3.5% FCM, and ECM were higher during the carryover period for cows that received FA-supplemented diets compared with CON during early postpartum, but no differences were observed among the FA-supplemented diets. Interestingly, Piantoni et al. (2015a) observed that feeding a saturated FA supplement (C16:0 + C18:0) did not affect the yield of 3.5% FCM, and ECM in cows in the immediate postpartum period (1 to 29 DIM), but FA supplementation had a pronounced carryover effect (30 to 71 DIM) decreasing both 3.5% FCM and ECM in a low forage diet. With grazing cows, supplementing a Ca-salts of palm FA supplement from 3 to 16 wks of lactation increased cumulative milk yield throughout lactation by 8.0 to 12% (Batistel et al., 2017; de Souza et al., 2017). Possible explanations for the carryover effect on milk yield may involve an increase in mammary cell number (Akers, 2002) or cell secretory activity (Nørgaard et al., 2005). Also, the development of epithelial cell populations in the mammary gland is mainly regulated by ovarian steroids including estrogen (Arendt and Kuperwasser, 2015). Flaxseed oil was shown to alter mammary development, modify mammary gland morphology, and increase the number of estradiol receptor binding sites in the mammary gland of mice (Hilakivi-Clarke et al., 1998). Feeding prepubertal heifers with soybean oil slightly improved the mammary development but did not affect the yields of milk and milk components during their first lactation (Thibault et al., 2003). However, further studies are needed to comprehend factors associated with carryover effects, and to determine the duration and magnitude of this under different dietary conditions including individual FA and combinations of FA.

Conclusion

Our results indicate that feeding FA supplements containing C16:0 and *cis*-9 C18:1 during the immediate postpartum period increased milk yield and ECM compared with a non-fat control diet. Increasing *cis*-9 C18:1 in the FA supplement reduce plasma NEFA and BW and

BCS losses and tended to increase DMI and plasma insulin. Additionally, the diets fed during the immediate postpartum period had a tremendous carryover effect during early lactation, when cows were fed a common diet. The yield of milk and milk components, 3.5% FCM, and ECM were higher during the carryover period for cows that received FA-supplemented diets compared with CON during early postpartum.

APPENDIX

APPENDIX

Table 8.1. Proportion of each FA supplement and FA profile for treatment blends.

	Treatment[1]		
	80:10	70:20	60:30
% of each FA supplement in treatment blends			
Prilled FA Supplement[2]	89.5	58.0	29.2
Ca-salts FA Supplement[3]	9.5	42.0	70.8
FA profile of each FA blend, g/100g FA			
C14:0	0.67	0.78	0.88
C16:0	81.2	70.7	60.1
C18:0	1.82	1.91	2.00
cis-9 C18:1	10.8	20.0	29.3
cis-9, *cis*-12 C18:2	2.95	4.72	6.49
cis-9, *cis*-12, *cis*-15 C18:3	0.11	0.17	0.22

[1]Treatments were: 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 70:20 (1.5% of FA supplement blend to provide ~ 70% C16:0 and 20% *cis*-9 C18:1); 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1).

[2]Palmitic acid-enriched FA supplement (Nutracor; Wawasan Agrolipids, Johor, Malaysia). The supplement contained (g/100 g of fatty acid) 0.64 of C14:0, 84.5 of C16:0, 1.80 of C18:0, 7.88 of C18:1 *cis*-9, and 99.0% total fatty acids.

[3]Ca salts of palm FA supplement (Nutracal; Wawasan Agrolipids, Johor, Malaysia). The supplement contained (g/100 g of fatty acid) 1.0 of C14:0, 48.0 of C16:0, 2.10 of C18:0, 39.8 of C18:1 *cis*-9, and 83.2% total fatty acids.

Table 8.2. Ingredient and nutrient composition of close up diet, treatment diets, and carryover diet.

	Diet[1]					
	Close up	Treatment[1]				Carryover
		CON	80:10	70:20	60:30	
Ingredient, % DM						
Corn Silage	42.0	29.7	29.7	29.7	29.7	26.5
Alfalfa Silage	-	10.9	10.9	10.9	10.9	13.8
Alfalfa Hay	-	12.4	12.4	12.4	12.4	-
Grass Hay	35.5	-	-	-	-	-
Wheat Straw	-	-	-	-	-	2.65
Ground Corn	7.09	19.6	19.6	19.6	19.6	14.7
High Moisture Corn	-	5.03	5.03	5.03	5.03	16.1
Soybean Meal	8.11	13.9	13.9	13.9	13.9	13.9
Soyhulls	-	3.10	1.55	1.47	1.39	3.00
SoyChlor[2]	2.52	-	-	-	-	-
Whole Cottonseed	-	-	-	-	-	4.66
Protein supplement[3]	1.13	1.42	1.42	1.42	1.42	1.19
Prilled FA supplement[4]	-	0.00	1.38	0.94	0.50	-
Ca-salts FA supplement[5]	-	0.00	0.16	0.69	1.21	-
Mineral and Vitamin mix[6,7,8]	2.60	3.95	3.95	3.95	3.95	3.52
Nutrient Composition, % DM[9]						
NDF	38.5	30.7	29.5	29.5	29.4	28.8
Forage NDF	34.9	23.0	23.0	23.0	23.0	20.3
CP	14.6	16.9	16.7	16.7	16.7	16.9
Starch	17.2	24.6	24.6	24.6	24.6	27.6
FA	1.82	2.49	4.01	4.01	3.99	2.94
16:0	0.28	0.36	1.57	1.43	1.26	0.30
18:0	0.06	0.07	0.10	0.10	0.10	0.09
cis-9 18:1	0.29	0.46	0.64	0.77	0.90	0.40
cis-9, *cis*-12 18:2	0.82	1.22	1.26	1.28	1.31	1.00
cis-9, *cis*-12, *cis*-15 18:3	0.17	0.15	0.15	0.15	0.15	0.15

[1]Treatments were: 1) CON (control; no supplemental fat); 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 70:20 (1.5% of FA supplement blend to provide ~ 70% C16:0 and 20% *cis*-9 C18:1); 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1).

[2] West Central Soy, Ralston, IA.

[3] Spectrum Agriblue (Perdue Agribussiness, Salisbury, MD).

[4]Palmitic acid-enriched FA supplement (Nutracor; Wawasan Agrolipids, Johor, Malaysia). The supplement contained (g/100 g of fatty acid) 0.64 of C14:0, 84.5 of C16:0, 1.80 of C18:0, 7.88 of C18:1 *cis*-9, and 99.0% total fatty acids.

[5]Ca salts of palm FA supplement (Nutracal; Wawasan Agrolipids, Johor, Malaysia). The supplement contained (g/100 g of fatty acid) 1.0 of C14:0, 48.0 of C16:0, 2.10 of C18:0, 39.8 of C18:1 *cis*-9, and 83.2% total fatty acids.
[6]Vitamin-mineral mix for the close-up diet contained (DM basis): 54.8% SoyChlor, 13.9% limestone, 10.0% rumen-protected choline, 8.8% di- calcium phosphate, 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).
[7]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).
[8]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).
[9] Expressed as percent of as fed.

Table 8.3. Health incidents during the experiment for each treatment diet.

	Treatment[1]			
	CON	80:10	70:20	60:30
During treatment period				
Ketosis	2	3	2	2
Metritis	1	-	-	-
Milk fever	1	1	1	-
Retained Placenta	3	1	1	3
Displaced abomasum	1	-	-	-
During carryover period				
Lame	1	-	-	-
Mastitis	-	2	-	1

[1]Treatments were: 1) CON (control; no supplemental fat); 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 70:20 (1.5% of FA supplement blend to provide ~ 70% C16:0 and 20% *cis*-9 C18:1); 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1).

Table 8.4. Milk production, milk composition, BW, and BCS for cows fed treatment diets during the treatment period (d 1 to 24 postpartum).

	Treatment (Trt)[1]				SEM	Contrast[2]			*P value*	
	CON	80:10	70:20	60:30		CON vs. FAT	Linear	Quadratic	Time	Trt x Time
DMI, kg	20.3	20.7	20.9	21.8	0.48	0.15	0.09	0.44	<0.01	0.95
Milk Yield, kg/d										
Milk	46.5	48.6	48.8	49.7	1.39	0.05	0.95	0.58	<0.01	0.84
3.5% FCM[3]	50.1	54.8	54.1	54.7	1.27	<0.01	0.74	0.97	<0.01	0.52
ECM[4]	50.2	54.8	53.5	54.3	1.18	0.01	0.41	0.71	0.04	0.50
Milk Composition										
Fat, kg/d	1.90	2.15	2.08	2.09	0.06	<0.01	0.61	0.59	0.03	0.27
Fat, %	4.06	4.45	4.26	4.21	0.12	0.03	0.01	0.28	<0.01	0.51
Protein, kg/d	1.41	1.56	1.50	1.52	0.05	0.06	0.57	0.59	0.83	0.63
Protein, %	3.13	3.25	3.19	3.22	0.06	0.21	0.78	0.48	<0.01	0.64
Lactose, kg/d	2.11	2.34	2.25	2.25	0.09	0.11	0.48	0.68	<0.01	0.55
Lactose, %	4.80	4.88	4.82	4.80	0.03	0.26	0.02	0.44	<0.01	0.82
BW, kg	697	678	705	715	16.1	0.71	0.10	0.69	<0.01	<0.01
BW change, kg/d	-1.55	-2.54	-1.63	-1.48	0.37	0.38	0.02	0.58	NA[5]	NA
BCS	3.46	3.33	3.35	3.38	0.06	0.12	0.54	0.92	<0.01	0.19
BCS change, units/wk	-0.09	-0.14	-0.12	-0.10	0.004	0.09	0.04	0.61	NA	NA

[1]Treatments were: 1) CON (control; no supplemental fat); 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 70:20 (1.5% of FA supplement blend to provide ~ 70% C16:0 and 20% *cis*-9 C18:1); 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1).
[2] P values associated with contrasts of treatment effects: CON vs. FAT [control vs. FA-supplemented diets; (80:10 + 70:20 + 60:30)/3]; Linear and quadratic effects of *cis*-9 C18:1 inclusion in supplemental fat.
[3] Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].
[4] Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)].
[5] NA= Not applicable.

Table 8.5. Milk production, milk composition, BW, and BCS for cows fed a common diet during the carryover period (d 25 to 60 postpartum).

	Treatment (Trt)[1]				SEM	Contrast[2]			*P value*	
	CON	80:10	70:20	60:30		CON vs. FAT	Linear	Quadratic	Time	Trt x Time
DMI, kg	26.7	27.2	27.7	27.9	0.75	0.21	0.42	0.91	<0.01	0.76
Milk Yield, kg/d										
Milk	57.8	59.5	60.9	60.8	1.65	0.08	0.46	0.34	<0.01	0.76
3.5% FCM[3]	56.1	59.2	61.2	61.2	1.94	0.02	0.36	0.66	0.030	0.54
ECM[4]	55.6	58.7	60.7	60.3	1.88	0.02	0.42	0.61	<0.01	0.46
Milk Composition										
Fat, kg/d	1.91	2.06	2.11	2.13	0.08	0.02	0.35	0.95	0.10	0.82
Fat, %	3.32	3.48	3.38	3.55	0.11	0.19	0.26	0.59	<0.01	0.93
Protein, kg/d	1.68	1.76	1.81	1.77	0.06	0.10	0.91	0.48	<0.01	0.09
Protein, %	2.90	2.96	2.92	2.92	0.06	0.65	0.59	0.75	<0.01	0.40
Lactose, kg/d	2.84	2.97	3.05	3.03	0.11	0.14	0.71	0.64	<0.01	0.07
Lactose, %	4.88	4.98	4.97	4.92	0.03	<0.01	0.12	0.45	0.04	0.07
BW, kg	668	657	676	686	16.3	0.76	0.18	0.80	<0.01	0.10
BW change, kg/d	0.38	0.45	0.32	0.32	0.16	0.63	0.73	0.85	NA	NA
BCS	3.23	3.11	3.06	3.07	0.07	0.02	0.42	0.85	0.61	0.84

[1]Treatments were: 1) CON (control; no supplemental fat); 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 70:20 (1.5% of FA supplement blend to provide ~ 70% C16:0 and 20% *cis*-9 C18:1); 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1).

[2] P values associated with contrasts of treatment effects: CON vs. FAT [control vs. FA-supplemented diets; (80:10 + 70:20 + 60:30)/3]; Linear and quadratic effects of *cis*-9 C18:1 inclusion in supplemental fat.

[3] Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].

[4] Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)].

[5] NA= Not applicable.

Table 8.6. Plasma insulin and metabolites for cows fed treatment diets during the treatment period (d 1 to 24 postpartum).

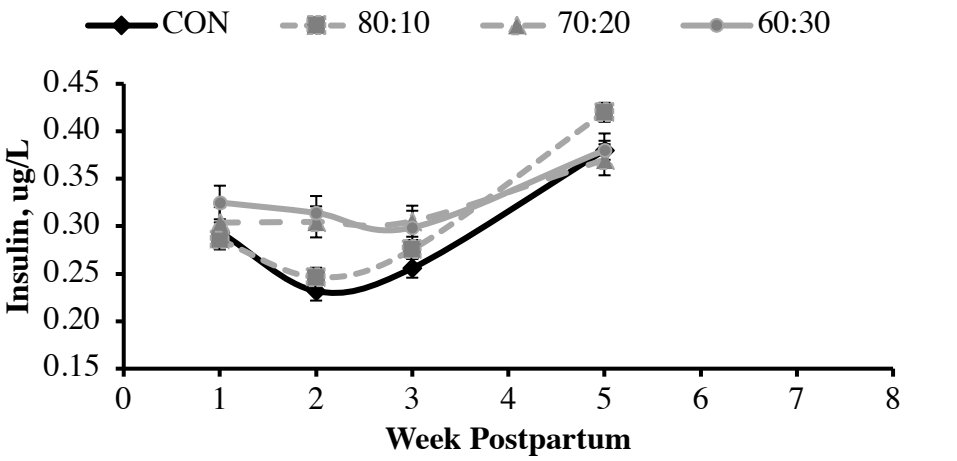
	Treatment (Trt)[1]				SEM	Contrast[2]			*P value*	
	CON	80:10	70:20	60:30		CON vs. FAT	Linear	Quadratic	Time	Trt x Time
Insulin, ug/L	0.26	0.27	0.31	0.31	0.01	0.02	0.07	0.55	0.04	0.21
NEFA, mEq/L	0.72	0.84	0.75	0.67	0.05	0.57	0.03	0.87	<0.01	0.56
BHB, mg/dL	10.6	11.5	13.3	14.8	1.53	0.14	0.10	0.91	0.37	0.15
Albumin, g/dL	3.08	3.27	3.17	3.18	0.06	0.11	0.26	0.48	<0.01	0.96

[1]Treatments were: 1) CON (control; no supplemental fat); 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 70:20 (1.5% of FA supplement blend to provide ~ 70% C16:0 and 20% *cis*-9 C18:1); 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1).
[2] P values associated with contrasts of treatment effects: CON vs. FAT [control vs. FA-supplemented diets; (80:10 + 70:20 + 60:30)/3]; Linear and quadratic effects of *cis*-9 C18:1 inclusion in supplemental fat.

Table 8.7. Plasma insulin and metabolites for cows fed a common diet during the carryover period.

	Treatment (Trt)[1]				SEM	Contrast[2]		
	CON	80:10	70:20	60:30		CON vs. FAT	Linear	Quadratic
Insulin, ug/L	0.38	0.42	0.37	0.38	0.02	0.81	0.38	0.58
NEFA, mEq/L	0.40	0.37	0.41	0.41	0.07	0.76	0.49	0.68
BHB, mg/dL	6.49	5.54	5.64	7.36	0.86	0.75	0.13	0.44
Albumin, g/dL	3.34	3.41	3.41	3.39	0.06	0.43	0.80	0.96

[1]Treatments were: 1) CON (control; no supplemental fat); 80:10 (1.5% of FA supplement blend to provide ~ 80% C16:0 and 10% *cis*-9 C18:1); 70:20 (1.5% of FA supplement blend to provide ~ 70% C16:0 and 20% *cis*-9 C18:1); 60:30 (1.5% of FA supplement blend to provide ~ 60% C16:0 and 30% *cis*-9 C18:1).
[2] P values associated with contrasts of treatment effects: CON vs. FAT [control vs. FA-supplemented diets; (80:10 + 70:20 + 60:30)/3]; Linear and quadratic effects of *cis*-9 C18:1 inclusion in supplemental fat.



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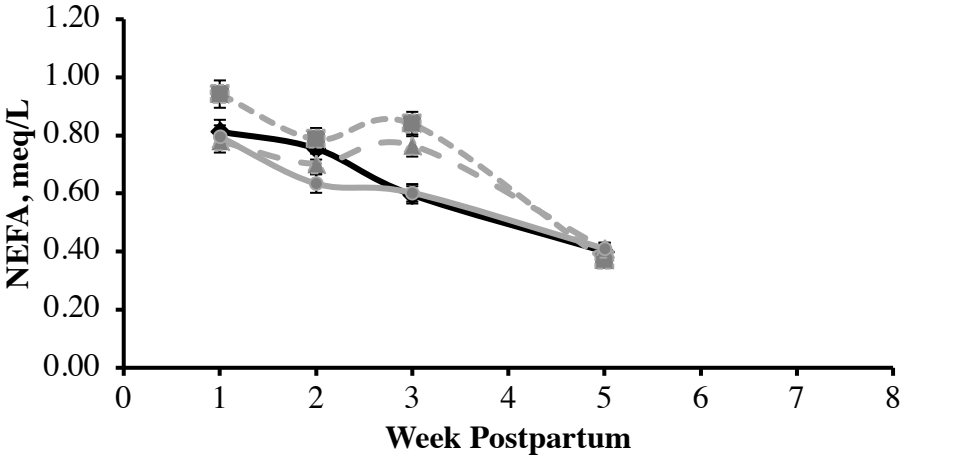

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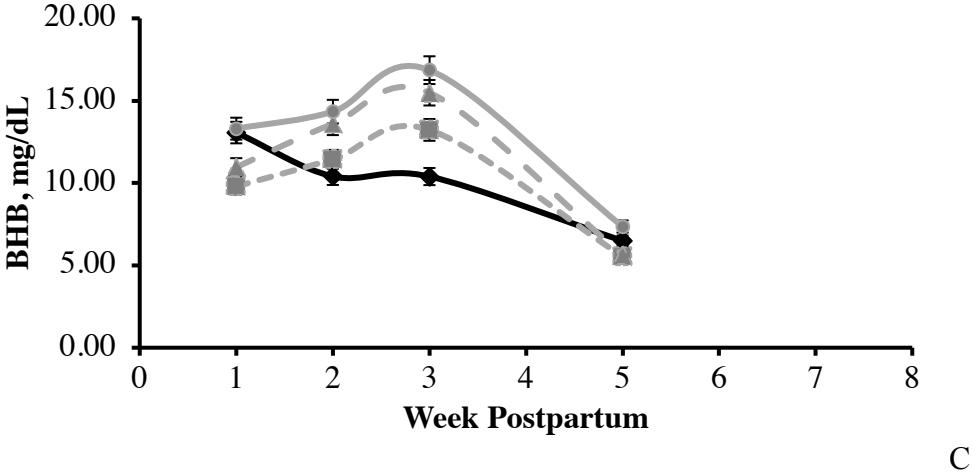

C

Figure 8.1. Effects of dietary treatments on plasma insulin (A), NEFA (B) and BHB (C) over time during the treatment (weeks 1-3) and carryover (weeks 4-8) periods.
Diets fed during the treatment period were CON (control; non-FA supplemented diet; black line); 2) 80:10 (80% C16:0 + 10% *cis*-9 C18:1; grey short-broke line); 3) 70:20 (70% C16:0 +

20% *cis*-9 C18:1; grey long-broke line); and 4) 60:30 (60% C16:0 + 30% *cis*-9 C18:1; grey line). The line on wk 3 indicates the start of the carryover period, when all cows were fed a common diet with no supplemental fat added. During the treatment period, compared with CON, FA-supplemented diets increased plasma insulin ($P = 0.02$). Also, we tended to observe an interaction between treatment and time for BHB ($P = 0.15$) due to FA-supplemented diets increasing BHB compared with CON at wk 3. Increasing *cis*-9 C18:1 in FA treatments linearly decreased plasma NEFA ($P = 0.03$), and tended to linearly increased insulin ($P = 0.07$) and BHB ($P = 0.10$). Error bars indicate SEM.

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CONCLUSIONS

Results reported in this dissertation have examined the effects of altering dietary FA ratios on digestion, metabolism, and production of dairy cows. In chapter 3, we observed that diet with whole cottonseed increased milk fat yield and energy partitioning to BW, without reducing milk energy output. Among the combinations of C16:0, C18:0 and *cis*-9 C18:1 evaluated, fat supplements with more C16:0 increased energy output in milk, while fat supplements with more *cis*-9 C18:1 increased energy storage as body weight. The fat supplement with more C18:0 reduced nutrient digestibility, which most likely explained its lower performance compared with the other treatments. Our results suggested that C16:0 and *cis*-9 C18:1 are able to alter energy partitioning between the mammary gland and adipose tissue, which may allow for different FA supplements to be used in different situations according to the metabolic priority of dairy cows and management needs. In response to these results, we further explored the effects of altering dietary C16:0 and *cis*-9 C18:1 under different physiological conditions in dairy cows.

We evaluated the effects of C16:0 supplements on energy partitioning of lactating cows during different stages of lactation. In Chapter 4, since most studies feeding C16:0 supplements have been conducted on short-term feeding (maximum 21 d of feeding), we evaluated the effects of long-term C16:0 supplementation and parity on nutrient digestibility and the yield of milk and milk components of mid-lactation dairy cows. Our long-term results were consistent with those reported previously in the literature from shorter term studies. We observed that feeding a C16:0 supplement consistently increased NDF digestibility, milk fat yield, energy-corrected milk, and energy partitioning to milk of mid-lactation dairy cows. In addition, C16:0 supplementation interacted with parity with production responses increased to a greater extent in multiparous than

primiparous cows when C16:0 was fed. Also, C16:0 increased BW change and plasma insulin in primiparous but not in multiparous cows. Interestingly, we consistently observed increases in NDF digestibility as previously reported in other studies, though the mechanisms need further research.

In Chapters 5 and 6, we evaluated the effects of C16:0 supplements on production and metabolic responses of early lactation cows. Feeding a C16:0-enriched supplement to early lactation cows consistently increased the yield of ECM throughout early lactation compared with a non-fat control diet. Our results suggest that feeding C16:0 during early lactation increased milk fat yield to a greater extent than previous studies with post-peak cows, but this is also partially related to an increase in the yield of preformed milk FA likely coming from adipose tissue. Feeding C16:0 during the fresh period (1-24 DIM) increased milk energy output but also increased negative energy balance, plasma NEFA, BW and BCS losses, and reduced plasma insulin concentration. Further studies are needed to evaluate the potential effects of greater BW and BCS losses on health and reproduction of dairy cows. For some variables, the effect of feeding C16:0 were affected by timing of supplementation since milk yield only increased after the fresh period while BW was reduced to a greater extent in the fresh period when C16:0 supplement was fed. For FA digestibility, the effect of feeding C16:0 were affected by the timing of supplementation since feeding C16:0 during the peak period (25 to 67 DIM) only reduced total FA digestibility and 16-carbon FA digestibility in cows that received the control diet during the fresh period.

Chapters 7 and 8 evaluated the effects of altering the dietary ratio of C16:0 and *cis*-9 C18:1 in post-peak and early lactation cows, respectively. Similar to the results in Chapter 3, we observed that increasing dietary *cis*-9 C18:1 increased FA digestibility and BW change (Chapter

7) and reduced BW losses in fresh cows (Chapter 8). Interestingly, we consistently that plasma insulin increased as we increased dietary *cis*-9 C18:1, which may indicate a role for this FA in mediating insulin and energy partitioning. The mechanisms relating *cis*-9 C18:1 and insulin deserves further investigation. Furthermore, in Chapter 7 we observed that high producing dairy cows (averaging 60 kg/d) responded better to FA supplements containing more *cis*-9 C18:1, while lower producing cows (averaging 45 kg/d) responded better to FA supplements containing more C16:0. This interaction is intriguing since a previous study feeding C18:0 observed similar tendencies. Based on this, we are unsure if these results are directly associated with *cis*-9 C18:1 or an indirect effect of increasing dietary 18-carbon FA. Further studies are needed to determine whether high producing cows may respond better to supplements containing 18-carbon FA, or to a specific 18-carbon FA. Finally, in Chapter 8 we also observed that the diets fed during the immediate postpartum period (1-24 DIM) had a tremendous carryover effect during early lactation, when cows were fed a common diet. Although carryover effects of different nutrition strategies were previously reported, the magnitude of these differences and the mechanisms related with it deserves future investigation.

Altogether, these studies increased our understanding of FA metabolism in the rumen, small intestine, adipose tissue, and mammary gland. Our results indicate that different combinations of FA should be used according to production level and stage of lactation in dairy cows. Our results will help nutritionists and dairy farmers determine whether they should feed FA supplements, which FA supplement or combination of FA should they feed, and to which groups of cows.