THE EFFECTS OF SUPPLEMENTAL FATTY ACIDS ON PRODUCTION AND NUTRIENT DIGESTIBILITY RESPONSES OF LACTATING DAIRY COWS

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Animal Science – Master of Science

2018

ABSTRACT

THE EFFECTS OF SUPPLEMENTAL FATTY ACIDS ON PRODUCTION AND NUTRIENT DIGESTIBILITY RESPONSES OF LACTATING DAIRY COWS

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Addition of fat supplements to dairy rations is becoming more common due to the increases in milk yield and milk fat yield that have been observed. This thesis contains two studies that evaluated the effects of palmitic (C16:0), stearic (C18:0), and oleic (C18:1) acids in the form of commercially available supplements (C16:0 and C18:0-enriched) or as custom blends (C16:0 and C18:1) on lactating dairy cows. The first experiment used two commercially-available products enriched in either C16:0 (PA) or C18:0 (SA) supplied at 1.5% diet dry matter (DM) and a control diet with no added fat. Fat supplementation increased milk yield, but decreased total FA digestibility when compared to control. PA increased digestibility of total, 16- and 18-carbon FA as well as NDF digestibility, energy corrected milk (ECM), and milk fat yield when compared to SA. In the second experiment, the effect of differing ratios of C16:0 and C18:1 (fed at 1.5% diet DM) was determined using blends that consisted of 80% C16:0 + 10% *cis*-9 C18:1 (80:10) or 60% C16:0 + 30% C18:1 (60:30) across a wide range in production level. Interactions between preliminary milk yield and treatment were observed for dry matter intake (DMI), and yields of ECM and 3.5% fat-corrected milk (3.5% FCM), indicating that higher producing cows responded better to the 60:30 and lower producing cows responded better to the 80:10. 60:30 increased digestibilities of total, 16- and 18- carbon FA compared with 80:10. Together, this work will provide information that can be used to guide feeding decisions to maximize performance and farm income while using commercial FA supplements.

ACKNOWLEDGEMENTS

First, I would like to thank Dr. Adam Lock for all his help throughout my academic endeavors. His mentorship and guidance throughout my time at Michigan State University have helped shaped my scientific and professional career. I can't thank him enough for his assistance in building my confidence in a scientific setting and learning how to use my skills in different ways including writing manuscripts, completing on-farm trials, composing presentations and public speaking.

I would also like to thank Dr. VandeHaar, Dr. Contreras, and Dr. Rowntree for their assistance on my graduate committee. Learning from all of you through class, experiments, or other meetings have helped me gain other exposure to the animal science industry and appreciate the diversity of our program. I am also thankful for their help on the completion of my program that couldn't have been done without their edits and critiques that helped me grow as a scientist.

Our lab group deserves more thanks than I could ever express to them. Lynn Worden, our lab manager, helped me in ways in I never imagined I would need help in. She is truly the foundation of the lab group and keeps everything on track for success. I would also like to thank Jonas de Souza for his mentorship during my time as an undergraduate and Master's student. I truly believe I wouldn't be completing this document if it wasn't for him. Also, thank you to all current and previous Lock Lab employees for their guidance, assistance, and friendship.

I also extend a sincere thank you to the MSU dairy farm staff for all their hard work every day with the cows that allow all of us to do the research that we do. Their constant thoughtfulness and care for the animals does not go unnoticed.

I would also like to thank Vita Plus Corporation for their financial, emotional, and educational support during my Master's program. None of this would have been possible without all of you and I can't wait to join the team!

The biggest thank you I extend to my family and friends for supporting me through this process. Thank you for always supporting me and believing in me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

BCS	Body condition score
BHBA	Beta-hydroxybutyric acid
BH	Biohydrogenation
BW	Body weight
CCK	Cholecystokinin
CLA	Conjugated linoleic acid
CP	Crude protein
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
ECM	Energy-corrected milk
EE	Ether extract
FA	Fatty acids
FAME	Fatty acid methyl ester
FAS	Fatty acid synthase
FCM	Fat-corrected milk
FFA	Free fatty acids
GLC	Gas liquid chromatography
GLUT	Glucose transporter
G3P	Glycerol-3-phosphate
ME	Metabolizable energy

MFD	Milk fat depression
MUFA	Monounsaturated fatty acids
MUN	Milk urea nitrogen
NADPH	Nicotinamide adenine dinucleotide phosphate
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acids
OA	Oleic acid
PA	Palmitic acid treatment
60:30	60% palmitic, 30% oleic acid treatment
PL	Phospholipid
PUFA	Polyunsaturated fatty acids
SA	Stearic acid treatment
SEM	Standard error of the mean
SD	Standard deviation
SFA	Saturated fatty acids
TG	Triglycerides
TMR	Total mixed ration
UFA	Unsaturated fatty acids

CHAPTER 1

INTRODUCTION

FA supplementation has been studied for many years, and many studies have shown beneficial effects as highlighted in the 100-year review completed by Palmquist and Jenkins (2017). For example, FA supplementation has been shown to increase the yield of milk and milk fat (Rabiee et al., 2012), alleviate heat stress (Wang et al., 2010), improve reproductive efficiency (R. Rodney et al., 2015), and modulate energy metabolism (Staples et al., 1998; Hutchinson et al., 2012). Currently, milk component yield is the principal driver of variation in producer milk price, which underlies the importance of focusing on increasing the yields of these components. Milk fat can be manipulated through diet and management, which has increased overall feeding of fat supplements. These benefits are increasingly important to maximize production with lactating dairy cows and increase profitability. However, great variation has been observed between studies possibly due to type of fat supplement, level of supplementation, stage of lactation, or production level of cows.

We propose that the FA profile of a fat supplement and the production level of the cow are most likely the major factors affecting the response to it. Therefore, there is increased interest in the role of individual FA and combinations of FA and how they could be included in rations for dairy cows. Commonly used fat sources include oilseeds, like cottonseed or soybeans, animal fat, and FA supplements. Palmitic (C16:0), stearic (C18:0) and oleic (*cis-9* C18:1) acids are three primary FA found in commercial fat supplements as well as in milk fat and adipose tissue (Palmquist et al., 2006; Douglas et al., 2007). These three FA have been studied extensively to assess their individual effects on milk production, digestibility, and metabolism in dairy cows.

To our knowledge, few studies have evaluated the effects of commercially-available FA supplements or ratios of different FA and their effects on production and nutrient digestibility across production level or over longer term. It is crucial to understand how these supplements impact performance to ensure proper nutrition for the cow. This will advance understanding of supplementation and the functionality of FA supplementation and nutrition. Therefore, the main objective of this dissertation was to examine the effects of C16:0, C18:0 and *cis*- C18:1 on production responses and nutrient digestibility.

CHAPTER 2

LITERATURE REVIEW

Importance of Milk Components

Milk fat and protein yield are the major price indicators used by the Federal Milk Order Program when establishing milk prices. Therefore, increasing milk component yields increases farm income. Importantly, milk fat is easier to influence than milk protein through dietary manipulation both positively and negatively. As a result, dietary strategies have become a topic examined by researchers to increase yield of milk fat and farm profitability.

Rumen Metabolism of Dietary Fats

For ruminant animals, fatty acids (FA) are extensively metabolized in the rumen. This metabolism has major effects on the FA that are later absorbed and utilized throughout the body (Doreau et al., 2017). FA in feeds commonly fed to ruminant animals are present mainly in triglycerides, phospholipids and glycolipids (Lock et al., 2005). Grasses contain mostly linolenic acid (*cis*-9, *cis*-12, *cis*-15, C18:3) while grains contain mostly linoleic acid (*cis*-9, *cis*-12, C18:2), meaning most dietary FA are unsaturated (UFA). However, the main FA reaching the intestine are saturated due to biohydrogenation (BH) in the rumen (Harfoot and Hazlewood, 1997).

The main processes that contribute to FA metabolism in the rumen are hydrolysis and BH (Figure 2-1). Harfoot and Hazlewood (1997) concluded the limiting step for BH is exposing plant lipids from their surrounding matrix. Once this step is completed, the lipids go through hydrolysis (Palmquist et al., 2005). This step releases energy and the FA from the glycerol backbone to

allowing the FA to be biohydrogenated (Buccioni et al., 2012). BH is the reduction of double bonds on FA carbon chains (Buccioni et al., 2012). BH occurs primarily to ensure rumen bacterial health by mediating negative effects of UFA due to the toxicity, which can be more or less toxic depending on the individual FA (Maia et al., 2010). Saturated FA found in the rumen do not change, while UFA undergo BH by ruminal microbes. 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.

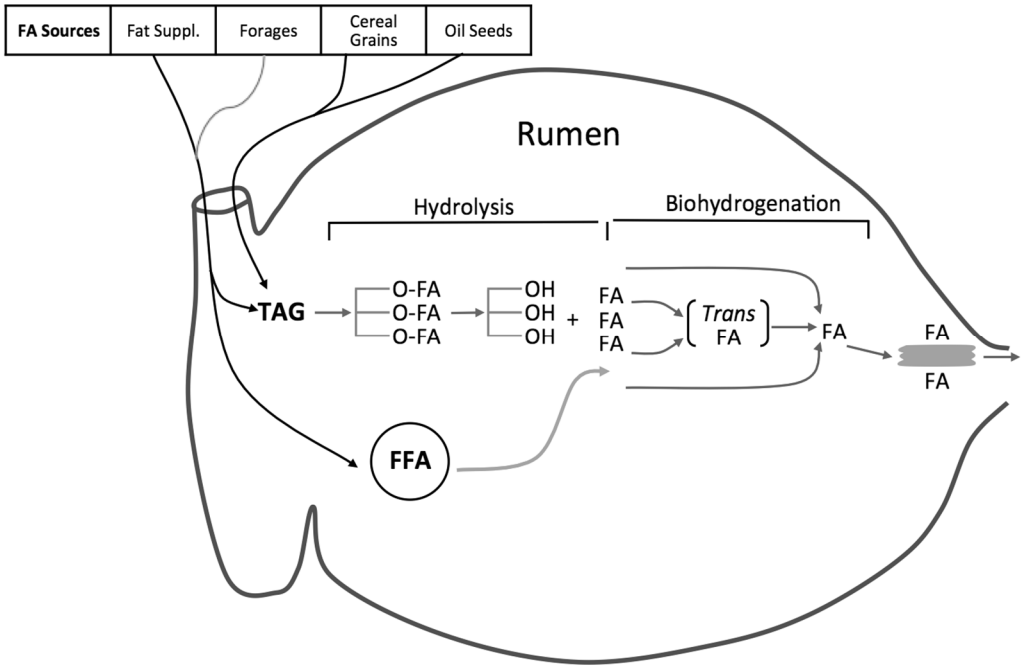

Figure 2-1. Metabolism of dietary lipids in the rumen. Triglycerides (TG), glycolipids (GL), phospholipids(PL), trans fatty acids (trans FA), mixture of fatty acids (FAs), and volatile fatty acids (VFA). Adapted from Lock et al., 2006.

BH requires many steps, but ends with the formation of SFA (mostly C18:0) (Harfoot and Hazlewood, 1997). Since most of the FA found in rations for dairy cows are 18-carbon UFA, the main FA exiting the rumen is C18:0 after BH (Palmquist, 2006). UFA are toxic to certain bacterial

species in the rumen (Maia et al., 2010) and can affect BH by shifting fermentation to alter native pathways (e.g. Figure 2-2). Altered fermentation pathways of BH can produce intermediates, including CLA, that cause milk fat depression (MFD) (Bauman et al., 2011). The main factors that can alter BH include changes in rumen pH, UFA content, and fermentability of the diet. Allen (1997) summarized the relationship between milk fat and rumen pH; as ruminal pH decreased, milk fat concentration also decreased. Low pH in the rumen inhibits microbial growth and changes the population of the bacteria that are present (Russell and Willson, 1996). These bacteria are extremely sensitive and can be altered by even small changes in pH. UFA are toxic in the rumen and can increase BH to compensate for this. However, the structure of these FA have been proposed to disrupt rumen metabolism because of the double bonds in their structure (Maia et al., 2010). This effect appears to increase with increased unsaturation of FA. Increasing dietary fermentability is negatively related with milk fat in a meta-analysis by Ferraretto et al. (2013). Causes for this include the fermentation of starches inhibiting BH and shifting pathways to include MFD intermediates, mostly because of a change in ruminal pH.

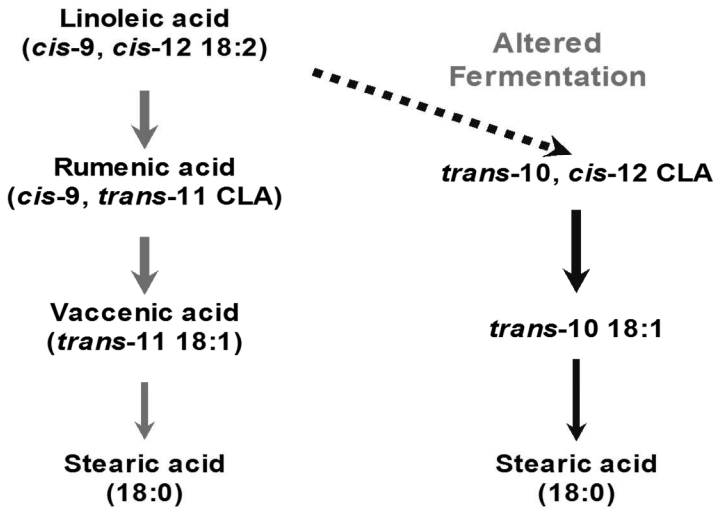

Figure 2-2. Biohydrogenation pathways of dietary lipids in the rumen. Adapted from Bauman et al., 2003.

Digestion and Absorption of Dietary FA

Under typical feeding situations, C18:0 is the predominant FA available for absorption by the dairy cow, regardless of the diet fed. Most absorption of FA takes place in the small intestine, specifically the jejunum. The FA that are transported from the rumen include mostly free FA attached to feed particles and microbial phospholipids (Doreau et al., 1994). For the FA to be absorbed, they must be solubilized in the surrounding environment. The low pH (< 2.5) in the ruminant small intestine keeps the FA in a protonated state, and attached to feed particles (Drackley, 2005). Bile and pancreatic secretions supply bile salts, lecithin, and pancreatic juice (Bauman and Lock, 2006) to aid in the formation of micelles to be absorbed (Doreau et al., 1994). Lecithin is converted to lysolecithin by pancreatic phospholipase A_2 and increases the pH with bicarbonate (Figure 2-3). Lysolecithin, an amphiphile, desorbs the FA from feed particles and bacteria to begin the transfer to a micellar phase (Moore and Christie, 1984; Bauman and Lock, 2006). This step is required for FA absorption. Importantly, lysolecithin is the most effective amphiphile at increasing the distribution of C18:0 to the micellar phase (Freeman, 1969 and 1984). Since C18:0 is the main FA exiting the rumen, this explains the efficiency of the FA digestion and absorption process with the assistance of lysolecithin. Once micelles are formed, the water-soluble FA molecule diffuse across the lipid bilayer through an energy-independent process for absorption into the enterocyte (Lock et al., 2006).

Once FA are absorbed in intestinal cells, they are incorporated back into triglycerides (Drackley, 2004). The triglycerides are then combined into lipoproteins. Free polyunsaturated FA (PUFA) from the intestine are incorporated into phospholipids and cholesterol to prevent oxidation (Moore and Christie, 1984).

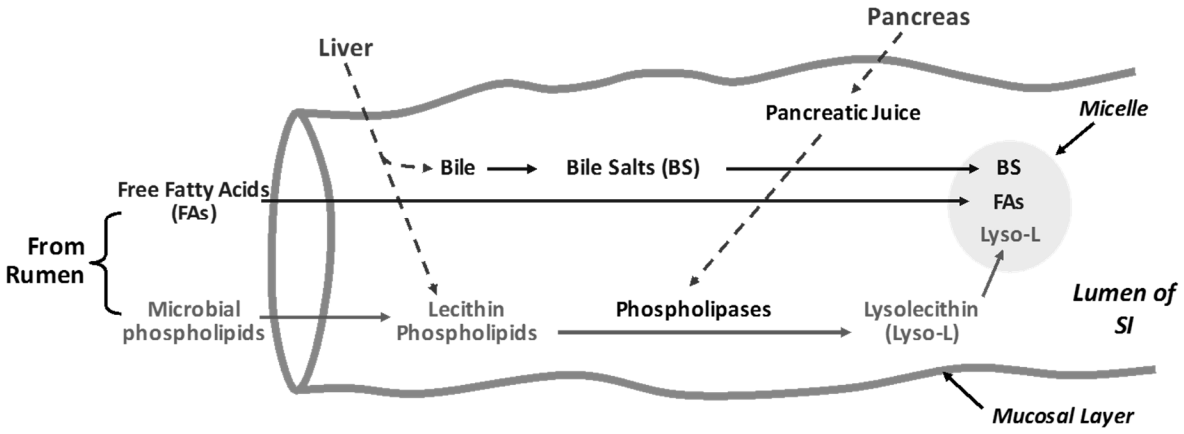

Figure 2-3. Fat digestion in the small intestine of ruminants. Adapted from Lock et al., 2006.

As mentioned above, lysolecithin is an amphiphile (Freeman, 1969). Amphiphiles combine hydrophobic and hydrophilic molecules to aid in digestion and absorption. Compared to other amphiphiles, lysolecithin was the most effective to increase C18:0 transformation to micelle phase (Freeman, 1984). Even though, this is the same process that takes place in all ruminants, there is variation in FA digestibility. Boerman et al., (2015) examined results from 15 publications and calculated intestinal FA digestibility. Interestingly, the range of total FA intestinal digestibility ranged from 71.5% to 78.0%, with the average being 74.7%. Differences were observed between individual FA with higher digestibility for UFA and lower digestibility with SFA.

Most studies evaluate digestibility of lipids measuring the entire digestive tract using total tract digestibility measurements. As expected, FA digestibility decreases with increased fat content in diets (Piantoni et al., 2015; de Souza et al., 2018). There have been differences detected between individual FA in the few studies where flow of FA through the duodenum was observed. Boerman et al. (2015) observed as the flow of C18:0 through the duodenum increased, FA digestibility

decreased linearly. In a dose response study completed by Rico et al. (2017) he observed as supplementation of C16:0 increased, digestibility decreased, but not at the same rate as observed by Boerman et al. (2017). These decreases in digestibility suggest that absorption of FA in the small intestine may be limited when the supply of FA increases (Bauchart et al., 1993). Specifically, it has been proposed that the amount of lysolecithin or the increased competition for absorption sites when the amount of FA in the small intestine increases, could be a potential limiting factor (Drackley, 2000). Once absorbed, FA are re-esterified to be transferred and used throughout the body as precursors for milk fat or stored as adipose tissue.

Recently, research has examined the effects of dietary C16:0, C18:0 and *cis*-9 C18:1 on FA digestibility. Analyzing results from Rico et al. (2017), and Boerman et al. (2017), outflow of C18:0 decreases digestibility of FA at a more pronounced rate compared to C16:0 (Figure 2-3). However, supplementary, C18:1 increased FA digestibility compared with C16:0 and C18:0 (de Souza et al., 2018). This increase in FA digestibility with C18:1 was also observed in an abomasal infusion study which increased FA digestibility and tended to increase milk yield, ECM, and 3.5% FCM as amount of C18:1 increased (Prom, 2018 ADSA Abstract). More research is required to assess the exact mechanisms between the individual FA and what drives increases and decreases in digestibility.

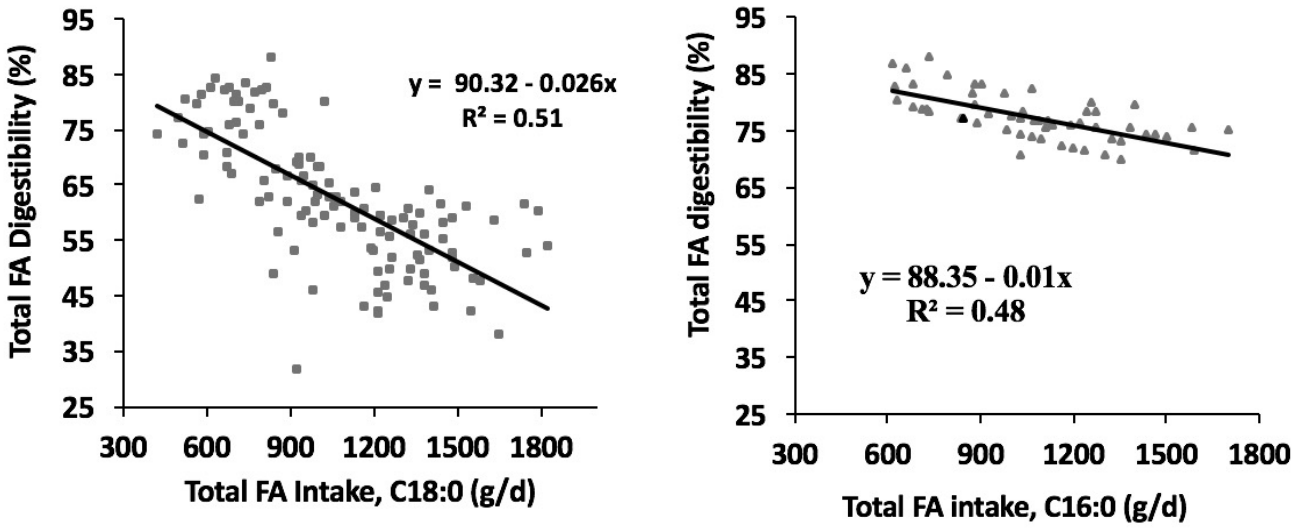

Figure 2-4. Total FA digestibility of C16:0 and C18:0. Adapted from Boerman et al., 2017 and Rico et al., 2017.

Milk Fat Synthesis

Milk fat is a major component in milk and is the component with the highest energy content, but also constitutes the major 'energetic investment' of milk synthesis (Harvatine and Bauman, 2008). Milk fat can be effected by many factors and therefore, is the most variable component of milk (Harvatine and Bauman, 2009). As mentioned above, most dietary FA are unsaturated, but due to BH in the rumen, mainly SFA are present in milk fat and adipose tissue. As a result, ruminant milk fat is different from others due to increased SFA. Bovine milk fat concentration typically ranges from 3.7% to 4.1% and it is mostly composed of triglycerides (98%), and phospholipids, diglycerides, and cholesterol (Jensen, 2002). The FA found in milk fat can exceed 400 different FA (Jensen, 2002) with different structures, chain length and configuration. Milk fat is the most variable component in milk and can be effected by genetics, physiological state, environment and especially nutrition (Bauman and Griinari, 2003). Milk FA synthesis is produced in two ways; 1) uptake from circulating FA and 2) de novo synthesis and 2

(Bauman and Griinari, 2003). Mixed source FA (C16:0 and C16:1) originate from de novo synthesis in the mammary gland, and extraction from plasma.

De Novo FA Synthesis

Synthesis of short and medium chain FA in the mammary gland occurs by de novo synthesis. In ruminants, most glucose originates from gluconeogenesis and is used in combination with acetate to initiate lipogenesis in adipose tissue and the mammary gland (Laliotis et al., 2010). Most of the reduction products are generated as NADH and originate from the pentose phosphate pathway (Emery et al., 1973). It is important to point out that although several precursors can initiate FAS, Acetyl-CoA is the principal building block that is used by the complex of FAS generating palmitate and carbon sources include acetate and betahydroxybutyrate. In ruminants, a major metabolic difference is the limited carbon from glucose contributing to FA synthesis (Palmquist, 2006). Hansen and Knudsen (1980) postulated that the more relaxed specificity of the acyl transferase in ruminants, relative to non-ruminants, was responsible for the release of significant amounts of short and medium-chain FA.

Preformed FA

Preformed FA for milk fat synthesis mainly come from the absorption of dietary FA, thus making most them saturated 18-carbon FA. 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), but FA can be utilized from the mobilization of FA from body reserves

in the form of NEFA, but this only accounts for a small percentage (Bauman and Griinari, 2003) when cows are not in negative energy balance. The mammary gland takes up FA released from the TAG-rich lipoproteins or FA within the albumin-FA pool. These FA for TAG synthesis in the mammary gland.

Triglyceride Synthesis

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 on the glycerol-3 phosphate backbone (Dils, 1983). Glycerol phosphate acyl transferase (GPAT) adds 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 the FA in the glycerol backbone is not random due to different specificty by individual FA (Jensen, 2002). SFA are predominantly esterified at the *sn*-1, UFA at the *sn*-2 position, and short and medium chain FA at *sn*-3 (Jensen, 2002). One product of de novo synthesis, C16:0, is key for this process and has a higher preference as a substrate for GPAT than C18:0 and C18:1 in the mammary gland (Kinsella and Gross, 1973). C16:0 esterification is distributed uniformly between *sn*-1 and *sn*-2, while C18:0 and C18:1 are primarily esterified at *sn*-1 and *sn*-3. The control of placement of FA allows the mammary gland to secret TAG into lipid droplets to be incorporated into milk fluidly (Jensen, 2002). 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). C18:0 can also be converted to C18:1 by Δ9-desaturase enzyme, which is important in regulation (Bauman and Griinari, 2003).

Effects of Fatty Acid Supplementation

This section will discuss the effects of FA supplementation on lactating dairy cows. Recent meta-analyses completed by Rabiee et al. (2012), Boerman et al. (2015), Weld and Armentano (2017) and a short-communication from de Souza and Lock (2018) are suggested if additional information is requested.

Fat supplementation is a common way to increase the energy density of the ration and sustain production measures in dairy cows. Interest in FA supplementation has increased as FA supplements become more available in the to the dairy industry. Extensive research has been completed to review the effects of feeding FA to dairy cows and differences have been reported depending on feeding rate, production level, and FA profile. Recent focus in our lab has been on individual FA and their specific effects on performance of lactating dairy cows.

Increases in the yield of milk and milk fat have all been associated with supplementation of FA, but results have varied including negative impacts on DMI (Rabiee et al., 2012). There are differences in effects across FA supplements, but variation also exists across studies when the same supplement was used. These results have led to research assessing FA profile, inclusion rate, and effects of production level of FA being supplemented.

Effects on DMI

Variable results on DMI when supplementing FA to dairy rations can been seen in a meta-analysis by Allen (2000). It was concluded that overall, adding fat decreases DMI, but there were many impacting factors. Supplemented FA levels in the rations varied and different results were observed depending on the profile of the supplement. Unprocessed animal fat and Ca-salts of palm FA had negative linear effects when supplemented to cows, but there was no effect on DMI when supplementing hydrogenated saturated fats. The largest decrease was observed with Ca-Salts of palm FA, as dietary FA inclusion increased (Allen, 2000).

As mentioned, as the degree of unsaturation increases, the hypophagic effects become more elevated. With the decreases in DMI, decreases in milk yield have also been associated with supplementation of UFA (Christensen et al., 1994). These effects are likely due to an altered environment in the rumen when UFA are present (Maia et al., 2007). The presence of UFA in the rumen may have negative effects on nutrients, and consequently milk production due to shift in the rumen bacterial population. SFA have minimal effects on rumen microbial activity (Palmquist and Jenkins, 1980). Because of this, more rumen inert FA supplements have become available to reduce ruminal effects.

Effects on FA Digestibility

FA digestibility when supplementing FA has been of increasing importance due to the impact it has on energy intake of the cow. Generally, supplementing fat results in a decrease in FA digestibility. A meta-analysis by Boerman et al. (2015) observed that as C18:0 duodenal flow

increased, FA digestibility decreased. Another study by Boerman et al. (2017) supplemented C18:0 at increasing levels and saw no effect on production responses, but a linear decrease in total FA digestibility as FA intake increased. Rico et al. (2017) completed a similar study, but with increasing levels of C16:0, and observed a positive effect on production up to 1.5% diet DM. Even though there was an increase in production, there was still a decrease in total FA digestibility as FA intake increased. However, when comparing these two studies, the decrease in FA digestibility is more prominent when supplementing C18:0 compared to C16:0 (Figure 2-3).

The exact mechanisms for this are unknown, but may be due to lower solubility of C18:0 (Palmquist and Jenkins, 2017). The meta-analysis by Boerman et al. (2015) reported that C18:1 had greater digestibility than C16:0 or C18:0. This has been related to amphiphilic properties of C18:1 that have positive effects on micelle solubility of C18:0 (Freeman, 1969). Due to ruminal biohydrogenation, the main FA flowing out of the rumen is C18:0. The increase in digestibility with C18:1 may be due to the ability to effect solubility of the major FA present in the small intestine.

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. Currently, it is believed that different supplemental FA have similar energy values. Previously, the majority of research determined digestible energy intake through calculations (NRC, 2001) which has mostly been measured during early lactation. Bomb calorimetry can be used to determine actual energy intake, however, there is limited research using bomb calorimetry. Moallem et al. (2007) fed a supplement enriched in palmitic and stearic acid and reported that there was no difference in predicted energy intake compared with no supplemental fat. Bomb

calorimetry can be used to correctly determine potential energy differences between supplemental FA sources.

Effects on Nutrient Digestibility

It is generally thought that fat supplementation decreases NDF digestibility. Older studies concluded that addition of vegetable oils have negative effects on fiber digestion (Palmquist and Jenkins, 2017), however, recent research has challenged this general dogma.

A meta-analysis concluded that although supplements high in medium-chain FA, and vegetable oil decreased NDF digestibility, there was a tendency for saturated fats to increase NDF digestibility (Weld and Armentano, 2017). Increases in NDF digestibility have been observed when supplementing C16:0 (Piantoni et al., 2013; de Souza et al., 2018, de Souza and Lock, 2018), even when there is no decrease in DMI. Piantoni et al. (2013) suggested that increases in NDF digestibility could be due to increased secretion of cholecystokinin (CCK), leading to an increased retention time in the rumen. The increase in NDF digestibility when feeding C16:0 may also be due to reduced ATP usage if dietary C16:0 could be included to order membrane of buty species in the rumen (Vlaeminck et al., 2006; Hackmann and Firkins, 2015).

Increases in NDF digestibility has not been observed with C18:0 (Piantoni et al., 2015; Boerman et al., 2017; de Souza et al., 2018). de Souza et al. (2018) observed increased NDF digestibility with blends including mostly C16:0 and C18:1 compared to a supplement including mostly C18:0. Most of the increases observed with C18:1 have been associated with the decrease in DMI, but C16:0 has increased NDF digestibility typically when having no effect on DMI.

Increasing NDF and FA digestibility with C16:0 has been associated with positive impacts on production measurements.

Effects on Production Responses

The effect of individual FA on production responses of dairy cows has recently received renewed attention. Milk yield and especially milk fat yield increases are common with fat supplementation. A recent meta-analysis reported over 1 kg/cow/d increase in milk yield and a tendency to increase milk fat yield when fat was supplemented (Rabiee et al., 2012). Most studies used in that meta-analysis supplemented tallow and Ca-salts of Palm, with few studies using prilled FA supplements. The few studies that used SFA supplements resulted in all prilled supplements being grouped together, not separated by FA profile.

Milk fat concentration and yield decreases were observed as the degree of unsaturation increased (Harvatine and Allen, 2006). As expected, this effect was more pronounced in high producing cows, most likely due to the increased passage rates. UFA have negative effects on ruminal fermentation, which can result in the accumulation of specific BH intermediates (Coppock et al., 1991). These specific intermediates have been associated with reduced milk fat synthesis in the mammary gland (Bauman et al., 2011).

Supplemental fat including C16:0 and C18:1 have been associated with increased milk yield, milk fat yield and concentration, ECM and 3.5% FCM (Rico et al., 2014; Piantoni et al., 2013, de Souza et al., 2018). de Souza et al. (2018) evaluated the effects of altering the ratio C16:0, C18:0, and C18:1 and determined C16:0 was associated with more milk energy output than the

other FA and C18:1 partitioned more energy to body reserves. Decreased digestibility measurements, and therefore lower milk performance, were observed with C18:0.

de Souza et al. (2017 ADSA Abstract) altered the ratio of C16:0 and C18:1 in supplement fat on production and digestibility measurements. The study used production groups and four ratios from 80% C16:1 and 10% C18:1 up to 60% C16:0 and 30% C18:1 and observed that increased C18:1 increased ECM, 3.5% FCM and milk yield in the high group while in the low group, these variables were increased with increased C16:0. A large magnitude of change in this study between treatments was observed through increases in ECM in the high group of 7 kg/d between those two treatments. Interestingly, in this study as well, increased C18:1 increased BW and BW changes. Additionally, it was observed that plasma insulin increased with increased C18:1, which is consistent with de Souza et al. (2018). Insulin is an antilipolytic hormone and elevated insulin concentrations may reduce lipolysis or increase lipogenesis in adipose tissue (Vernon, 2005). It was proposed that increased insulin reducing lipolysis was the primary factor for repartitioning of energy towards body reserves. Therefore, the effect of C18:1 on energy partitioning to body reserves could be linked to increased insulin concentrations and/or production of biohydrogenation intermediates in milk.

Supplementation of C16:0, C18:0, and C18:1 also have impacts on milk FA. Supplementation of long chain FA increase yields of preformed milk FA while C16:0 supplementation increases the yield of de novo and mixed source FA (Boerman et al., 2017, de Souza et al., 2018). The effects of feeding FA supplements depend on many variables including inclusion rate, production level, and type of FA in the supplement.

Conclusion

Through multiple processes and mechanisms from ingestion through excretion, cows are excellent at utilizing energy from feeds to use for production, reproduction, and other metabolic needs. Continued interest in FA supplementation to increase milk yield, component yields, and overall cow productivity warrants further research to investigate effects of commercial FA supplements and potential blends of FA and how they increase production and can increase farm profitability. We propose that the FA profile of a fat supplement is most likely the major factor affecting the response to it. Understanding how and which FA affect production, digestibility and energy partitioning in lactating dairy cows should allow the development of nutritional management strategies that reduce the risk of overconditioning cows and improve milk component yields and milk income and possibly reproductive performance.

Our objectives were to determine the effects of commercially-available and blended FA products on production and digestibility measures with cows at different levels of production. Determining these factors through this thesis will advance overall knowledge about FA digestion and metabolism in lactating dairy cows and allow for more informed decision making for nutritionists and dairy farmers.

CHAPTER 3

EFFECTS OF COMMERCIALLY AVAILABLE PALMITIC AND STEARIC ACID-ENRICHED SUPPLEMENTS ON NUTRIENT DIGESTIBILITY AND PRODUCTION RESPONSES OF LACTATING DAIRY COWS

Abstract

We evaluated the effects of commercially available fatty acid (FA) supplements enriched with palmitic (C16:0) or stearic acid (C18:0) on nutrient digestibility and milk production of dairy cows. Thirty-six Holstein cows (146 ± 84 DIM) were used in a truncated Latin square design of treatments with two consecutive 35-d periods, with the final 5 d used for sample and data collection. Treatments were: 1) control (CON; diet containing no supplemental FA); 2) C16:0-supplement (PA; 84% C16:0, 4% C18:0, 9% C18:1); and 3) C16:0 and C18:0-supplement (SA; 33% C16:0, 53% C18:0, 5% C18:1). Supplements were fed at 1.5% DM and replaced soyhulls in CON. The statistical model included the random effect of cow nested within square and the fixed effects of treatment, period, square, and their interactions. Preplanned contrasts were: 1) overall effect of FA treatments [CON vs. FAT; 1/2 (PA + SA)]; and 2) effect of FA supplement (PA vs. SA). There were no effects of treatments on DMI, BW, or BW change. Compared with CON, FAT decreased digestibilities of total FA (CON = 76.7, SA = 76.3, (PA = 67.6%, $P < 0.01$), 16-carbon FA (74.3, 69.0, 68.0%, $P < 0.01$), and 18-carbon FA (CON = 78.3, SA = 82.1, PA = 67.2%, $P < 0.01$). Compared to SA, PA increased DM and NDF digestibilities by 3.6 and 4.8% units, respectively ($P<0.01$). PA also increased total FA and 18-carbon FA digestibilities ($P < 0.01$) but did not alter 16-carbon FA digestibility ($P = 0.55$) compared with SA. Using a Lucas test, apparent digestibility coefficients were 0.768 and 0.553 for the PA and SA supplements, respectively.

Compared with CON, FAT increased milk yield (CON = 43.1, SA = 45.7, PA = 44.8 kg/d, P = 0.01), tended to increase ECM (CON = 44.8, SA = 46.4, PA = 44.5 kg/d, P = 0.08), but did not affect yield of milk fat (CON = 1.55, SA = 1.65, PA = 1.52 kg/d P = 0.19) or milk protein (CON = 1.43, SA = 1.44, PA = 1.46 kg/d, P = 0.32). Compared to SA, PA increased ECM (P = 0.03) and milk fat yield, ($P < 0.01$) but had no effect on milk protein yield (P = 0.47). Our results indicate that high producing dairy cows respond better to a FA supplement enriched in C16:0 compared with a supplement containing both C16:0 and C18:0 which is likely due in part to PA increasing FA and NDF digestibility compared with SA.

Introduction

FA supplementation is commonly used to increase the energy density of the diets for dairy cows. Interest in FA supplementation has increased due to benefits observed from inclusion including increased milk yield and increased the yield of milk components. Palmitic (C16:0), stearic (C18:0) and oleic acid (*cis*-9 C18:1) are the three main FA that are found in milk fat, adipose tissue (Palmquist, 2006 and Douglas et al., 2007) as well as in commercially-available supplements. Extensive research has been completed to review the effects of feeding FA to dairy cows and differences have been reported depending on the feeding rate, production level and FA profile. We have recently carried out research with blends of FA using commercial supplements, but there is limited research evaluating commercial sources of C16:0 and C18:0.

Substantial research has been completed with saturated FA. C16:0 and C18:0 enriched-supplements have been investigated to examine production as well as nutrient digestibility results. A recent meta-analysis by Rabiee et al. (2012) reviewed 59 papers and their effects on FA supplementation, but grouped all prilled FA supplements together, not allowing assessment of

individual FA. However, differences have been observed between C16:0 and C18:0. It is well documented that C16:0 supplementation increases milk fat concentration and yield, and NDF digestibility (Piantoni et al., 2013; Mathews et al., 2016; de Souza et al., 2018). Rico et al. (2017) fed increasing levels of C16:0 to dairy cows over 21 days and observed that positive responses on production up to 1.5% with only small decreases in FA digestibility, even though intake of C16:0 was increasing. Boerman et al. (2017) completed a similar study but supplemented C18:0 at increasing levels for 21 days. They observed that FA digestibility decreased as FA intake increased and FA supplementation had no effect on milk production. FA digestibility decreased in both studies, but supplementation with C16:0 decreased FA digestibility to a much lesser degree than did supplementation with C18:0. A meta-analysis by Boerman et al. (2015) studied intestinal digestibility of C18:0 across 15 studies and concluded C18:0 flow through the duodenum reduces digestibility of many FA.

Rico et al. (2014) compared supplementation with nearly pure C16:0 and C18:0 over 21 days on post peak cows and observed that C16:0 increased milk fat and 3.5% fat-correct milk but had no effect on body weight or DMI compared to C18:0. The FA profile of supplements used in this study are not commonly found on farms today due to the cost of nearly pure supplements, as well as the FA profile of the byproducts that are available to make FA supplements. Recently, a review by Loften et al. (2014) suggested that feeding a combination of C16:0 and C18:0 is needed to optimize their utilization for milk production and overall performance of the dairy cow. In contrast, in a short-term study (21-d periods), de Souza et al. (2018) found no support for this theory because feeding a FA blend (40% C16:0 and 40% C18:0) reduced nutrient digestibility and NE_L intake and animal performance compared with other treatments with ratios or pure FA supplements. Our study will assess this suggestion to feed a blend of C16:0 and C18:0 and add to

the understanding of supplements available for producers on production and digestibility measurements through the direct comparison of supplements, but will also track this across longer period lengths (5 weeks) than have been measured before. There is limited research completed using actual energy intake and digestibility instead of predicted values determined using a calculation from NRC (2001). Our analyses on gross energy intake and digestibility will explain energy found in the treatments and their impact on the overall production of the cow.

Therefore, the objective of our study was to determine the effects of commercially-available C16:0 and C18:0-enriched supplements on nutrient digestibility and production responses in high producing lactating dairy cows. Our hypothesis was that a C16:0-enriched supplement will increase milk production, whereas a C18:0-enriched supplement will decrease FA digestibility and milk production.

Materials and Methods

Design and Treatments

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University. Thirty-six mid-lactation (146 ± 84 DIM) multiparous Holstein cows, from the Michigan State Dairy Teaching and Research Center were randomly assigned to treatment in a truncated Latin square design experiment with two 35-d treatment periods. The study was completed from June 2017, through August 2017. All animals received a common diet with no FA supplementation during a 10-d preliminary period to obtain baseline values. Cows were blocked by production level based on the data collected during the preliminary

period and assigned to one of three treatments. Treatments were: 1) a control diet containing no supplemental FA (CON); 2) a control diet supplemented with a commercially available C16:0-enriched supplement (PA, Spectrum Fusion; Perdue Agribusiness, Salisbury, MD); and 3) a control diet supplemented with a commercially available C18:0-enriched supplement (SA, Energy Booster 100; Milk Specialties Global, Eden Prairie, MN). Supplements were added at 1.5% of diet DM, replacing 1.5% of soyhulls in the diet. Characterization and FA profile of the supplements are shown in Table 3-1. Truncated Latin Square designs have been used previously in experiments in dairy science (Clark and Armentano, 1999; Weiss et al., 2013; de Souza, 2017 ADSA Abstract); we chose this design to allow longer periods for data measurements compared to more traditional 2-3 week periods for complete Latin square experiments.

The ingredient and nutrient composition of the diets fed as TMR are described in Table 3-2. Dry matter concentration was determined twice weekly for forages, and diets were adjusted accordingly. All cows remained in the same tie-stall throughout the experiment. Cows were milked twice per day (0300 and 1400 h). Access to feed was blocked from 0800 to 1000 to allow for collection of orts and offering feed. Cows were fed at 115% of expected intake at 1000 h daily. Water was available ad libitum in each stall which was bedded with sawdust and cleaned twice per day.

Data and Sample Collection

Preliminary milk yield was determined the last 3 d of the preliminary period. Throughout the study, we collected milk samples twice per week for milk component analysis. Sampling and data collection for production variables, nutrient digestibility, and plasma metabolites and

hormones occurred during the last 5d of each treatment period (d 31 to 35). During sampling periods, one milk sample was collected for component analysis as well as another sample without preservative stored at -20°C until analyzed for FA composition. Samples of all diets ingredients and orts from each cow were collected daily and composited by period for analysis. One blood sample was collected in the middle of the sampling period (d 33, 0700). Fecal samples were collected every 15 hours during the sampling period to total eight samples per cow per period. The 15 h interval over 5 d simulated sampling every 3 hours over a 24-h period. Feces were stored in a sealed plastic cup at -20°C until dried, ground and composited per cow per period. Blood was stored on ice until centrifugation at 3,000 × g for 15 minutes at 4°C (within 30 minutes of sample collection). Plasma was transferred into microcentrifuge tubes and stored at -20°C.

Body weight was measured three times per week throughout the study, following the afternoon milking. Body weight change was calculated according to Boerman et al (2015b). Body condition score was determined on the last day of the preliminary period as well as the last day of each treatment period by three trained investigators on a 5-point scale (0.25 point increments; Wildman et al., 1982).

Sample Analysis

Feed ingredients, orts, and fecal samples were dried at 55°C in a forced-air oven for 72 h for DM analysis. Dried samples were ground with a Wiley mill (1 mm screen; Arthur H. Thomas, Philadelphia, PA). Diet ingredients, orts and feces were analyzed by Cumberland Valley Analytical Services for NDF, indigestible NDF, CP, and starch as described by Boerman et al. (2017). Indigestible NDF was used as an internal marker to predict fecal output to determine

apparent total-tract digestibility (Cochran et al. 1986). Indigestible NDF was estimated as NDF after 240-h in-vitro fermentation (Goering and Van Soest, 1970) and total tract digestibility was calculating using total tract disappearance. Gross energy was assayed by bomb calorimeter (Parr Instrument Inc. Moline, IL). Calculations for intake of ME and NE_L were calculated using DE to ME and ME to NEL as per NRC (2001). The commercial FA supplements were analyzed for melting point (method Cc 1-25; AOCS, 2013), iodine value (method Cd 1d-92; AOCS, 2013), and percentage of free FA (method Ca 5a-40; AOCS, 2013) by Eurofins Global Inc. (Des Moines, IA). FA concentrations of feed ingredients were determined as described by Lock et al. (2013).

Plasma non-esterified fatty acids (NEFA) and ß-hydroxybutyrate (BHB) were analyzed using an Olympus AU640e chemistry analyzer (Olympus America, Center Valley, PA) at the Diagnostic Center for Population and Animal Health at Michigan State University (East Lansing). Plasma insulin concentrations were determined by ELISA (Bovine Insulin ELISA; Mercodia AB, Uppsala, Sweden). Individual milk samples were analyzed for fat, true protein and lactose concentration by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan Herd Improvement Association (Universal Lab Services, Grand Ledge, MI). Yields of 3.5% fat-corrected milk [(0.4324 × kg milk) + (16.216 × kg milk fat)], energy-corrected milk [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)] and milk components were calculated using milk yield and component concentrations for each milking, summed for a daily total and averaged for each period. Milk samples for FA analysis were composited based on milk fat yield (d 31 to 35 of each treatment period). Milk lipids were extracted and FA methyl esters (FAME) prepared 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

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

$Y_{ijkl} = \mu + S_i + A_j (S_i) + P_k + T_l + P_k \times T_l + e_{ijkl}$

Where Y_{ijkl} = dependent variable, μ = overall mean, F_i = fixed effect of square, $A_j (S_i)$ = random effect of cow within square , P_k = fixed effect of period, T_l = fixed effect of treatment (l = 1 to 3), $P_k \times T_l$ was the fixed effect of the interaction between period and treatment, and e_{ijkl} = residual error. Normality of the residuals was check with the normal probability, box plots, and homogeneity of variances with plots of residuals vs. predicted values. Two pre-planned contrasts were evaluated: 1) the overall effect of FA supplements [CON vs. FAT (½ (SA + PA))]; and 2) the effect of PA versus SA supplements (SA vs. PA). Contrast were declared significant at $P \leq 0.05$, and tendencies were declared at $0.05 < P \leq 0.10$. All data was expressed as least square means and standard error of the means, unless otherwise specified.

Results

Nutrient Intake and Total-tract Digestibility

Compared with CON, FAT did not affect DMI ($P = 0.83$, Table 3-4), NDF intake ($P = 0.42$), DM digestibility ($P = 0.53$), or NDF digestibility ($P = 0.11$). FAT increased total FA intake

($P < 0.01$), 16-carbon FA intake ($P < 0.01$), and 18-carbon FA intake ($P < 0.01$) compared with CON, but decreased total ($P < 0.01$), 16-carbon ($P < 0.01$) and 18-carbon FA digestibility ($P < 0.01$). FAT increased absorbed total ($P < 0.01$), 16-carbon ($P < 0.01$), and 18-carbon FA ($P < 0.01$) compared with CON.

FA intake was not different between PA and SA ($P = 0.32$). Compared with SA, PA increased 16-carbon ($P < 0.01$) and decreased 18-carbon FA intake ($P<0.01$). There was no difference between PA and SA for 16-carbon digestibility ($P = 0.55$), but PA increased the digestibility of DM ($P < 0.01$), NDF ($P < 0.01$), total FA ($P < 0.01$), and 18-carbon FA ($P < 0.01$) digestibility compared with SA. PA increased total ($P < 0.01$), and 16-carbon FA ($P < 0.01$) absorption, compared to SA, and tended to decrease 18-carbon FA absorption ($P = 0.09$). By using a Lucas test, we estimated a ~20 percentage point increase for the digestibility of total FA in the PA supplement compared with the SA supplement (77% vs. 55%, respectively, Figure 3-1).

Gross Energy Intake and Digestibility

There was no difference between CON and FAT for DE intake ($P = 0.19$), ME intake ($P = 0.19$), NEL intake ($P = 0.19$), or gross energy digestibility ($P = 0.75$). However, compared to SA, PA increased DE intake ($P = 0.05$), ME intake ($P = 0.04$), NE_L intake ($P = 0.03$), and gross energy digestibility ($P < 0.01$).

Production Results

Compared with CON, FAT increased milk production ($P < 0.01$) and tended to increase 3.5% fat-corrected milk (FCM) ($P = 0.07$) and ECM ($P = 0.08$). FAT decreased protein concentration ($P = 0.03$), and tended to decrease fat concentration ($P = 0.09$). We observed no differences in BW, BW change, BCS, or BCS change between CON and FAT.

No differences were observed between PA and SA for milk yield ($P = 0.35$). However, compared with SA, PA increased 3.5% FCM ($P = 0.01$) 2.1 kg/d, ECM ($P = 0.03$) 1.9kg/d, fat yield ($P < 0.01$), and fat concentration ($P < 0.01$). No differences were observed for protein concentration ($P = 0.87$), protein yield ($P = 0.47$), lactose concentration ($P = 0.83$), or lactose yield ($P = 0.49$). There was a tendency for PA to decrease BCS compared to SA ($P = 0.08$), but no differences were observed between PA and SA for BW ($P = 0.44$), BW change ($P = 0.36$), or BCS change ($P = 0.21$).

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. Compared with CON, FAT decreased the concentration of de novo milk FA and increased the concentration of mixed milk FA ($P < 0.01$), but had no effect on preformed milk FA. On a yield basis, FAT decreased de novo milk FA ($P < 0.01$) and increased mixed milk FA ($P < 0.01$) and preformed milk FA ($P < 0.01$).

Compared to SA, PA decreased the concentration of de novo milk FA ($P < 0.01$) and preformed milk FA ($P < 0.01$), and increased the concentration of mixed milk FA ($P < 0.01$). The increase in mixed milk FA was predominantly due to the increased concentration of C16:0 in milk fat for PA ($P < 0.01$). Compared to SA, PA decreased concentrations of C18:0 ($P < 0.01$) and *cis*-9 C18:1 ($P < 0.01$) in milk fat. On a yield basis, PA increased mixed milk FA ($P < 0.01$) and tended to decrease preformed milk FA compared with SA ($P < 0.06$). There was no effect of treatment on the yield of de novo milk FA ($P = 0.67$). PA increased the yield of C16:0 ($P < 0.01$) and had no effect on C18:0 ($P = 0.13$) or C18:1 ($P = 0.12$) compared with the SA treatment.

Blood Metabolites

With the blood samples collected at 0700, FAT had no effect on plasma insulin or BHBA ($P < 0.23$ and $P < 0.15$, respectively), but increased NEFA concentration ($P < 0.01$) when compared with CON. Compared to SA, PA increased NEFA ($P < 0.01$), but there was no difference between PA and SA for insulin or BHBA ($P < 0.96$ and $P < 0.13$).

Discussion

Maximizing the yield of milk and milk components allows for increasing milk income on dairy farms. Commercially available FA supplements have commonly been fed to dairy cows to increase energy density of the ration to accomplish these goals; however there is limited research on production and nutrient digestibility. C16:0, C18:0 and *cis*-9 C18:1 are the predominant FA found in commercial FA supplements. These three FA are also the most prevalent in milk fat

making up on average 51 to 79% of milk FA (Jensen, 2002). Recent research showed the effects of differing ratios of these three FA on nutrient digestibility, nutrient partitioning, and production (de Souza et al., 2018). In that study, the ratios that were used were similar to commercial products, but were made with blends of different FA supplements to achieve pre-determined ratios. The goal of our study was to use commercially-available products and evaluate their effects on nutrient digestibility and production measurements across 5 weeks, which is longer than most previous research. Our study was completed during the summer months from June to August and consisted of 35 d periods to look at long term supplementation and impacts on production and digestibility measures.

Previous research has shown that the amount and FA profile of FA supplements impacts feed intake (Rabiee et al., 2012); however great variation has been detected in DMI when supplementing different FA supplements. Rico et al. (2014) supplemented cows with nearly pure palmitic or stearic acid at 2% of diet DM, and they found no effect on DMI. Similarly, de Souza et al. (2018) observed no differences in DMI in post peak lactating cows fed different ratios of palmitic and stearic acid at 1.5% of DMI compared to cows fed a control diet with no added FA. Consistent with these findings, we also observed no difference in DMI for CON vs FAT or for PA vs SA when supplemental FA was included in the diet at a low level (1.5%).

A recent meta-analysis concluded that there was a tendency for saturated FA supplements to increase total-tract NDF digestibility (Weld and Armentano, 2017). Increases in NDF digestibility have been observed in studies supplementing C16:0 (Piantoni et al., 2013; de Souza et al., 2018; de Souza and Lock, 2018). C16:0 supplements increased NDF digestibility in these studies from a 3.3% to 5.0%. In contrast, C18:0 supplements did not alter NDF digestibility in two reports (Piantoni et al., 2015; Boerman et al., 2017). In our study, there was no difference in DM

or NDF digestibility when comparing CON to FAT, but we saw an increase in both DM and NDF digestibility for PA compared with SA. The lack of difference between no added FA and supplemented FA was due to a numeric decrease in NDF digestibility for the SA treatment in relation to control and a 4.0%-unit increase over control with the PA treatment, which is similar to what was observed by de Souza et al. (2018). Piantoni et al. (2013) did not observe a difference in DMI and suggested that increases in NDF digestibility could be due to increased secretion of cholecystokinin (CCK), leading to an increased retention time in the rumen. The increase in NDF digestibility when feeding C16:0 may also be due to reduced ATP usage if dietary C16:0 could be included in the rumen bacterial membranes instead of having to be synthesized (Vlaeminck et al., 2006; Hackmann and Firkins, 2015), or due to a slightly lower level of NDF in the diets due to the subtraction of soyhulls (de Souza et al., 2018). Despite this, we did not see the increase in NDF digestibility with the C18:0-enriched supplement. Due to these findings, this increase is specific to supplemental C16:0. Further research is required to determine the mechanisms for increased NDF digestibility when C16:0 is supplemented.

FA supplementation has been linked to decreases in total FA digestibility due to the increase in overall FA in the diet (Piantoni et al., 2013, de Souza and Lock., 2017). However, Rico et al. (2014) supplemented a C16:0-enriched supplement and saw increases in total and 16-carbon FA digestibilites in low producing cows. In a meta-analysis, Boerman et al. (2015) determined that as C18:0 reaching the duodenum increased, the digestibility of C18:0 linearly decreased compared to control diets with no added FA. In our study, FAT decreased total, 16- and 18-carbon digestibilities compared with CON, but PA increased total and 18-carbon digestibility compared with SA. The decrease in total FA digestibility when comparing CON and FAT was driven by the substantial decrease (15%-units) in 18-carbon digestibility for SA despite a numeric increase in

18-carbon FA digestibility for PA compared with CON. The increase in FA digestibility for PA compared to SA resulted in an increase of ~100 g/d absorbed total FA even when supplements were fed at the same inclusion rate. Although 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). With increasing FA flow through the small intestine, this emulsification could be a limiting step in FA digestibility (Drackley, 2000). It is possible that the decrease observed in FA absorption is limited by the supply of lysolecithin, an amphiphile and natural emulsifier that assists in the formation of micelles (Freeman, 1969). Further research is needed to examine the means related to FA absorption and its limitations.

Most research that reports energy intake uses predicted values that are generated through a calculation and have observed cows during the fresh period of lactation. 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 (de Souza, 2017 ADSA Abstract). In our study, we used bomb calorimetry to determine energy in feed, feces, and orts to calculate gross energy digestibility and digestible energy intake. Moallem et al. (2007) fed a supplement enriched in C16:0 and C18:0 acid and reported that there was no difference in predicted energy intake compared with no supplemental FA. We observed no difference between CON and FAT for gross energy digestibility or digestible energy intake, however, PA increased both variables compared to SA. Digestible energy intake for the treatments indicate no increase with SA (CON = 86.2 Mcal/d, SA = 86.5 Mcal/D, and PA = 91.6 Mcal/d). Our findings are different what has been seen in past research with FA supplements all having

similar energy values, but actual energy values are different than calculated values that have been previously reported. Bomb calorimetry gives the actual energy values instead of predicted from calculations. This increase in digestible energy intake in PA compared to SA along with overall lower digestibility of the commercially-available supplement used for the SA treatment as seen in the Lucas test does not support Loften et al. (2014) which suggested feeding a combination of C16:0 and C18:0 would be most beneficial to performance measures.

Feeding palmitic acid increases milk yield when compared with a no added fat control (de Souza et al., 2018), and stearic acid compared to a no added fat control also increased milk production (Piantoni et al., 2015). In our study, FAT increased milk yield by 2.1 kg/d as well as 3.5% FCM and ECM compared with CON. The increases in 3.5% FCM and ECM with FAT were driven by the increases with PA. These results are consistent with de Souza and Lock (2018) where a similar increase in ECM was seen when comparing PA to CON across 10 weeks of supplementation. Short-term feeding studies with increased levels of palmitic acid have shown similar results (Piantoni et al., 2013, de Souza et al., 2018). de Souza et al. (2018) observed no differences between C16:0 treatment and C18:0 treatment on body weight or body condition score and their respective changes. Similarly, we saw no differences in body weight changes or body condition score (BCS). Increased plasma NEFA concentrations have been seen when adding saturated FA to dairy rations (Choi et al., 2000, Piantoni et al., 2013). We did see increases in plasma NEFA concentrations between CON and FAT and PA and SA, but no differences in BW or BCS were observed meaning this is not due to mobilization of body reserves (Rico et al., 2014) and most likely due to more plasma triglyceride FA absorbed with PA.

It is well established that feeding FA supplements can alter the FA profile of milk. A recent review by Dorea and Armentano (2017) indicated that supplementation of C16:0 increased total

milk fatty acids. This increase is mainly due to the increase in mixed source FA. Similarly, we found that PA increased the secretion of mixed FA compared to SA. Likewise, compared to SA, PA decreased de novo and preformed yields with no effect on concentrations of FA. Additionally, PA increased yield of C4:0 in milk, which has been seen previously (Piantoni et al. 2013, Lock et al. 2013, Rico et. al 2014), and likely is linked to mechanisms in the mammary gland to regulate milk fluidity as more longer chain FA enter the mammary gland (Barbano et al. 1980).

Conclusion

Feeding a supplement enriched with C16:0, but not C18:0, increased milk fat concentration and yield. Dry matter digestibility, 16- 18- and total FA digestibility, NDF digestibility, and digestible energy intake increased when supplementing C16:0 in a supplement compared to C18:0. These increases with PA on digestibility resulted in increased ECM, 3.5% FCM, fat yield and fat content, without influencing BW compared to SA.

Table 3-1. Composition of fatty acid (FA) supplements fed during the treatment periods[1].

	FA Supplement	
	Energy Booster 100[2]	Spectrum Fusion[3]
Melting point, °C	57.3	74.8
Iodine value	11.4	7.60
FFA, %	87.3	80.5
FA profile of each treatment, g/100g FA		
C14:0	2.28	0.66
C16:0	33.1	84.3
C18:0	53.3	4.06
cis-9 C18:1	5.17	8.70
cis-9, *cis*-12 C18:2	0.65	1.58
cis-9, *cis*-12, *cis*-15 C18:3	0.01	0.04

[1]Average (n=2) composition of FA supplements based on samples taken during the collection period.
[2]Energy Booster 100; Milk Specialties Global, Eden Prairie, MN.
[2]Spectrum Fusion; Perdue Agribusiness, Salisbury, MD.

Table 3-2. Ingredient and nutrient composition diets fed during the treatment periods.

	Treatments[1]		
	CON	SA	PA
Ingredient, % DM			
Corn Silage	36.8	36.8	36.8
Ground Corn	17.9	17.9	17.9
Wheat Straw	6.00	6.00	6.00
High Moisture Corn	4.08	4.08	4.08
Soybean Meal	16.7	16.7	16.7
Soyhulls	10.9	9.37	9.37
Cottonseed	2.57	2.57	2.57
Protein supplement[2]	1.15	1.15	1.15
C16:0-enriched FA supplement[3]	0.00	0.00	1.49
C18:0-enriched FA supplement[4]	0.00	1.49	0.00
Mineral and Vitamin mix[5]	3.94	3.94	3.94
Nutrient Composition, % DM[6]			
NDF	30.9	29.9	29.9
Forage NDF	19.1	19.1	19.1
CP	16.9	16.6	16.6
Starch	27.0	26.8	26.8
FA	2.71	4.22	4.29
16:0	0.46	0.97	1.96
18:0	0.09	0.93	0.17
cis-9 18:1	0.50	0.59	0.55
cis-9, *cis*-12 18:2	1.46	1.42	1.41
cis-9, *cis*-12, *cis*-15 18:3	0.10	0.09	0.10

[1]Treatments were: 1) CON (No supplemental fat) 2) SA (1.5% of FA supplement of a C18:0-enriched supplement); 3) PA (1.5% of FA supplement of a C16:0-enriched supplement).

[2] Protein supplement (Perdue Agribussiness, Salisbury, MD).

[3]Palmitic acid-enriched FA supplement (Spectrum Fusion; Perdue Agribusiness, Salisbury, MD). The supplement contained (g/100 g of fatty acid) 0.66 of C14:0, 84.3 of C16:0, 4.06 of C18:0, 8.70 of C18:1 *cis*-9, and 94.4% total fatty acids.

[4]Stearic acid-enriched FA supplement (Energy Booster 100; Milk Specialties Global, Eden Prairie, MN). The supplement contained (g/100 g of fatty acid) 2.28 of C14:0, 33.1 of C16:0, 53.3 of C18:0, 5.17 of C18:1 *cis*-9, and 89.2% 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 3-3. Nutrient intake and digestibility for cows fed treatment diets (n = 36).

Variable	Treatments[1]			SEM	P value[2]	Contrast[3]	
	CON	SA	PA		Trt	CON vs. FAT	SA vs. PA
Intake, kg/d							
DM	30.6	30.8	30.6	0.49	0.83	0.90	0.55
NDF	9.43	9.61	9.43	0.15	0.42	0.51	0.26
Intake, g/d							
Total FA	0.86	1.36	1.34	19.2	<0.01	<0.01	0.32
16-carbon	0.13	0.30	0.51	6.11	<0.01	<0.01	<0.01
18-carbon	0.67	0.97	0.77	12.6	<0.01	<0.01	<0.01
Digestibility, %							
DM	64.3	63.0	66.6	0.67	<0.01	0.53	<0.01
NDF	39.1	38.3	43.1	0.92	<0.01	0.12	<0.01
Total FA	76.7	67.6	76.3	1.00	<0.01	<0.01	<0.01
16-carbon	74.3	68.0	69.0	1.18	<0.01	<0.01	0.55
18-carbon	78.3	67.2	82.1	1.00	<0.01	<0.01	<0.01
Absorbed, kg/d							
Total FA	666	919	1021	16.6	<0.01	<0.01	<0.01
16-carbon	100	202	354	5.52	<0.01	<0.01	<0.01
18-carbon	528	648	628	11.3	<0.01	<0.01	0.09

[1]Treatments were: 1) CON (No supplemental fat) 2) SA (1.5% of FA supplement of a C18:0-enriched supplement); 3) PA (1.5% of FA supplement of a C16:0-enriched supplement).
[2] P values associated with treatment differences and contrasts between control and fat supplements, and stearic and palmitic acid treatments.
[3] Contrasts evaluated refers to the treatment effects of different supplemental fats.

Table 3-4. Gross energy intake and digestibility for cows fed treatment diets (n=36).

Variable	Treatments[1]			SEM	P value[2]	Contrast[3]	
	CON	SA	PA		Trt	CON vs. FAT	SA vs. PA
Digestibility, %							
Gross Energy	64.5	62.6	66.9	0.70	<0.01	0.75	<0.01
Energy intake, Mcal/d							
DE	86.2	86.5	91.6	2.15	0.07	0.19	0.05
ME	73.5	73.6	78.7	1.93	0.04	0.19	0.04
NE_L	45.9	45.9	49.5	1.25	0.03	0.19	0.03

[1]Treatments were: 1) CON (No supplemental fat) 2) SA (1.5% of FA supplement of a C18:0-enriched supplement); 3) PA (1.5% of FA supplement of a C16:0-enriched supplement).
[2] P values associated with treatment differences and contrasts between control and fat supplements, and stearic and palmitic acid treatments.
[3] Contrasts evaluated refers to the treatment effects of different supplemental fats.

Table 3-5. Milk yield, milk composition, BW, and BCS of cows fed treatment diets (n = 36).

Variable	Treatments[1]			SEM	P value[2]	Contrast[3]	
	CON	SA	PA		Trt	CON vs. FAT	SA vs. PA
Milk Yield, kg/d							
Milk	43.1	44.8	45.7	1.36	0.02	0.01	0.35
3.5% FCM[4]	44.1	43.9	46.2	1.21	0.01	0.07	0.01
ECM[5]	44.8	44.5	46.4	1.21	0.02	0.08	0.03
Milk Composition							
Fat, kg/d	1.55	1.52	1.65	0.05	<0.01	0.19	<0.01
Fat, %	3.63	3.41	3.69	0.10	<0.01	0.09	<0.01
Protein, kg/d	1.43	1.46	1.44	0.04	0.47	0.32	0.47
Protein, %	3.32	3.27	3.26	0.05	0.10	0.03	0.87
Lactose, kg/d	2.10	2.16	2.14	0.07	0.17	0.08	0.49
Lactose, %	4.79	4.80	4.79	0.03	0.96	0.84	0.83
BW, kg	744	738	742	11.7	0.47	0.34	0.44
BW change kg/d	0.42	0.35	0.67	0.25	0.63	0.76	0.36
BCS	3.51	3.58	3.47	0.07	0.21	0.90	0.08
BCS change	0.09	0.09	0.14	0.02	0.36	0.53	0.21

[1]Treatments were: 1) CON (No supplemental fat) 2) SA (1.5% of FA supplement of a C18:0-enriched supplement); 3) PA (1.5% of FA supplement of a C16:0-enriched supplement).
[2] P values associated with treatment differences and contrasts between control and fat supplements, and stearic and palmitic acid treatments.
[3] Contrasts evaluated refers to the treatment effects of different supplemental fats.
[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 3-6. 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]	
	CON	SA	PA		Trt	CON vs FAT	SA vs PA
Summation by Source[4], g/100g							
De Novo	27.8	26.3	24.4	0.39	<0.01	<0.01	<0.01
Mixed	37.5	36.8	42.5	0.49	<0.01	<0.01	<0.01
Preformed	34.7	36.9	33.1	0.55	<0.01	0.47	<0.01
Summation by Source[4], g/d							
De Novo	404	380	376	14.8	<0.01	<0.01	0.67
Mixed	551	525	649	21.0	<0.01	<0.01	<0.01
Preformed	499	524	500	14.2	0.08	0.21	0.06

[1]Treatments were: 1) CON (No supplemental fat) 2) SA (1.5% of FA supplement of a C18:0-enriched supplement); 3) PA (1.5% of FA supplement of a C16:0-enriched supplement).

[2] P values associated with treatment differences and contrasts between control and fat supplements, and stearic and palmitic acid treatments.

[3] Contrasts evaluated refers to the treatment effects of different supplemental fats.

[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).

Table 3-7. Plasma insulin and blood metabolites for cows fed treatment diets (n=36).

Variable	Treatments[1]			SEM	P value[2]	Contrast[3]	
	CON	SA	PA		Trt	CON vs. FAT	SA vs. PA
Insulin, ug/L	0.77	0.82	0.83	0.05	0.49	0.23	0.96
NEFA, (mEq/L)	0.09	0.10	0.11	0.00	<0.01	<0.01	0.03
BHB, (mg/dL)	6.71	6.14	6.58	0.27	0.11	0.15	0.13

[1]Treatments were: 1) CON (No supplemental fat) 2) SA (1.5% of FA supplement of a C18:0-enriched supplement); 3) PA (1.5% of FA supplement of a C16:0-enriched supplement).

[2] P values associated with treatment differences and contrasts between control and fat supplements, and stearic and palmitic acid treatments.

[3] Contrasts evaluated refers to the treatment effects of different supplemental fats.

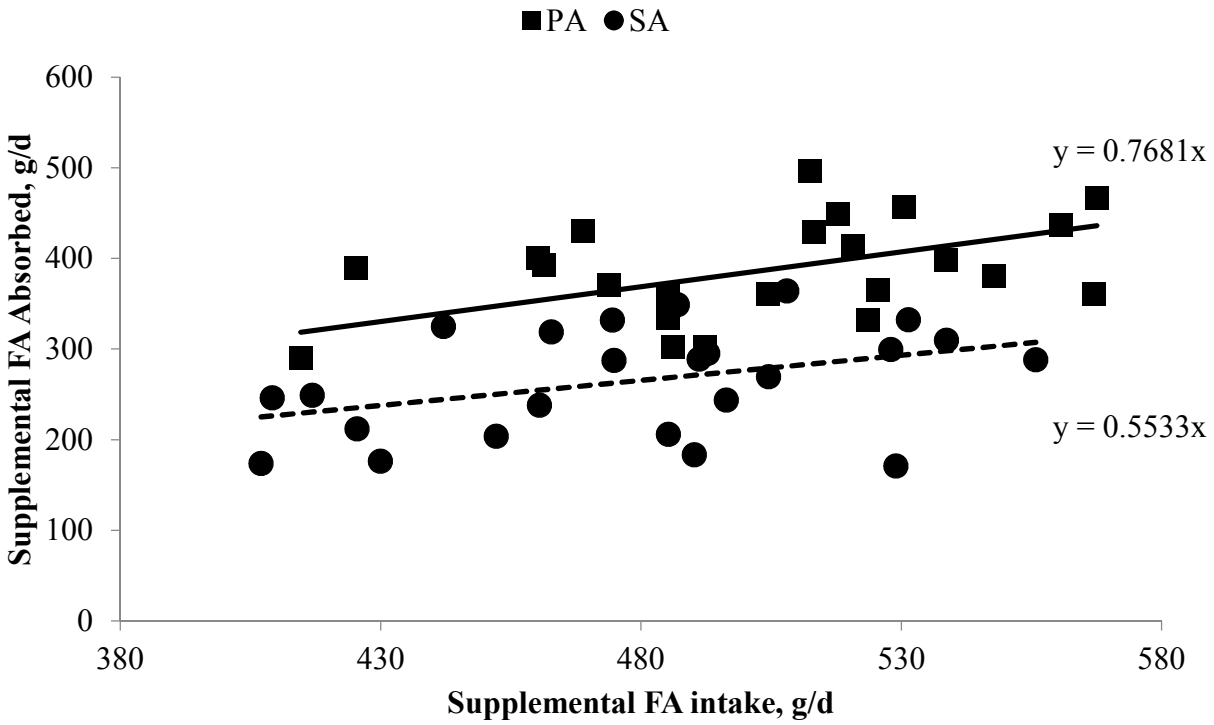

Figure 3-1. Lucas test of fat supplement digestibility. The intake of FA from the basal diet was subtracted from that in each supplemented diet to calculate the supplemental FA intake. Fecal output of undigested basal FA was estimated using FA digestibility measured when cows were fed the control diet and the average value was used. Fecal output of basal FA was subtracted from fecal output of total FA when cows were fed the supplemented diets. Digestibility of supplemental fat was estimated with a Lucas test by regressing supplemental FA intake on supplemental FA absorbed. The slope of each regression is the estimated apparent digestibility coefficient of each fat supplement.

Table 3-8. Milk fatty acid concentration for cows fed treatment diets (n=36).

Variable	Treatments[1]			SEM	P Value[2]	Contrast[3]	
	CON	SA	PA		Trt	CON vs FAT	SA vs PA
Selected Individual FA[4], g/100g							
C4:0	2.49	2.42	2.51	0.06	0.43	0.69	0.22
C6:0	1.82	1.74	1.71	0.04	0.03	0.01	0.48
C8:0	1.18	1.11	1.03	0.03	<0.01	<0.01	0.01
C10:0	3.52	3.24	2.90	0.09	<0.01	<0.01	<0.01
C12:0	4.37	3.98	3.53	0.10	<0.01	<0.01	<0.01
C14:0	13.6	12.9	12.0	0.16	<0.01	<0.01	<0.01
C14:1	0.81	0.88	0.79	0.05	0.35	0.65	0.17
C16:0	35.9	35.1	40.8	0.46	<0.01	<0.01	<0.01
cis-9 C16:1	1.55	1.68	1.62	0.09	0.48	0.3	0.55
C18:0	8.34	8.88	7.77	0.22	<0.01	0.89	<0.01
trans-6 to 8 C18:1	0.20	0.23	0.20	0.01	0.01	0.07	0.01
trans-9 C18:1	0.16	0.17	0.16	0.01	0.07	0.11	0.09
trans-10 C18:1	0.37	0.58	0.46	0.08	0.08	0.06	0.20
trans-11 C18:1	0.31	0.29	0.26	0.02	<0.01	0.01	0.02
cis-9 C18:1	16.9	18.4	16.6	0.3	<0.01	0.01	<0.01
cis-11 C18:1	0.54	0.60	0.52	0.02	0.01	0.41	0.01
cis-9, *cis*-12 C18:2	2.37	2.33	2.13	0.06	<0.01	0.02	<0.01
cis-9, *trans*-11 C18:2	0.31	0.29	0.26	0.02	<0.01	0.01	0.02
cis-9, *cis*-12, *cis*-15 C18:3	0.27	0.25	0.23	0.01	<0.01	<0.01	0.04

[1]Treatments were: 1) CON (No supplemental fat) 2) SA (1.5% of FA supplement of a C18:0-enriched supplement); 3) PA (1.5% of FA supplement of a C16:0-enriched supplement).

[2] P values associated with treatment differences and contrasts between control and fat supplements, and stearic and palmitic acid treatments.

[3] Contrasts evaluated refers to the treatment effects of different supplemental fats.

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

Table 3-9. Milk fatty acid yields for cows fed treatment diets (n = 36).

Variable	Treatments[1]			SEM	P Value[2]	Contrast[3]	
	CON	SA	PA		Trt	CON vs FAT	SA vs PA
Selected Individual FA[4], g/d							
C4:0	36.1	35.2	38.1	1.43	0.07	0.63	0.03
C6:0	26.6	25.4	26.3	1.15	0.30	0.28	0.27
C8:0	17.1	16.2	16	0.76	0.05	0.01	0.67
C10:0	51.1	47.5	45.1	2.32	<0.01	<0.01	0.09
C12:0	63.4	58	54.8	2.76	<0.01	<0.01	0.04
C14:0	197	186	184	6.81	<0.01	<0.01	0.66
C14:1	11.8	12.2	11.9	0.64	0.85	0.68	0.7
C16:0	528	502	624	20.40	<0.01	<0.01	<0.01
cis-9 C16:1	22.6	23.2	24.8	1.12	0.11	0.13	0.13
C18:0	120	128	118	4.81	0.04	0.41	0.02
trans-6 to 8 C18:1	2.86	3.15	3.02	0.13	0.09	0.06	0.29
trans-9 C18:1	2.23	2.41	2.4	0.08	0.11	0.04	0.87
trans-10 C18:1	5.23	7.42	6.7	0.91	0.11	0.05	0.50
trans-11 C18:1	8.25	7.19	7.04	0.41	0.01	<0.01	0.73
cis-9 C18:1	243	260	251	6.6	0.01	0.01	0.12
cis-11 C18:1	7.76	8.43	7.92	0.40	0.22	0.23	0.21
cis-9, *cis*-12 C18:2	33.7	33.2	32.1	1.19	0.44	0.34	0.40
cis-9, *trans*-11 C18:2	4.33	4.11	3.86	0.21	0.12	0.07	0.27
cis-9, *cis*-12, *cis*-15 C18:3	3.79	3.54	3.47	0.13	0.07	0.03	0.64

[1]Treatments were: 1) CON (No supplemental fat) 2) SA (1.5% of FA supplement of a C18:0-enriched supplement); 3) PA (1.5% of FA supplement of a C16:0-enriched supplement).

[2] P values associated with treatment differences and contrasts between control and fat supplements, and stearic and palmitic acid treatments.

[3] Contrasts evaluated refers to the treatment effects of different supplemental fats.

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

CHAPTER 4

MILK PRODUCTION RESPONSES TO A CHANGE IN THE DIETARY RATIO OF PALMITIC AND OLEIC ACIDS VARY BY PRODUCTION LEVEL IN DAIRY CATTLE

Abstract

We evaluated the effects of altering the dietary ratio of palmitic (C16:0) and oleic (*cis*-9 C18:1) acids on production responses of cows with a wide range of milk production (32 to 65 kg/d) in a crossover design experiment. Thirty-two multiparous Holstein cows (144 ± 94 DIM) were assigned randomly within level of milk yield to treatment sequence. Treatments were diets supplemented with FA blends (1.5% of diet DM) that provided 80% C16:0 + 10% C18:1 (80:10) and 60% C16:0 + 30% C18:1 (60:30). The corn silage and alfalfa-based diets contained 20.0% forage NDF, 28.5% starch and 17.1% CP. Treatment periods were 21 d with the final 5 d used for data and sample collection. The statistical model included the random effect of cow, the fixed effect of treatment, period, preliminary milk yield (PMY), and two-way interactions. Linear effects for the interaction between PMY and treatments were added to evaluate responses to treatment by level of milk yield. There were no effects of treatments on DMI (P=0.34), milk yield (P=0.38), ECM (P=0.35), body weight (BW) (P=0.74), or BW change (P=0.54). 60:30 increased total, 16- and 18-carbon FA digestibility compared to 80:10 (all P<0.01). Compared with 60:30, 80:10 increased fat yield (1.92 vs. 1.83 kg/d, P<0.01) and protein yield (1.61 vs. 1.55 kg/d, P=0.03). 80:10 also increased the yield of de novo (448 vs. 428 g/d, P<0.05) and mixed (749 vs. 669 g/d, P<0.01) milk FA and decreased the yield of preformed FA (605 vs. 627 g/d, P<0.05)

compared with 60:30. Interactions were detected between treatment and PMY for DMI, total FA intake, 16-carbon FA intake, ECM, 3.5% FCM (linear interaction both $P<0.05$), and a tendency for milk yield (linear interaction $P=0.12$); lower producing cows (less than 45 kg/d) had increased DMI and ECM on the 80:10 diet whereas higher producing cows (over 55 kg/d) had increased DMI and ECM on 60:30. A linear interaction was also detected between treatment and PMY for mixed milk FA yield (linear interaction both $P<0.10$) and a tendency for de novo milk FA yield (linear interaction $P<0.15$). Our results demonstrate that production responses (DMI, milk yield, and ECM) of high producing cows were better with a fat supplement containing more C18:1, while lower producing cows responded better to a supplement containing more C16:0.

Introduction

Supplemental fat has been added to dairy cow rations to increase energy density and improve milk production and milk fat yields (Palmquist and Jenkins, 2017). Recent research has focused on the effects of supplemental fat on milk production, nutrient digestibility, and metabolism. Great variability has been observed between individual FA across studies (Rabiee et al., 2012), but one of the main differences observed is the effects that individual fatty acids (FA) have on production and digestibility measurements. Palmitic (C16:0), stearic (C18:0) and oleic acid (C18:1) are the three main fatty acids present in milk fat and adipose tissue (Palmquist, 2006 and Douglas et al., 2007). Understanding the differences between each individual FA is crucial to determining ideal FA profiles for cows.

Recent research has focused on C16:0, C18:0 and C18:1 to evaluate their effects on production, nutrient digestibility and energy metabolism. While Chapter 3 focused on C16:0 and

C18:0, Chapter 4 will focus on C16:0 and C18:1. C16:0 supplementation increases milk fat concentration and yield as well as NDF digestibility (de Souza et al., 2018) while C18:1 increases FA digestibility (Boerman et al., 2015, de Souza et al., 2018). We recently determined the impact of different FA ratios of C16:0, C18:0 and C18:1 compared to a control diet with no added fat on production, digestibility and metabolic responses. Overall, higher C16:0 (80% C16:0) increased milk yield and milk fat, while a blend of C16:0 and C18:1 (45% C16:0 and 35% C18:1) increased FA digestibility and body weight (BW) (de Souza et al., 2018). These different effects of different FA profiles could lead to the opportunity to feed specific FA to cows depending on production goals and metabolic demands.

The optimal diet for a cow depends on her level of milk production; this idea was highlighted in a recent publication by Piantoni et al. (2015), who showed that the response to supplemental FA varies with production level of lactating dairy cows (Piantoni et al., 2015). Effects of supplemental C18:0 on yields of milk and milk components in that study were more pronounced in higher producing cows. de Souza (2017 ADSA Abstract) evaluated different ratios of C16:0 and C18:1 in dairy cows with different production levels (with 4 treatments), and observed that higher producing cows produced more milk with a blend containing more C18:1, while lower producing cows produced more milk with a blend containing more C16:0. Though that study included three production groups, they were all high-producing animals with the low group milk yield average was 45 kg/d and the high group at 60 kg/d. Therefore, the objective of the current study was to evaluate the effects of different ratios of C16:0 and C18:1 on production responses, nutrient digestibility and milk FA profile of lactating cows across a wide range of production. Our hypothesis was that increasing C18:1 would increase production measurements in higher producing cows while not having an effect in lower 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 study was designed to test feeding fat blends with varying ratios of C16:0 and C18:1 to cows across a wide range of milk production. Thirty-two mid-lactation multiparous Holstein cows from the Michigan State Dairy Field Laboratory used in a crossover design from September to November 2017. Prior to the study, all animals started with a 14-d preliminary period and fed a common diet with no supplemental fat to obtain baseline values. Cows were paired by production level based on the data collected during the preliminary period. Treatment periods were two consecutive 21-d periods. The two treatments were combinations of two commercially available FA supplements that differed in FA profile and blended to achieve different ratios of C16:0 and C18:1 in the FA supplement blends (Table 4-1). The FA treatments were 1) 80:10 (80% C16:0, 10% C18:1); and 2) 60:30 (60% C16:0, 30% C18:1). The supplement blends were fed at 1.5% FA (% diet DM). Crossover studies with a wide range of production have been previously used to assess interactions (Bradford and Allen 2004; Harvatine and Allen 2005; Piantoni et al., 2015).

The ingredient and nutrient composition of the diets fed as a total mixed ration are presented in Table 4-2. 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. Cows were milked twice per day (0300 and 1400 h). Access to feed was blocked daily from 0800 to 1000 h to allow for the collection of orts and offering of new feed. Cows were fed 115%

of expected daily intake, and feed intake was recorded daily. Water was available ad libitum and each stall was bedded with sawdust and cleaned twice per day.

Data and Sample Collection

Preliminary milk yield was determined the last 3 d of the preliminary period. Samples and data for production and digestibility variables, as well as plasma metabolites, were collected during the last 5 d of each treatment period (d 17 to 21). Samples (0.5 kg) of all diet ingredients and orts (12.5%) from each cow were collected daily during the sampling period and composited by period for analysis. Fecal (~400g) and blood (~15mL) samples were collected every 15 hours during the last 5 d of each sampling period totaling eight samples per cow per period. The 15-h interval over 5 d stimulates sampling every 3 h hour a 24-h period. Feces were stored in a sealed plastic container at -20°C. Blood was stored on ice until centrifugation at 3,000 x g for 15 min at 4°C (within 30 minutes of sample collection). Plasma was transferred into microcentrifuge tubes and stored at -20°C until composited by period. Milk yield was recorded and two milk samples were collected at each milking. One aliquot was collected and sealed in a 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 afternoon milking, and BW change was calculated according to Boerman et al. (2015). On the last day of the preliminary period and the last day 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 by Cumberland Valley Analytical Services for NDF, CP, starch and FA concentration as described by Boerman et al. (2017). Indigestible NDF was determined after 240 h of in vitro fermentation (Goering and Van Soest, 1970). FA concentrations of feed ingredients were determined as described by Lock et al. (2013).

Plasma non-esterified fatty acids (NEFA) and ß-hydroxybutyrate (BHB) were analyzed using an Olympus AU640e chemistry analyzer (Olympus America, Center Valley, PA) at the Diagnostic Center for Population and Animal Health at Michigan State University (East Lansing). Plasma insulin concentrations were determined by ELISA (Bovine Insulin ELISA; Mercodia AB, Uppsala, Sweden).

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 17 to 21 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).

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:

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

where Y_{ijk} = dependent variable, μ = overall mean, C_i = random effect of cow (i = 1 to 32), P_j = fixed effect of period (j = 1 to 2), T_k = fixed effect of treatment (k = 1 to 2), $P_j \times T_k$ = interaction between period and treatment, pMY = preliminary milk yield used as covariate, pMY × T_k = interaction between pMY and treatment, pMY × pMY = pMY squared, pMY × pMY × T_k = interaction between pMY × pMY and treatment, and e_{ijk} = residual error. Linear and quadratic effects for the interaction between pMY and treatment were added to evaluate responses to treatment by level of milk yield. Normality of the results were tested using box plots, normal probability, and homogeneity of variances. Main effects were declared significant at $P \leq 0.05$, and tendencies were declared at $0.05 < P \leq 0.10$. Interactions were deemed significant at $P \leq 0.10$ and tendencies were $0.10 < P \leq 0.15$.

Results

Nutrient Intake and Total-tract Digestibility

There was an interaction between treatment and PMY for DMI ($P = 0.04$), NDF intake ($P = 0.05$), total fatty acid intake ($P = 0.06$), and absorbed 16-carbon FA ($P = 0.08$) (Table 4-3). All interactions followed the same trend in our study. Higher producing cows responded more positively to 60:30, while lower producing cows responded more positively to 80:10. The relationship between treatment and DMI can be seen in Figure 4-1 as an example. Compared with the 60:30 treatment, 80:10 decreased digestibilities of total FA (74.9 vs. 77.6 %, $P < 0.01$), 16-carbon FA (74.2 vs. 76.9 %, $P < 0.01$) and 18-carbon FA (73.0 vs. 79.3 %, $P < 0.01$). The 60:30 treatment increased total FA absorption (787 vs. 828g/d, $P = 0.03$), decreased 16-carbon FA absorption (352 vs. 296g/d, $P < 0.01$), and increased 18-carbon FA absorption (354 vs. 508g/d, $P < 0.01$), compared to 80:10. No differences were observed for NDF digestibility ($P = 0.71$) or dry matter ($P = 0.81$) digestibility between the treatments.

Production Results

Interactions between treatment and PMY were observed for 3.5% fat-corrected milk (FCM) ($P = 0.05$), energy corrected milk (ECM) ($P = 0.04$) (Figure 4-2), and there was a tendency for an interaction for milk yield ($P = 0.12$) (Table 4-3). Higher producing cows responded more positively to 60:30, while lower producing cows responded more positively to 80:10. Overall, compared with 60:30, 80:10 increased milk fat content (4.10 vs. 4.05%, $P = 0.03$), fat yield (1.92

vs. 1.85kg/d, $P < 0.01$), and protein yield (1.61 vs. 1.55kg/d, $P = 0.03$), with a tendency to increase protein concentration (3.40 vs. 3.35%, $P = 0.06$). No differences were observed for body weight (BW), BW change, body condition score (BCS) or change in BCS between the treatments.

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 C16:1) originate from de novo synthesis in the mammary gland and extraction from plasma. Treatment interacted with PMY for the yield of mixed source FA ($P = 0.08$). Compared to 60:30, 80:10 increased de novo (448 vs. 428 g/d, $P < 0.01$) and mixed (749 vs. 669g/d, $P < 0.01$) source FA yield and decreased preformed source FA yield (605 vs. 627g/d, $P < 0.01$). 80:10 increased the concentration of mixed source FA (41.5 vs 38.7%, $P < 0.01$) in the milk and tended to decrease the concentration of preformed milk FA (33.6 vs. 36.4%, $P = 0.08$), compared to 60:30 (Table 4-5). These changes were driven by increases of 16-carbon FA seen on the 80:10 treatment while 60:30 increased or tended to increase all 18-carbon FA.

Blood Metabolites

We observed no effect of treatments on plasma insulin ($P = 0.36$) or BHBA ($P = 0.17$). Compared with 60:30, 80:10 decreased NEFA concentration ($P = 0.04$). No interactions were observed between treatment and PMY for blood measures.

Discussion

Increasing the energy density of the ration through the supplementation of FA can enhance milk and component yields (Rabiee et al., 2012). Determining the ideal FA or FA profile needed for a cow is one way to maximize milk yield and production efficiency. Recently, research has focused on different FA and their effects on animal performance. C16:0 and C18:1 are two FA that are commonly found in commercially available products (de Souza et al., 2018) and are also the main FA found in milk fat and adipose tissue (Jensen, 2002). C16:0 has been shown to increase milk yield and milk components while C18:1 has been shown to increase energy partitioning to body reserves (de Souza et al., 2018). We recently observed an interaction between production level and performance of cows as the ratios of C16:0 and C18:1 change (de Souza, 2017 ADSA Abstract). In that study, the low group averaged 45 kg/d, the medium group averaged 53 kg/d and the high group averaged 60 kg/d. Results from that study showed cows averaging 60 kg/d responded better to increased C18:1, while cows averaging 45 kg/d responded better to increased C16:0. Comparing the high and low groups only, more C16:0 in a supplement blend increased ECM 2.7 kg/d over more C18:1. Additionally, in the high group, more C18:1 increased ECM 6.7 kg/d compared to more C16:0. For the current study, we included cows from a wide range of production (32 to 65 kg/d), and therefore, cows were selected for this and distributed evenly instead of dividing cows into production level groups.

Previous research has observed variable results on DMI due to fat supplementation. Saturated fat supplements have not altered DMI compared to control diets with no added fat (Harvatine and Allen, 2006; Rico et al., 2014; de Souza et al., 2018). However, as the degree of unsaturation increases, DMI linearly decreases (Harvatine and Allen, 2006). de Souza (2018)

observed that a blend of C16:0 and C18:1 (45% C16:0 and 35% C18:1) did not decrease intake in a diet containing soyhulls, but DMI decreased in a diet containing cottonseed. In our study, we saw an interaction between DMI and PMY. Interestingly, in our current study, higher producing cows increased DMI on the 60:30 treatment while lower producing cows increased DMI on the 80:10 treatment. This interaction between production level and treatment was not observed in our previous C16:0 and C18:1 ratio study (de Souza, 2017 ADSA Abstract). Piantoni (2015) however, observed increased intake with supplemental C18:0 with higher-producing cows responding more favorably to C18:0 than lower-producing cows. Boerman et al. (2017) found that C18:0 supplementation increased DMI. This increase in intake could be related to higher producing cows having a greater energy requirement which may have led to an increase in DMI. However, previous research indicates increasing the amount of C18:1 reaching the small intestine would have increased risk of reducing DMI, which is different than what we observed in the current study. Further research needs to be conducted to determine the exact effects of FA profile on DMI.

The 60:30 treatment increased total, 16- and 18-carbon FA digestibility compared with the 80:10 treatment. Similar to our results, de Souza et al. (2017 ADSA Abstract) observed increases in total, 16- and 18-carbon FA digestibility with no interactions seen based on production group. Total, 16- and 18-carbon digestibility has been seen to increase with increased unsaturated FA at the small intestine compared C18:0 as shown in a meta-analysis (Boerman et al., 2015). C18:1 increasing total FA is likely due to C18:1 having amphiphilic properties which can assist in micelle solubility of C18:0 (Freeman, 1969; Moate et al., 2004). Additionally, unsaturated FA permit faster uptake and re-esterification compared with saturated FA (Ockner et al., 1972). Our results show that not only does the flow of FA to the duodenum effect the FA digestibility (Boerman et al.,

2015), but most likely the FA profile reaching the duodenum also influences FA digestibility. However, the mechanisms of how C18:1 increase FA digestibility require further research.

We saw no differences between treatments on NDF digestibility. Similarly, de Souza (2018; 2017 ADSA Abstract) also saw no treatment differences when feeding different ratios of C16:0 and C18:1 that ranged from a treatment similar to our 80:10 treatment, up to a treatment similar our 60:30 treatment. Supplemental C16:0 has been shown to increase NDF digestibility when compared to a no added fat control (Piantoni et al., 2013; Rico et al., 2017; de Souza et al., 2018; Chapter 3). Results are variable for C18:1 impact on digestibility which can be related to different supplements inclusion rates and the impacts on DMI. The major FA in both of our treatments was C16:0, possibly increasing NDF digestibility, resulting in no change between treatments. Similar to Piantoni et al. (2013; 2015) studies when C16:0 and C18:0 were supplemented across production levels, we also did not observe an interaction between PMY and treatment on DM digestibility. de Souza (2017 ADSA Abstract) determined energy intake from 4 different blends of FA supplements and observed that although FA digestibility increased with no interaction based on production group, that higher producing cows increased digestible, metabolizable, and net energy of lactation energy intake when compared to lower producing cows.

We observed interactions between treatment and PMY for 3.5% FCM and ECM higher producing cows to increase measurements on the 60:30 treatment while lower producing cows increased both on the 80:10 treatment. C16:0 supplementation has been shown to increase ECM (Lock et al., 2013, de Souza et al., 2017). Rico et al. (2014) did not see differences in ECM when comparing C16:0 supplement to a Ca-salt of palm FA supplement, but this could be due to a higher feeding rate (2.3% DM), shorter period length, or the milk production of the cows. de Souza et al. (2017 ADSA Abstract) altered FA ratio and observed higher producing cows increased milk

energy output when supplemented a FA higher in C18:1. Although our study saw similar trends compared to de Souza et al. (2017 ADSA Abstract), we did not see the magnitude of change that was observed in that study. This could be because our wide range of production did not cover the span of the previous study, but covered lower producing cows. Piantoni et al. (2013) supplemented C16:0 across production levels and saw no interactions, but increased yields of milk and milk components overall when supplementing C16:0 compared to a no added fat control. Despite no interactions with C16:0 observed in high and low producing cows (Rico et al., 2014), supplementation of C18:0 increased milk production and DMI in higher producing cows compared to lower producing cows (Piantoni et al., 2015). Increasing the amount of 18-carbon FA in the ration through the supplementation of C18:1 has presented interactions across production groups for ECM (de Souza, 2017 ASDA Abstract), but it is unclear if this is due to an overall effect of 18-carbon FA or a specific FA. Rico et al. (2014), however, compared C16:0 and C18:0 supplementation and concluded C16:0 improved milk fat concentration and yield across all production levels more effectively than C18:0. de Souza et al. (2018) used blends of C16:0+C18:0 and C16:0+C18:1 and observed including C18:1 increased FA digestibility and body weight compared to C18:0 which decreased DM, NDF and FA digestibility and performance overall. While our current study and previous results suggest the increase in ECM in high cows is due to increasing C18:1 in the supplement, we cannot rule out this is due to C18:0 from biohydrogenation of C18:1 in the rumen. Further research is needed to assess 18-carbon FA on production levels of lactating cows.

There were no treatment effects or interactions observed for BW or BCS. Previous research observed increased BW and BCS change with supplemented C18:1, regardless of production level. (de Souza, 2017 ADSA Abstract; 2018). This could be due to the shorter period length in our study

at 21 d compared to 35 d in that study. Increasing C16:0 increased milk fat content and yield, compared to increased C18:1. The increased milk fat content and yield observed in our study is similar to previous research (Piantoni et al., 2013; Rico et al., 2017; de Souza et al., 2018). C4:0 yield was increased in the 80:10 treatment compared with 60:30, which is consistent with previous research (Piantoni et al. 2013, Lock et al. 2013, Rico et. al 2014). This increase in C4:0 is a mechanism in the mammary gland to assist in milk fluidity as more longer chain FA enter the mammary gland (Barbano et al. 1980). Increases seen in long chain FA (>16-carbons) account for the decrease in de novo and mixed and increased preformed FA sources on the 60:30 treatment. Similarly, increases in 16-carbon FA on the 80:10 treatment increased mixed FA sources therefore decreasing de novo and preformed sources,

The interactions between PMY and treatments on ECM and DMI with observed in our study were similar to the previous study completed by de Souza et al. (2017 Abstract), however, our differences were not as pronounced as what was observed in that study. Our wide range of milk production included cows from 30kg/d to 65kg/d. This group compared to the previous study would have been in the mid-production level group (Averaged 53 kg/d), where no difference in ECM between the 80:10 and 60:30 treatments was observed. This could be why our differences were not as pronounced as what we observed previously.

Conclusion

Feeding fat supplements higher in oleic acid increased ECM and DMI, and tended to increase milk yield as preliminary milk yield of cows increased while supplements higher in palmitic acid increased these variables in lower producing cows. Overall, oleic acid increased total,

16- and 18-carbon FA digestibility compared with palmitic acid, but no differences were observed on body weight or body condition score. The increases in production variables observed across a wide range of production suggest supplementing more C18:1 in a ratio to high producing cows and more C16:0 in a ratio to low producing cows.

Table 4-1. Composition of fatty acid (FA) supplements to make FA blends fed during the treatment periods.

	Treatment[1]	
	80:10	60:30
% of each FA supplement in the treatment blends		
C16:0-enriched FA supplement[2]	93.0	41.0
Ca-Salt of Palm FA supplement[3]	7.00	59.0
FA profile of each treatment, g/100g FA		
C14:0	0.74	0.82
C16:0	82.2	59.7
C18:0	1.51	2.93
cis-9 C18:1	12.5	29.5
cis-9, *cis*-12 C18:2	2.44	5.89
cis-9, *cis*-12, *cis*-15 C18:3	0.07	0.16

[1]Average (n=2) composition of FA supplements based on samples taken during the collection period.
[2]Palmitic acid-enriched FA supplement (Nutracor; Wawasan Agrolipids, Johor, Malaysia).
[3]Ca salts of palm FA supplement (Nutracal; Wawasan Agrolipids, Johor, Malaysia).

Table 4-2. Ingredient and nutrient composition of treatment diets.

	Treatments[1]	
	80:10	60:30
Ingredient, % DM		
Ground Corn	27.9	27.9
Corn Silage	24.0	24.0
Haylage	18.9	18.9
Soybean Meal	12.8	12.8
Soyhulls	4.28	4.16
Cottonseed	3.43	3.43
Vitamin Mineral Mix[2]	3.27	3.27
Wheat Straw	2.57	2.57
Amino Acid Supplement[3]	1.29	1.29
C16:0-enriched FA Supplement[4]	1.42	0.63
Ca-Salt Palm FA Supplement[5]	0.13	1.08
Nutrient Composition, % DM[6]		
NDF	27.9	27.8
Forage NDF	20.0	20.0
CP	17.1	17.0
Starch	28.5	28.5
FA	4.41	4.41
16:0	2.00	1.61
18:0	0.11	0.13
cis-9 18:1	0.69	0.99
cis-9, *cis*-12 18:2	1.31	1.35
cis-9, *cis*-12, *cis*-15 18:3	0.20	0.20

[1]Treatments were: 1) 80:10 (1.5% of FA blend of 80% C16:0-enriched supplement and 10% Ca-Salt of Palm FA); 2) 60:30 (1.5% of FA supplement of 60% C16:0-enriched supplement and 30% Ca-Salt of Palm FA).
[2]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.
[3] Protein supplement (Perdue Agribusiness, Salisbury, MD).
[4]Palmitic acid-enriched FA supplement (Nutracor; Wawasan Agrolipids, Johor, Malaysia). The supplement contained (g/100 g of fatty acid) 0.73 of C14:0, 85.2 of C16:0, 1.32 of C18:0, 10.2 of C18:1 *cis*-9, and 98% total fatty acids.
[5]Ca salts of palm FA supplement (Nutracal; Wawasan Agrolipids, Johor, Malaysia). The supplement contained (g/100 g of fatty acid) 0.88 of C14:0, 41.9 of C16:0, 4.04 of C18:0, 42.9 of C18:1 *cis*-9, and 82% total fatty acids.
[6] Expressed as percent of as fed.

Table 4-3. Nutrient intake and digestibility for cows fed treatment diets (n=32).

	Treatments[1]		SEM	P value[2]		
Variable	80:10	60:30		Trt	PMY	Trt*PMY
Intake, kg/d						
DM	23.6	23.3	0.32	0.34	<0.01	0.04
NDF	9.79	9.84	0.13	0.80	<0.01	0.05
Intake, g/d						
Total FA	1050	1069	14.3	0.37	<0.01	0.06
16-carbon	475	384	6.09	<0.01	<0.01	0.04
18-carbon	468	643	10.2	<0.01	0.06	0.26
Digestibility, %						
DM	63.1	63.2	0.36	0.81	0.27	0.69
NDF	38.1	38.4	0.64	0.71	0.01	0.88
Total FA	74.9	77.6	0.94	<0.01	0.86	0.38
16-carbon	74.2	76.9	0.79	<0.01	0.96	0.87
18-carbon	73.0	79.3	1.35	<0.01	0.92	0.30
Absorbed, g/d						
Total FA	787	828	14.1	0.03	0.01	0.21
16-carbon	352	296	5.61	<0.01	<0.01	0.08
18-carbon	345	508	10.9	<0.01	0.18	0.62

[1]Treatments were: 1) 80:10 (1.5% of FA blend of 80% C16:0-enriched supplement and 10% Ca-Salt of Palm FA); 2) 60:30 (1.5% of FA supplement of 60% C16:0-enriched supplement and 30% Ca-Salt of Palm FA).
[2] P values associated with treatment, preliminary milk yield and interaction.

Table 4-4. Milk yield, milk composition, BW and BCS of cows fed treatment diets (n=32).

	Treatments[1]		SEM	P value[2]		
Variable	80:10	60:30		Trt	PMY	Trt*PMY
DMI, kg/d	23.6	23.3	0.32	0.34	<0.01	0.04
Milk Yield, kg/d						
Milk	46.2	47.1	0.72	0.38	<0.01	0.12
3.5% FCM[3]	51.9	51.3	0.79	0.37	<0.01	0.05
ECM[4]	52.0	51.3	0.71	0.35	<0.01	0.04
Milk Composition						
Fat, kg/d	1.92	1.85	0.04	<0.01	<0.01	0.41
Fat, %	4.10	4.05	0.07	0.03	<0.01	0.95
Protein, kg/d	1.61	1.55	0.03	0.03	<0.01	0.15
Protein, %	3.40	3.35	0.05	0.06	<0.01	0.75
Lactose, kg/d	2.33	2.27	0.04	0.10	<0.01	0.28
Lactose, %	4.87	4.86	0.03	0.61	<0.01	0.90
BW, kg	733	734	9.5	0.74	0.43	0.36
BW change kg/d	0.35	0.45	0.11	0.54	0.02	0.97
BCS	3.41	3.40	0.06	0.87	<0.01	0.62
BCS change	0.03	0.01	0.02	0.54	0.01	0.93

[1]Treatments were: 1) 80:10 (1.5% of FA blend of 80% C16:0-enriched supplement and 10% Ca-Salt of Palm FA); 2) 60:30 (1.5% of FA supplement of 60% C16:0-enriched supplement and 30% Ca-Salt of Palm FA).
[2] P values associated with treatment, preliminary milk yield and interaction.
[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)].

Table 4-5. FA concentration and yield by source of milk FA for cows fed treatment diets (n=32).

	Treatments[1]		SEM	P value[2]		
Variable	80:10	60:30		Trt	PMY	Trt*PMY
Summation by Source[3], g/100 g FA						
De Novo	24.8	24.8	0.26	0.22	0.22	0.38
Both	41.5	38.7	0.31	<0.01	0.66	0.97
Preformed	33.6	36.4	0.31	0.08	0.81	0.26
Summation by Source[3], g/d						
De Novo	448	428	10.5	0.01	<0.01	0.14
Both	749	669	16.4	<0.01	<0.01	0.08
Preformed	605	627	11.4	0.01	<0.01	0.84

[1] Treatments were: 1) 80:10 (1.5% of FA blend of 80% C16:0-enriched supplement and 10% Ca-Salt of Palm FA); 2) 60:30 (1.5% of FA supplement of 60% C16:0-enriched supplement and 30% Ca-Salt of Palm FA).
[2] P values associated with treatment, preliminary milk yield and interaction.
[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).

Table 4-6. Plasma insulin and blood metabolites for cows fed treatment diets (n=32).

	Treatments[1]		SEM	P value[2]		
Variable	80:10	60:30		Trt	PMY	Trt*PMY
Insulin, ug/L	0.97	0.94	0.04	0.36	0.01	0.58
NEFA, mEq/L	0.12	0.13	<0.01	0.04	<0.01	0.17
BHB, mg/dL	8.18	8.43	0.21	0.17	0.03	0.86

[1] Treatments were: 1) 80:10 (1.5% of FA blend of 80% C16:0-enriched supplement and 10% Ca-Salt of Palm FA); 2) 60:30 (1.5% of FA supplement of 60% C16:0-enriched supplement and 30% Ca-Salt of Palm FA).
[2] P values associated with treatment, production level and interaction.

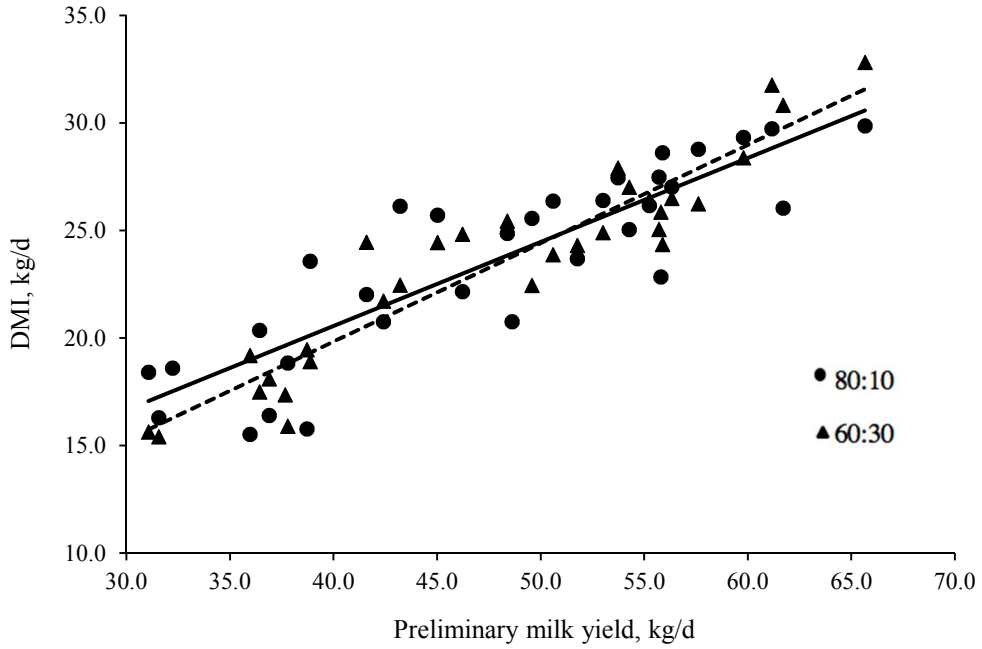

Figure 4-1. Relationship between DMI and PMY of cows fed either higher palmitic acid-supplemented diet or an increased oleic acid-supplemented diet. (80:10; 1.5% of FA blend of 80% C16:0-enriched supplement and 10% Ca-Salt of Palm FA); ECM (kg/d) = 4.93 + 0.39x × PMY (kg/d); $R^2 = 0.76$; $P < 0.04$; solid line) (60:30 (1.5% of FA supplement of 60% C16:0-enriched supplement and 30% Ca-Salt of Palm FA; ECM (kg/d) = 1.55 + 0.457x × PMY (kg/d); $R^2 = 0.88$; $P < 0.04$; broken line).

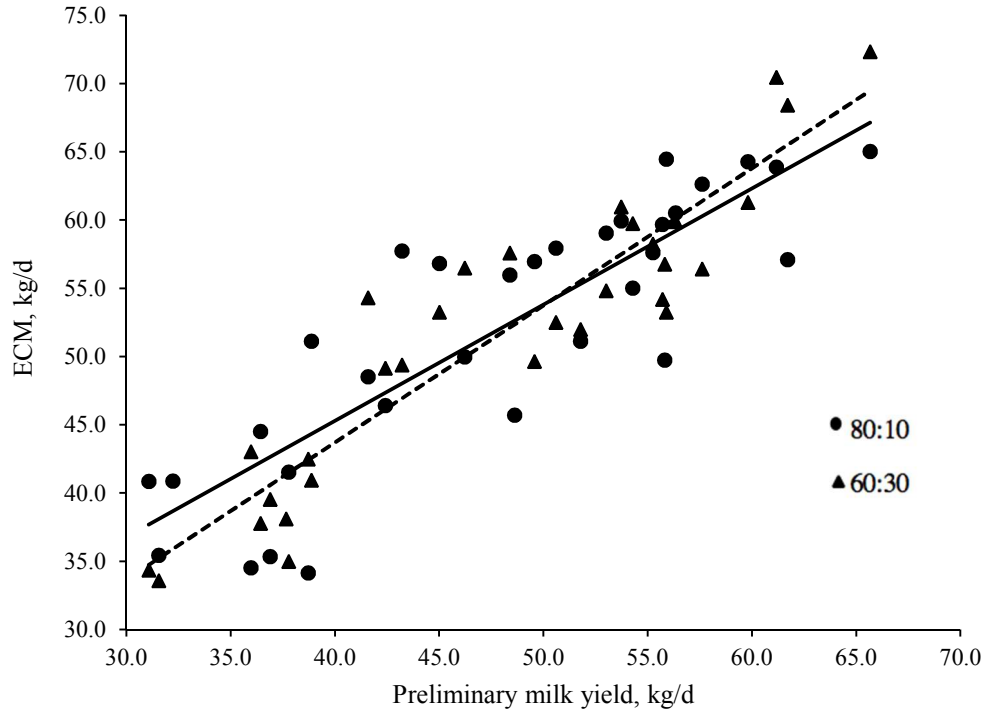

Figure 4-2. Relationship between ECM and PMY of cows fed either higher palmitic acid-supplemented diet or an increased oleic acid-supplemented diet. (80:10; 1.5% of FA blend of 80% C16:0-enriched supplement and 10% Ca-Salt of Palm FA; ECM (kg/d) = 11.2 + 0.852x × PMY (kg/d); $R^2 = 0.74$; $P < 0.04$; solid line) (60:30 (1.5% of FA supplement of 60% C16:0-enriched supplement and 30% Ca-Salt of Palm FA); ECM (kg/d) = 3.54 + 1.004x × PMY (kg/d); $R^2 = 0.86$; $P < 0.04$; broken line).

Table 4-7. Milk fatty acid concentration for cows fed treatment diets (n=32).

	Treatments[1]		SEM	P value[2]		
Variable	80:10	60:30		Trt	PMY	Trt* PMY
Selected Individual FA[3]						
C4:0	2.64	2.70	0.04	0.03	0.81	0.83
C6:0	1.82	1.85	0.03	0.21	0.38	0.76
C8:0	1.12	1.12	0.02	0.55	0.14	0.51
C10:0	3.11	3.09	0.06	0.54	0.1	0.34
C12:0	3.69	3.62	0.07	0.09	0.06	0.24
C14:0	11.7	11.7	0.11	0.45	0.84	0.13
C16:0	40.1	37.4	0.30	<0.01	0.66	0.97
cis-9 C16:1	1.40	1.28	0.04	<0.01	0.81	0.99
C18:0	7.70	8.49	0.17	<0.01	0.59	0.40
cis-9 C18:1	16.8	18.2	0.20	<0.01	0.24	0.60
cis-11 C18:1	0.48	0.50	0.02	0.08	0.01	0.49
trans-6 to 8 C18:1	0.21	0.27	0.01	<0.01	0.01	0.27
trans-9 C18:1	0.17	0.21	0.01	<0.01	0.03	0.23
trans-10 C18:1	0.35	0.41	0.02	<0.01	0.01	0.92
trans-11 C18:1	0.57	0.67	0.02	<0.01	0.12	0.23
cis-9, *cis*-12 C18:2	2.21	2.29	0.03	<0.01	0.03	0.98
cis-9, *trans*-11 C18:2	0.30	0.34	0.01	<0.01	0.26	0.34
cis-9, *cis*-12, *cis*-15 C18:3	0.29	0.30	0.01	0.01	0.09	0.32

[1] Treatments were: 1) 80:10 (1.5% of FA blend of 80% C16:0-enriched supplement and 10% Ca-Salt of Palm FA); 2) 60:30 (1.5% of FA supplement of 60% C16:0-enriched supplement and 30% Ca-Salt of Palm FA).

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

[3] g/100 g FA. A total of approximately 80 individual FA were quantified. Only select FA are reported in the table.

Table 4-8. Milk fatty acid yield for cows fed treatment diets (n=32).

Variable	Treatments[1]		SEM	P value[2]		
	80:10	60:30		Trt	PMY	Trt* PMY
Selected Individual FA[3]						
C4:0	47.6	46.5	1.16	0.02	<0.01	0.49
C6:0	33.0	31.9	0.89	0.08	<0.01	0.33
C8:0	20.2	19.4	0.60	0.06	<0.01	0.26
C10:0	56.0	53.1	1.70	0.02	0.01	0.17
C12:0	66.4	62.1	1.90	<0.01	0.01	0.11
C14:0	210	202	4.64	0.02	<0.01	0.10
C16:0	724	647	15.9	<0.01	<0.01	0.09
cis-9 C16:1	25.3	22.1	0.89	<0.01	<0.01	0.09
C18:0	138	146	4.13	<0.01	<0.01	0.87
cis-9 C18:1	301	312	5.73	0.01	<0.01	0.8
cis-11 C18:1	8.73	8.67	0.27	0.71	<0.01	0.86
trans-6 to 8 C18:1	3.88	4.73	0.12	<0.01	<0.01	0.23
trans-9 C18:1	3.09	3.64	0.08	<0.01	<0.01	0.18
trans-10 C18:1	6.38	7.21	0.38	<0.01	<0.01	0.93
trans-11 C18:1	10.3	11.6	0.43	<0.01	<0.01	0.39
cis-9, *cis*-12 C18:2	40.0	39.7	0.94	0.57	<0.01	0.33
cis-9, *trans*-11 C18:2	5.37	5.93	0.23	<0.01	<0.01	0.50
cis-9, *cis*-12, *cis*-15 C18:3	5.32	5.20	0.13	0.13	<0.01	0.21

[1] Treatments were: 1) 80:10 (1.5% of FA blend of 80% C16:0-enriched supplement and 10% Ca-Salt of Palm FA); 2) 60:30 (1.5% of FA supplement of 60% C16:0-enriched supplement and 30% Ca-Salt of Palm FA).
[2] P values associated with treatment, production level and interaction.
[3] g/d FA. A total of approximately 80 individual FA were quantified. Only select FA are reported in the table.

CHAPTER 5

OVERALL CONCLUSIONS

Fatty acid supplementation can help achieve farm goals of higher productivity and profitability through increasing milk components yields. The objectives of our studies were to determine nutrient digestibility and production responses of commercially-available C16:0 and C18:0-enriched supplements in high producing lactating dairy cows as well as the effect of different ratios of C16:0 and *cis*-9 C18:1 in lactating dairy cows across a wide range of milk production. Together, these studies examined the effects of common FA included in supplements and their effects on performance of lactating dairy cows.

In chapter 3, commercially-available C16:0 and C18:0-enriched supplements increased milk production compared to no added fat, but the C16:0 supplement increased milk fat yield, ECM and 3.5% FCM without increasing DMI or having effects on BW or BCS compared to the C18:0 supplement. C16:0 also increased digestibilities of gross energy, DM, NDF, and total FA compared with C18:0 when included at 1.5% of DM. Thus, C16:0 increased energy intake and milk energy output without increasing DMI, and therefore increased feed efficiency. Though our study examined two different types of SFA, we determined that they are not equivalent as only C16:0 increased digestible energy intake compared with C18:0 fed at the same rate.

In Chapter 4, we observed interactions between preliminary milk yield and treatment. Higher producing cows (>50 kg/d) increased DMI and ECM, and tended to increase milk yield with a supplement containing a higher proportion of C18:1, while lower producing cows (<50 kg/d) responded more positively when fed a supplement with a higher proportion of C16:0. These

differences indicate that cows respond differently to specific FA or ratios depending on production level.

Both chapters support recent research recently completed in our lab. Boerman et al. (2017) observed decreases in digestibility measures with C18:0 supplementation causing decreased production measures, but no differences were observed on BW. Interestingly, we observed similar results on nutrient digestibility, production measures, and BW with a commercially available supplement containing C18:0 even when examined over a longer period. Recently, a review by Loften et al. (2014) suggested that feeding a combination of C16:0 and C18:0 is needed to optimize their utilization for milk production and overall performance of the dairy cow; our results do not support that suggestion.

While the magnitude of response to feeding different ratios of C16:0 and C18:1 in our current study was less than that observed by de Souza et al. (2017 ADSA Abstract), the interaction of FA supplement and preliminary milk production was similar. In both studies, higher producing cows responded better to a supplement containing more C18:1, while lower producing cows responded better to a supplement containing more C16:0. In conclusion, individual SFA supplements enriched in C16:0 or C18:0 have different impacts on production as well as nutrient digestibility, and different ratios of C16:0 and C18:1 can be used at across production levels to increase nutrient digestibility and production responses. This work provides information that can be used to guide feeding decisions to maximize performance and farm income while using commercial FA supplements. Our results indicate that optimal feeding of FA depends on production level and the FA profile of the supplement or blend. Additional research on FA supplementation and individual FA is required to determine the ideal FA or ratio depending on production, stage of lactation, and metabolic requirements.

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