

**EFFECTS OF 16- AND 18-CARBON FATTY ACIDS ON NUTRIENT DIGESTIBILITY
AND PRODUCTION RESPONSES OF LACTATING DAIRY CATTLE**

By

Alycia Marie Burch

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ABSTRACT

EFFECTS OF 16- AND 18-CARBON FATTY ACIDS ON NUTRIENT DIGESTIBILITY AND PRODUCTION RESPONSES OF LACTATING DAIRY CATTLE

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Addition of fat supplements to dairy cow diets is common practice due to increases in yields of milk and milk fat. This thesis contains two studies that evaluated the effects of palmitic (C16:0), stearic (C18:0), and oleic acids (*cis*-9 C18:1) on lactating dairy cows. The first experiment used three commercially available products to make custom blends containing 60% C16:0 + 30% C18:0 (PA+SA) and 60% C16:0 + 30% *cis*-9 C18:1 (PA+OA) supplied at 1.5% diet dry matter (DM) and a non-FA supplemented control (CON) diet fed to low- and high-producing dairy cows. The PA+OA treatment increased yields of milk, milk fat, and energy corrected milk (ECM) compared with CON for high-producing cows. In contrast, the PA+SA treatment increased yields of milk fat, milk protein, ECM, and 3.5% fat-corrected milk (FCM) compared with the PA+OA treatment in low-producing cows. The second experiment evaluated different ratios of C16:0 + *cis*-9 C18:1 in low fat (LF; average 2.40% FA content) and high fat (HF; average 3.28% FA content) diets. Fatty acid (FA) treatments were products consisting of 80% C16:0 + 10% *cis*-9 C18:1 (PA) and 60% C16:0 + 30% *cis*-9 C18:1 (PA+OA) supplemented at 1.5% diet DM and a non-FA supplemented control (CON) diet. Interactions were observed between basal diet and FA treatment with FA treatments increasing yields of milk and lactose in the LF diet but not in the HF diet. The HF diet increased yields of milk fat and FCM and tended to increase ECM. FA treatments increased yields of milk fat, ECM, and FCM, but were not different from each other. Results from this work can help further feeding decisions for nutritionists and producers.

This thesis is dedicated to my mother, Shelly Burch.
I only wish you were here to cherish this moment with me.

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KEY TO ABBREVIATIONS

BCS	Body condition score
BH	Biohydrogenation
BW	Body weight
CCK	Cholecystokinin
CLA	Conjugated linoleic acid
CP	Crude protein
CON	Control treatment
CSH	Cottonseed hulls
CSM	Cottonseed meal
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	3.5% Fat-corrected milk
FFA	Free fatty acids
GLC	Gas liquid chromatography
GLUT	Glucose transporter

G3P	Glycerol-3-phosphate
HF	High fat
LF	Low fat
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
PA	80 % palmitic, 10% oleic acid treatment
PA+OA	60 % palmitic, 30% oleic acid treatment
PA+SA	60% palmitic, 30% stearic acid treatment
PUFA	Polyunsaturated fatty acids
SEM	Standard error of the mean
SD	Standard deviation
SFA	Saturated fatty acids
TAG	Triacylglyceride
TG	Triglycerides
TMR	Total mixed ration
WCS	Whole cottonseed
UFA	Unsaturated fatty acids

CHAPTER 1

INTRODUCTION

Dairy farmers are paid based on the yield of milk components produced which emphasizes the importance of increasing the yields of milk fat and milk protein. In comparison to other milk components, milk fat is typically the easiest to manipulate by nutritional strategies and management, which has increased the use of feeding fat supplements. Fat supplementation has been studied for many years, and a recent 100-year review highlighted the positive benefits of including fat supplements in lactating dairy cow diets (Palmquist and Jenkins, 2017).

In a meta-analysis, fatty acid (FA) supplementation increased the yield of milk and milk fat, with variation among different types of supplemental fat (Rabiee et al., 2012). This variation could be attributed to different FA profiles of fat supplements, the level of FA supplementation, and production level of cows. Increased attention is being given to individual FA and understanding the effects of the FA profile of supplements on production responses. Palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (*cis*-9 C18:1) are the most abundant FA present in milk fat and adipose tissue of dairy cows and are also the most common FA found in commercial fat supplements (Palmquist, 2006). These three FA are being studied extensively to understand their individual effects on milk production, nutrient digestibility, and metabolism in dairy cows.

Production level is a factor that interacts with nutrition and affects production (Harvatine and Allen, 2015). To our knowledge, there are few studies that have evaluated different blends of these individual FA on production responses and nutrient digestibility of lactating dairy cows across different production levels and how they interact with different basal fat levels. FA supplementation within different production levels have been evaluated but were not designed to

evaluate differences in C18:0 and *cis*-9 C18:1. It is critical to understand how these blends of FA affect overall production responses and how they impact production responses of cows at different production levels. This will advance understanding of tailoring FA supplementation to specific production level needs and thus increasing farm profitability. Also, understanding how FA supplementation interacts with other feed ingredients and basal fat content will allow nutritionists to fine tune dairy cow diets. It will also help to understand the functionality of FA supplementation and nutrition. Therefore, the main objective of this thesis was to examine the effects of C16:0, C18:0 and *cis*-9 C18:1 on production responses and nutrient digestibility of lactating dairy cows.

CHAPTER 2

LITERATURE REVIEW

Importance of Milk Production and Milk Components

In most milk market federal orders, dairy producers are paid for the yields of milk fat and milk protein produced. The Federal Milk Order Program uses milk fat and protein yield as the major price influencers when they are establishing the price of milk. Thus, an increase in the yield of these milk components will increase income for dairy producers. Milk fat is easier to manipulate than milk protein, but dietary strategies can either positively or negatively affect them. Therefore, strategies to increase milk fat and protein yield, and as a result increase farm profitability, is a topic that is becoming increasingly examined by researchers.

Fatty Acid Content of Feed Ingredients

Feedstuffs commonly fed to ruminants contain mostly unsaturated fatty acids (UFA), with grass and legume forages containing high levels of linolenic acid (*cis*-9, *cis*-12, *cis*-15-18:3) while corn silage and grains consist of linoleic acid (*cis*-9, *cis*-12-18:2). Oilseeds, such as whole cottonseed (WCS) and soybeans, are also high in UFA, and both predominantly contain linoleic acid, followed by oleic acid (*cis*-9 C18:1). Fatty acid (FA) supplements are abundant in palmitic (C16:0), stearic (C18:0), and *cis*-9 C18:1 but range in FA profile depending on the product. Percentages of individual FA and total FA content of common feedstuffs are presented in Table 2.1. It is important to note that corn, grass, and legume forages contain approximately 2-3% total FA as a percent of DM (Drackley, 2004). The FA% on a DM basis of grains range from 1 to 4%, although distiller grains can reach 10% (NRC, 2001). WCS, whole soybeans, and whole canola seeds range from 15 to 20% total FA, but other oilseeds can be as high as 40%, e.g.

sunflower seeds (NRC, 2001; Walker, 2006). FA supplements contain greater than 80% total FA on a DM basis, with Ca-salts containing approximately 80 to 85% total FA while prilled fats contain approximately 95% total FA. In order to increase the FA content of a diet, oilseeds and FA supplements are often used as they are both higher in FA content compared with forages and grains.

WCS is a by-product of the cotton-gin industry and is a feedstuff generally fed to higher-yielding dairy cattle due to its high level of fat, crude protein, and neutral detergent fiber (Arieli, 1998). The digestible fiber in WCS has been proposed to be able to replace that of forage, if needed (Arieli, 1998). WCS has high levels of UFA, with linoleic (*cis*-9, *cis*-12, C18:2) and oleic acids (*cis*-9 C18:1) comprising respectively 62 and 15% of the total lipid content (Smith et al., 1981). Even though there is indication of greater biohydrogenation of WCS in the rumen due to the digesta of the small intestine containing increased levels of saturated FA (SFA) when WCS are fed (Arieli, 1998), WCS may be released slowly in the rumen or even leave the rumen still partially enclosed within the seed (Sklan et al., 1992). Responses in digestibility and production vary when feeding WCS as some studies have reported increases in milk fat yield while others have reported negative effects on milk production and milk protein content, although these responses could happen simultaneously (Arieli, 1998).

Rumen Metabolism of Dietary Fats

Fatty acid composition of ruminant meat and milk is influenced by FA metabolism in the rumen. Although ruminant diets are high in UFA, biohydrogenation (BH) of dietary UFA in the rumen results in saturated fatty acids (SFA) being the main FA reaching the intestine, resulting in

ruminant meat and milk containing a much higher amount of SFA than UFA (Palmquist et al., 2005).

The two main processes that contribute to the metabolism of FA are lipolysis and BH (Figure 2.1; Buccioni et al., 2012), with lipolysis being the first step. Lipolysis is the process of microbial lipases hydrolyzing the ester linkages in lipids in order to release the FA and thus exposing the UFA to BH by ruminal microbes (Jenkins et al., 2008). The lipolysis process is a critical step, especially with esters, salts, and other modifications of UFA, since a requirement for BH is the presence of a free carboxyl group (Harfoot and Hazlewood, 1997). BH is the reduction of the double bonds on the carbon chain of an UFA to produce SFA (Buccioni et al., 2012) with the main end product being C18:0 (Harfoot and Hazlewood, 1997). The rumen bacteria perform BH as a detoxification mechanism against UFA on bacterial growth, as they are more toxic to the rumen bacteria than SFA (Maia et al., 2010). This toxicity could be due to the double bonds in the structure of the UFA and the level of toxicity differs depending on the individual FA (Maia et al., 2010). Bacteria that are responsible for BH can be categorized into two groups; Group A and Group B. Bacteria that are a part of Group A are responsible for the hydrogenation of linoleic and linolenic acids to vaccenic acid (*trans*-11 C18:1) and appear to be incapable of hydrogenating 18-carbon monounsaturated FA. Meanwhile Group B bacteria are able to complete the BH process of *trans*- and *cis*-octadecenoic acids and the only known hydrogenators of *cis*-9 C18:1 to C18:0 (Harfoot and Hazlewood, 1997; (Palmquist et al., 2005). Figure 2.2 shows how these two groups both must be present in order to fully BH UFA to C18:0. Since there is continual passage of digesta that is leaving the rumen, some UFA and BH intermediates escape the rumen and then are absorbed in the small intestine.

Predominant pathways and intermediates produced are shown in Figure 2.3, but there are alternative BH pathways that produce other intermediates, such as *trans*-10, *cis*-12 CLA, that can reduce milk fat synthesis in the mammary gland (Bauman et al., 2011). These altered BH intermediates can be the result of the content of UFA and a change in rumen pH (Maia et al., 2010). A low ruminal pH can inhibit and cause differences in growth of the microbial population thus producing altered BH pathway intermediates, such as transforming *cis*-9 C18:1 to *trans*-10 C18:1 instead of *trans*-11 C18:1 (Jenkins et al., 2008).

Fat supplements have been developed to increase dietary FA supply while minimizing the negative effects that UFA have on rumen fermentation and digestion. Calcium-salts are a product that have been shown to be effective in mitigating negative ruminal effects while also being efficiently digested by the cow (Jenkins and Palmquist, 1984). Prilled fats have also been developed, mostly containing C16:0 and C18:0, and are another means to provide FA without disrupting rumen fermentation or milk fat synthesis. In general, these products increase production (Rabiee et al., 2012).

Digestibility and Absorption of Dietary FA

Lipids that leave the rumen are predominantly free FA (85 to 90%) and phospholipids (10 to 15%). These free FA are found as potassium, sodium, or calcium salts of FA in the rumen, and will dissociate passing through the acidic conditions (pH ~ 2.0) in the abomasum to then be adsorbed to the surface of the feed particles that pass through as digestive contents (Drackley, 2004). Of the free FA, a majority of them are C18:0 due to the BH of UFA in the rumen. The FA enters the duodenum where secretions of bile and pancreatic juice are added to the digesta via the bile duct and are essential for FA digestion and absorption (Scarlet and Drackley, 2013). Bile

supplies bile salts and lecithin while pancreatic secretions provide enzymes to convert lecithin to lysolecithin, thus allowing for bile salts and lysolecithin to dissociate FA from feed particles to enable micelle formation (Lock et al., 2005). In ruminants, lysolecithin acts as an amphiphile, containing both hydrophobic and hydrophilic molecules that help aid formation of micelles for FA absorption. Lysolecithin is the most effective amphiphile at increasing the micellar solubility of C18:0 (Freeman, 1969) and is a requirement for FA absorption to occur (Moore and Christie, 1984). These micelles consist of water-insoluble lipids surrounded by bile salts and phospholipids that transport the lipids across the unstirred water layer of intestinal epithelial cells of the jejunum, where the FA and lysolecithin are absorbed (Lock et al., 2006). The bile salts are not absorbed by the jejunum and will continue to create micelles through the small intestine (Scarlet and Drackley, 2013). After absorption, the FA are re-esterified into triglycerides in the endoplasmic reticulum of the enterocyte and then combined into lipoprotein particles, such as chylomicrons or VLDL (Drackley, 2004; Cifarelli and Abumrad, 2018).

Effects of Fat Supplements on Rumen Fermentation

Rumen protozoa have been found to be the most affected by FA, followed by cellulolytic bacteria (Hino and Nagatake, 1993). As stated earlier, compared with SFA, UFA are more toxic to the rumen bacteria, but they can also inhibit fermentation to a greater extent (Jenkins, 1993). Rumen fermentation processes, such as digestibility and microbial cell synthesis and sites of digestion, are known to be affected by the FA concentration entering the rumen (Jenkins and Palmquist, 1984; Boggs et al., 1987). One theory proposed is FA inhibit fermentation by coating the microorganisms with a hydrophobic film that disrupts the adherence of bacteria to cellulose fibers and thus decreasing cellulose digestion (Jenkins, 1993). A free carboxyl group was

suggested to disrupt rumen fermentation and supplying the FA as calcium salts has been found to reduce this inhibitory effect (Jenkins and Palmquist, 1994).

Effects on Fat Supplements on NDF and DM Digestibility

Fiber digestibility can be impacted depending on the FA profile. Weld and Armentano (2017) concluded that vegetable oils decreased NDF digestibility while SFA tended to increase NDF digestibility. C16:0 has been observed to consistently increase NDF digestibility (Piantoni et al., 2013; de Souza et al., 2018) while C18:0 supplementation had no effect on NDF digestibility (Piantoni et al., 2015; Boerman et al., 2017). Supplementation of C16:0 and a blend of C16:0 + *cis*-9 C18:1 increased NDF digestibility and tended to increase DM digestibility compared with a blend of C16:0 + C18:0 (de Souza et al., 2018). The increase in digestibility of both NDF and DM with the blend of C16:0 + *cis*-9 C18:1 could be due to the decrease in DMI. Increasing the amount of dietary *cis*-9 C18:1 was observed to linearly increase DM digestibility with no effects on DMI and had no effect on NDF digestibility (de Souza et al., 2019). This increase in NDF digestibility when supplementing C16:0 and blend of C16:0 + *cis*-9 C18:1 could be attributed to an increase in the secretion of cholecystokinin (CCK), a gut peptide associated with satiety, that can lead to an increase in retention time (Piantoni et al., 2013). Also, if dietary C16:0 can be incorporated into bacterial membranes, it can alleviate the need to produce C16:0 *de novo*, thus sparing ATP for bacteria growth and increasing NDF digestibility (Wu and Palmquist, 1991; Hackmann and Firkins, 2015).

Effects of Fat Supplements on FA Digestibility

Rumen outflow of total FA is similar to dietary intake of total FA (Lock et al., 2006) but apparent digestibility of FA can decline as the supply of FA increases (Palmquist, 1991). In a meta-analysis evaluating intestinal digestibility, Boerman et al. (2015) concluded that total FA

digestibility was negatively affected by the flow of total FA in the duodenum. This decrease in digestibility could be attributed to limitations in secretion and activity of bile salts and pancreatic lipases that can affect absorption at elevated FA intakes (Bauchart, 1993). Even though Boerman et al (2015) reported total FA intake negatively affecting digestibility, the amount of C16:0 reaching the duodenum had positive impacts on total FA digestibility. These results indicate that profile of the FA reaching the duodenum is an important factor affecting FA digestibility.

Boerman et al. (2015) reported that UFA had a higher digestibility compared with SFA, with these values for individual FA being 76.5, 73.7, 80.8, 79.9, and 78.8% for C16:0, C18:0, C18:1, C18:2, and C18:3 respectively. Additionally, Boerman et al. (2015) reported that increased flow of C18:0 not only negatively affected C18:0 digestibility, it also negatively impacted the digestion of other FA. Supplementation of C16:0 and C18:0 have both been observed to decrease FA digestibility as the amount of FA reaching the duodenum increased, but this decrease was more pronounced for C18:0 (Figure 2.4) (Boerman et al, 2017; Rico et al., 2017). As stated, UFA increases digestibility, with *cis*-9 C18:1 having a higher digestibility than C16:0 and C18:0 (Boerman et al., 2015; de Souza et al., 2018). In an abomasal infusion study, this increase in digestibility with *cis*-9 C18:1 was also observed, and FA digestibility increased as the amount of *cis*-9 C18:1 increased (Prom, 2018 ADSA Abstract). de Souza et al. (2019) reported a linear increase in total and 16-carbon digestibility, and a quadratic increase in 18-carbon digestibility when increasing the amount of dietary *cis*-9 C18:1 in a C16:0 + *cis*-9 C18:1 supplemental blend. Reasons for these increases in digestibility could be related to amphiphilic properties of *cis*-9 C18:1 as it increases the saturation point of C18:0 in micelles (Freeman, 1969).

As expected from previous research, the literature highlights differences in the digestibility of individual and total FA. Ca-salts and vegetable oils increased individual FA digestibility compared with whole oilseeds (Boerman et al., 2015). These results are also similar to Wu et al., (1991) where Ca-salts of FA were more digestible than an animal-vegetable FA blend.

Effects of Oil Seeds on Digestibility

In a review of utilization of WCS, Coppock et al. (1987) reported that increasing the amount of WCS increased digestibility of ether extract without effecting DM or fiber digestibility. Comparing whole soybean seeds and cottonseeds, DM digestibility was not affected by addition WCS whereas incorporation of whole soybeans decreased DM digestibility (Mohamed et al., 1988). In a meta-analysis, Boerman et al. (2015) reported a decrease in FA digestibility with oilseeds compared with other forms of FA supplementation. Comparing level of basal FA, Rico et al. (2017) reported that a basal diet containing WCS increased 16-carbon and total FA digestibility while decreasing 18-carbon FA and NDF digestibility compared with a soyhull basal diet. In contrast, de Souza et al. (2018) evaluated a soyhull basal diet and WCS basal diet and observed that WCS decreased digestibility of 16-carbon, 18-carbon, total FA, and NDF digestibility compared with the soyhull diet. Rumen fermentation could play a role in the decrease of digestibility with WCS, and there is potential that the resistance of the seed coat to rumen degradation can be a factor for whole oilseeds (Mohamed et al., 1988).

Milk Fat Synthesis in the Mammary Gland

Milk fat is a major energy expense to the cow (Emery, 1973) and is also the most variable component of milk, both in concentration and composition and these can be influenced by diet

and stage of lactation (Palmquist, 2006). As mentioned earlier, due to rumen BH of UFA to SFA, bovine milk fat and adipose tissue are highly saturated even though most of the dietary FA a cow consumes are UFA. Lipid globules are emulsified in the aqueous phase of milk, with bovine milk fat concentration ranging from 3 to 5% and mostly comprised of triglycerides (98%), along with phospholipids, diglycerides, and cholesterol (Jensen, 2000). Bovine milk fat is complex, with more than 400 different FA that differ in chain length, structure, and configuration of double bonds (Jensen, 2002). Milk FA are derived from de novo synthesis of short-chain FA and uptake of long-chained FA from circulation (Palmquist, 2006). Mixed source FA (C16:0 and *cis*-9 C16:1) are derived from both de novo synthesis in the mammary gland and extraction from plasma.

De Novo Synthesis

The synthesis of short and medium chain FA (< 16-carbons) occurs by de novo synthesis in the mammary gland. In ruminants, de novo synthesis accounts for approximately half of the FA in milk (Bauman and Griinari, 2003). Requirements for ruminant milk FA synthesis include a carbon source (acetate and beta-hydroxybutyrate) and reducing equivalents in the form of NADPH (produced from glucose and acetate) (Palmquist, 2006). Acetate is produced during rumen fermentation and is the main carbon source for FA synthesis, where it is activated to acetyl-CoA in the cytosol and then incorporated into FA via the malonyl-CoA pathway (Bauman and Davis, 1974; Bauman and Griinari, 2003). Beta-hydroxybutyrate is also used for a carbon source, but at a lesser extent than acetate, and it contributes the first four carbon units of the FA produced (Bauman and Griinari, 2003). Glucose is required for milk synthesis, as it is a precursor for lactose, but for ruminants the carbons from glucose are limited and supply of glucose utilized for the mammary gland must be supplied by gluconeogenesis (Palmquist, 2006).

The reductive steps of fatty acid synthase (FAS) require NADPH, which can be sourced from glucose oxidation via the pentose phosphate pathway or acetate via the isocitrate pathway, although the demand for these reducing equivalents can be decreased due to high-fat diets that reduce the synthesis of de novo FA (Palmquist, 2006). This can spare glucose for the mammary gland and lactose synthesis (Cant et al., 1993). The chain length of the FA is determined by a series of decarboxylative condensation reactions, that add 2 carbon units in a continuous cycle until the FA reaches C₁₂ to C₁₆ when a thioesterase enzyme for a specific chain length terminates the cycle (Smith et al., 2003).

Preformed FA

Long-chain FA, >16-carbons in length, that are used for preformed milk FA synthesis mainly come from absorption of dietary FA, with these FA mostly being saturated 18-carbon FA. The primary sources of these FA are derived from TAG-rich lipoproteins from blood and account for greater than 95% of the 18-carbon and long-chain FA in milk fat (Palmquist, 2006). Thus, the mammary gland is supplied with long-chain FA that are used exclusively for fat synthesis. NEFA, derived from the mobilization of body reserves and lipolysis usually account for only a small fraction of the FA in milk fat (Bauman and Griinari, 2003). An exception to this is when cows are in negative energy balance, the contribution from NEFA is higher due to greater mobilization of body reserves (Palmquist, 2006). As stated earlier, 16-carbon FA come from blood lipids and de novo synthesis. C16:0 can be synthesized de novo in large quantities when dietary fat intake is reduced, but as FA intake is increased and thus uptake from circulation increases, the amount of C16:0 synthesized from de novo synthesis will decrease (Palmquist, 2006).

Triglyceride Synthesis

Both de novo and preformed FA are utilized for triacylglyceride (TAG) synthesis in the mammary gland. The primary path for this synthesis is through the *sn*-glycerol-3 phosphate pathway and incorporated on the glycerol-3 phosphate backbone (Dils, 1983). Glycerol-3 phosphate (G3P) is required for esterification of FA, generated through glycolysis or phosphorylation of free glycerol by glycerol kinase, and acylation of G3P is the first step in TG synthesis (Palmquist, 2006). G3P acyl transferase adds the first fatty-acyl CoA to the *sn*-1 position of the G3P, then acyl glycerol phosphate acyl transferase adds the second fatty-acyl CoA to the *sn*-2 position, and ends with diglycerol acyl transferase adding the final fatty-acyl CoA to the *sn*-3 position to form the TAG (Palmquist, 2006). Placement of individual FA to the 3 positions of the glycerol backbone are not random (Jensen, 2002) as short-chain FA are esterified at *sn*-3, medium-chain FA at *sn*-2, and long-chain FA predominantly at *sn*-1 (Mansson, 2008). C16:0 can be found equally at *sn*-1 and 2-positions, while C18:0 is mostly found at the *sn*-1 position but can be acylated at *sn*-3, and *cis*-9 C18:1 is located at *sn*-1 and 3-positions (Jensen, 2002). In a recent meta-analysis, Glasser et al. (2008) discussed the interdependence between short-/medium-chain FA and long-chain FA and postulated that milk fat synthesis is dependent on the simultaneous supply of these FA for milk fat synthesis. DGAT esterifies both short-chain and long-chain FA at the *sn*-3 position and is up-regulated with increasing amounts of FA (Palmquist, 2006). As a result, increases in the supply of exogenous long-chain FA can reduce de novo synthesis due to the competition for the diglycerol acyl transferase between short- and long-chain FA (Palmquist, 2006).

Impacts of Feed Ingredients with High Fatty Acid Content on Production Responses

Oilseeds are a rich source of UFA and are a way to increase the energy density of the diet and have also been reported to increase milk fat yield (Rabiee et al., 2012). There are numerous oilseeds that can be fed to dairy cattle, with a wide range in FA content and rumen fermentability. Commonly used oilseeds are WCS and soybeans. Besides these oilseeds, other cottonseed and soybean ingredients are cottonseed meal and hulls and soybean meal and hulls, that still contain the same FA profile but at a lower FA content. Banks et al. (1976) concluded that low-fat diets could limit the yields of milk and milk fat and increasing dietary FA supply increased these yields when different oils and oilseeds were fed (Virtanen, 1966; Banks et al., 1976). These low-fat diets potentially restricted energy supply to the cow and increasing the dietary FA content resulted in improved energy balance.

Effects on DMI

Increasing the fat content of diets can be achieved by utilizing oilseeds but this also means incorporating a higher level of UFA into the diet. Studies that have utilized WCS in the basal diet to increase fat content did not observe changes in DMI (Bernard et al., 1997; Smith et al., 1980; Johnson et al., 2002). Likewise, in a meta-analysis, Glasser et al. (2008) observed that soybean oil when in seeds did not alter DMI whereas the free soybean oil depressed DMI. In contrast, a meta-analysis by Rabiee et al. (2012) found that DMI was decreased with the use of oilseeds and other forms of FA (i.e. Ca-salts, prilled fat, and tallow) and increasing the amount of WCS decreased DMI linearly (Coppock et al., 1985). Allen (2000) proposed that the hypophagic effects of fat increase as the level of UFA are increased which could explain the decrease in DMI observed when oilseeds are fed. Results from these studies suggest that the effects on DMI are

variable depending on the type of oilseed used, the amount of UFA incorporated into the diet, and the profile of the FA leaving the rumen.

Basal diets with a low FA content usually contain no or low amounts of oilseeds. When comparing basal fat contents, both de Souza et al. (2018) and Rico et al. (2017) utilized WCS for a high-fat basal diet and soyhulls for a low-fat basal diet. There was no difference in DMI between the two basal diets for de Souza et al. (2018) while Rico et al. (2017) observed that the low-fat basal diet tended to increase DMI compared with the high-fat basal diet. Cottonseed hulls decreased intake compared with alfalfa hay, but increased intake compared with sunflower hulls, peanut hulls, corn silage, and alfalfa silage (Coppock et al., 1987). The lower content of FA, and thus lower content of UFA, of hulls could explain why they did not reduce DMI.

Effects on Milk Yield and Milk Components

Higher fat content in the basal diet, due to oilseeds, has been reported to increase the yield of milk and milk fat (Rabiee et al., 2012) while low-fat diets reduced these yields (Maynard and McCay 1929; Banks et al., 1976). Utilizing WCS to increase the fat content of the diet did not affect milk yield but rather increased milk fat content, milk fat yield, and FCM (Smith et al., 1981; Sklan et al., 1992) compared with lower levels of WCS or a basal diet without WCS. These results could be due to the greater intake of UFA and incorporation of preformed FA into milk fat. The addition of canola and WCS to a diet increased milk yield, milk fat yield, and FCM compared with a low-fat basal diet (Johnson et al., 2002). In contrast, a high-fat basal diet decreased milk yield, but increased milk fat yield and content compared with a low-fat basal diet (de Souza et al., 2018). Another study reported that a high fat basal diet did not alter milk yield, milk fat yield, ECM, or FCM when compared with a low-fat basal diet (Rico et al., 2017).

Milk protein concentration and yield responses with inclusion of WCS have been proposed to be related to microbial protein synthesis (Arieli, 1998). Milk protein content has been negatively affected by the addition of oilseeds while milk protein yield was not affected (Rabiee et al., 2012). Studies focusing on WCS have reported reductions in milk protein content compared with lower levels of WCS or no WCS (Smith et al., 1991; Harrison et al., 1995). When adding WCS and canola to increase the basal fat content, milk protein content was reduced (Johnson et al., 2002). A review by Arieli (1998) discussed that feeding WCS, in many cases, did not affect milk protein concentration and yield. Reasons for variation have been proposed to be related to the basal diet, as it has been found that the effect of WCS on milk protein is negative when the only forage utilized is corn silage (Adams et al., 1995).

Overall, increasing the dietary FA content of the diet is beneficial, as it can increase yields of milk and milk fat. Further research should be accessed to see how the range of FA content in the basal diet interacts with FA supplementation. This will expand knowledge on understanding if diets low in FA content are limiting energy to the cow and how to improve production responses by adding ingredients with a higher FA content.

Impacts of FA Supplements

This section will discuss the effects that FA supplementation has on production responses of lactating dairy cattle. Fat supplements are often included in dairy cattle diets to increase the energy density of the ration and help support milk production and milk fat yield (Rabiee et al., 2012). Previous research has reported that FA supplementation can have positive or negative effects due to differences in the FA profile of ingredients, feeding rates, and production level. Individual FA have been given renewed attention, and recent focus in our lab has been to better

understand the effects on performances of lactating dairy cattle supplemented with palmitic (C16:0), stearic (C18:0), and oleic acids (*cis*-9 C18:1).

Effects on DMI

The effects of fat supplements on DMI are variable and usually depend on the type, degree of saturation and profile of the fat being fed (Rabiee et al., 2012). Saturated fats have been reported to not affect DMI, while unprocessed animal fats and Ca-salts of palm FA were found to decrease DMI, with this decrease being greatest for Ca-salts of palm FA (Allen, 2000). In a meta-analysis, Rabiee et al. (2012) reported that oilseeds, Ca-salts, and tallow reduced DMI. A meta-analysis by Allen (2000) discussed how fat supplementation reduces DMI due to possible effects on fiber digestion although Weld and Armentano (2017) concluded that some fat supplements had positive effects on fiber digestion that was not related to a decrease in DMI. Fat supplements could trigger signals that affect gut motility and induce satiety. Compared with SFA, UFA decreased DMI and increased CCK (Relling and Reynolds, 2007; Bradford et al., 2008). As the degree of unsaturation increases, this hypophagic effect becomes more pronounced, and can result in a reduction of milk yield (Christensen et al., 1994; Relling and Reynolds, 2007).

It is unlikely that chain length plays a role in the hypophagic effect, as there is no evidence that 16-carbon SFA are more hypophagic than 18-carbon SFA (Allen, 2000). This is supported as highly enriched sources (>85%) of C16:0 and C18:0 fed between 1.5 to 2.3% of diet DM did not reduce DMI compared with a non-FA supplemented control diet (Piantoni et al., 2013; Boerman et al., 2017). The profile of the FA supplement plays a large role, as de Souza et al. (2018) reported that a blend of 45% C16:0 + 35% *cis*-9 C18:1 decreased DMI compared with blends of 80% C16:0 and 40% C16:0 + 40% C18:0. In contrast, altering the ratio of C16:0 + *cis*-

9 C18:1 did not affect DMI (de Souza et al., 2019) while a blend of 60% C16:0 + 30% *cis*-9 C18:1 increased DMI in high producing cows and a blend of 80% C16:0 + 10% *cis*-9 C18:1 increased DMI in low producing cows (Western et al, 2018).

Effects on Milk Yield and Milk Components

An increase in milk yield and a tendency for milk fat yield to increase with FA supplementation was reported in a recent meta-analysis, but the supplements used were not separated by FA profile and was instead grouped together by product type (Rabiee et al., 2012). Recently, the effects that individual FA have on production responses has been given renewed attention.

Decreases in milk yield, milk fat yield, and milk fat concentration have been observed as the degree of unsaturation of the FA supplement increases (Harvatine and Allen, 2006; Relling and Reynolds, 2007). As stated earlier, during BH of UFA alteration of BH pathways can produce intermediates that reduce milk fat synthesis in the mammary gland (Bauman et al., 2011).

Rico et al. (2017) increased C16:0 supplementation and observed that supplementing C16:0 up to 1.5% DM of the diet was the optimal feeding rate to increase yields of milk fat and FCM. Using highly enriched (> 97%) sources of C18:0 and C16:0, Rico et al (2014) reported increases in yields of FCM, ECM, and milk fat with C16:0 supplementation compared with C18:0. Additionally, a FA supplement containing 80% C16:0 tended to increase yields of FCM and ECM compared with a control diet (no FA supplementation) and a FA treatment of 30% C16:0 + 50% C18:0 (Western et al., 2020). de Souza et al. (2018) altered the ratio of individual FA to obtain FA treatments of 80% C16:0, 40% C16:0 + 40% C18:0 and 45% C16:0 + 35% *cis*-9 C18:1. They determined that a high level of C16:0 increased energy output in milk,

supplements with more *cis*-9 C18:1 partitioned more energy to body reserves and increasing the amount of C18:0 in the diet did not increase energy intake and thus decreased production. In a blend of C16:0 + *cis*-9 C18:1, increasing the amount of *cis*-9 C18:1 tended to increase milk yield and also increased body weight change (de Souza et al., 2019). Feeding of FA supplements can increase the yields of milk and milk components but the effect depends on feeding rate and FA profile of the supplement.

Effects on Production Level

Previous research has determined that cows at different milk production levels can have differing production and metabolic responses to FA supplementation (Harvatine and Allen, 2005). Across a wide range of milk production, a FA supplement highly enriched (99%) in C16:0 consistently increased yields of milk, milk fat, FCM, and ECM compared with a non-FA supplemented control (Piantoni et al., 2013). However, a FA supplement highly enriched (98%) in C18:0 only increased yields of milk, milk fat, FCM, and ECM in high producing cows compared with a non-FA control diet (Piantoni et al., 2014). Recently, de Souza et al. (2019) fed four ratios (from 80:10 to 60:30) of C16:0 and *cis*-9 C18:1 to three production groups described as low-, medium-, and high-producing. Increasing the proportion of *cis*-9 C18:1 increased FCM, ECM and milk yield in the high-producing group, while the low-producing group responded best to the treatment with more C16:0 and the medium-producing group had no response.

Additionally, Western et al. (2018) also reported that cows producing > 55 kg of milk yield increased ECM with a treatment of 60% C16:0 + 30% *cis*-9 C18:1 while cows producing below that threshold responded better to the treatment containing 80% C16:0 + 10% *cis*-9 C18:1.

Although de Souza et al. (2019) and Western et al. (2018) both concluded that higher producing cows responded best to a diet containing 30% *cis*-9 C18:1, neither included a treatment with

C18:0 to evaluate if production responses were due to *cis*-9 C18:1 or overall 18-carbon FA because of BH. Rumen-inert FA supplements are one way to deliver *cis*-9 C18:1 to the rumen and these products will have some degree of dissociation in the rumen (Chalupa et al., 1986). Even though a portion of the FA supplement will undergo BH, this dissociation in the rumen does not surpass $\geq 50\%$ at normal rumen pH levels (Sukhija and Palmquist, 1990). Overall, the response of cows to FA profile of the FA supplement depends on production level and increasing the level of *cis*-9 C18:1 could be beneficial for high-producing cows.

Conclusion

Considerable research has examined the impacts of FA supplementation to dairy cows and how different FA and blends of FA are utilized for production and metabolic needs. Although recent research indicates that the FA profile of the supplement is a major factor affecting production responses, there are still some gaps in our understanding. Higher producing cows could benefit from *cis*-9 C18:1, although at this time it is unclear if they are responding specifically to *cis*-9 C18:1 or 18-carbon FA in general. The impact of basal fat content and its impacts FA supplementation need to be further investigated to understand how the FA profile can interact with the basal diet.

Therefore, the objective for Chapter 3 was to determine if increases in production responses in high producing cows fed increasing levels of *cis*-9 C18:1 were due to *cis*-9 C18:1 or overall 18-carbon FA from biohydrogenation. In Chapter 4, our objective was to determine if the fat content of the basal diet interacts with FA supplementation, utilizing comparable ingredients to formulate the basal diets as similar as possible. Determining these factors will advance overall

knowledge about FA metabolism in lactating dairy cows that can allow for more strategized decision making for dairy farmers and nutritionists.

APPENDIX

Table 2.1. FA profile and FA % DM of common feed ingredients.

Feedstuff ¹	C16:0, % FA	C18:0, % FA	C18:1, % FA	C18:2, % FA	C18:3, % FA	FA, %DM
Alfalfa Hay	29.9	4.98	2.99	19.9	30.6	1.71
Alfalfa Silage	21.3	3.54	3.11	20.4	42.3	3.51
Corn Silage	17.3	2.51	22.7	43.9	4.87	3.01
Cottonseed	24.6	2.00	14.8	56.5	0.21	15.9
Distillers	14.0	2.40	24.6	56.1	1.70	7.76
Ground Corn	12.3	1.72	26.5	56.3	1.35	2.43
High Moisture Corn	14.7	1.86	23.1	58.4	1.37	4.90
Pasture Grass, cool	16.0	2.50	3.40	13.2	61.3	1.70
Soybeans, whole	11.4	4.10	22.3	53.5	7.0	18.8
Soyhulls	14.0	5.47	17.4	47.7	10.9	1.55
Tallow	28.7	10.3	46.2	9.50	0.20	53.7

¹Compilation of data from the Lock Laboratory and Caledonia Feed Elevator.

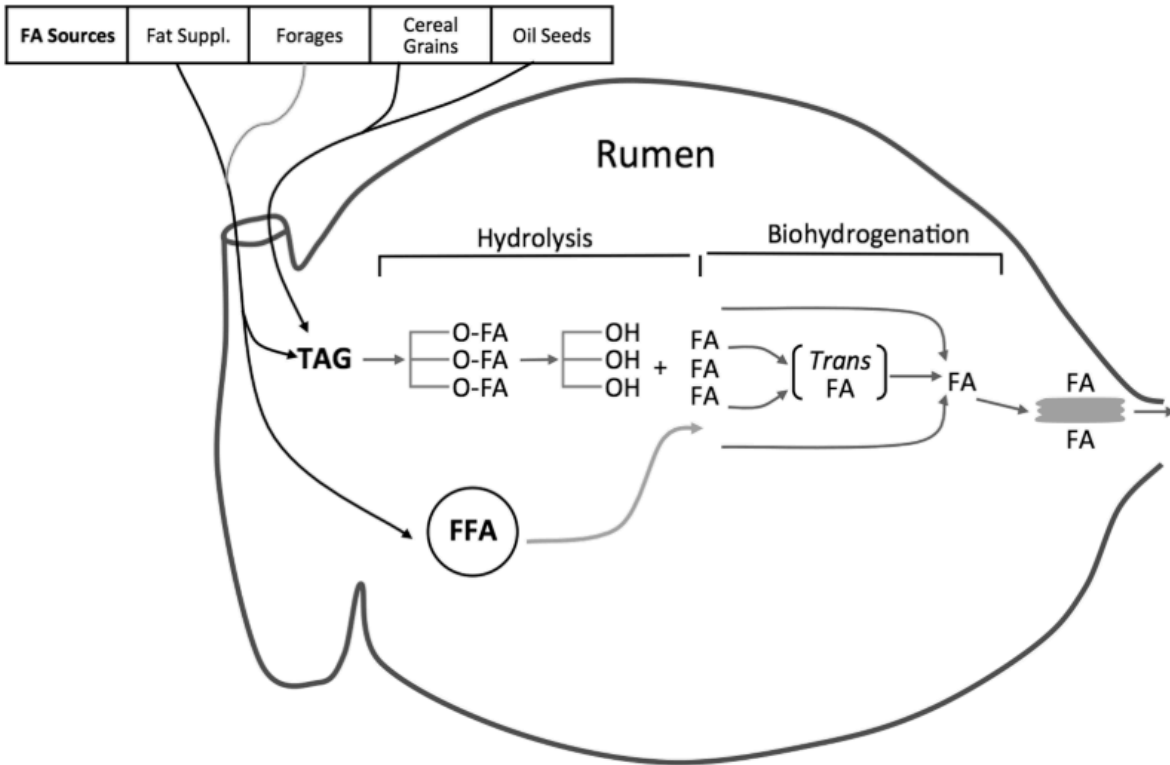


Figure 2.1. Metabolism of dietary lipids in the rumen. Triglycerides (TG), glycolipids (GL), phospholipids (PL), trans fatty acids (trans FA), mixture of fatty acids (FAs), and volatile fatty acids (VFA). Adapted from Lock et al., 2006.

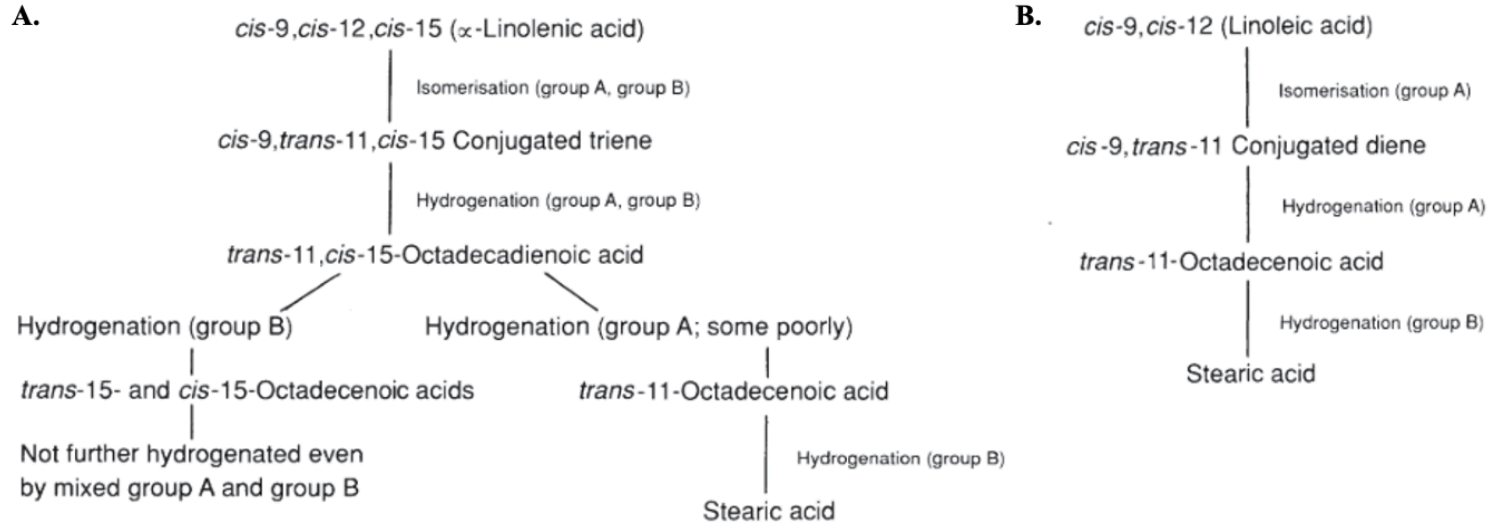


Figure 2.2 Scheme for the biohydrogenation of (A) linolenic acid and (B) linoleic acid. Group A and group B refer to the two classes of biohydrogenation bacteria. Adapted from Palmquist et al., 2005.

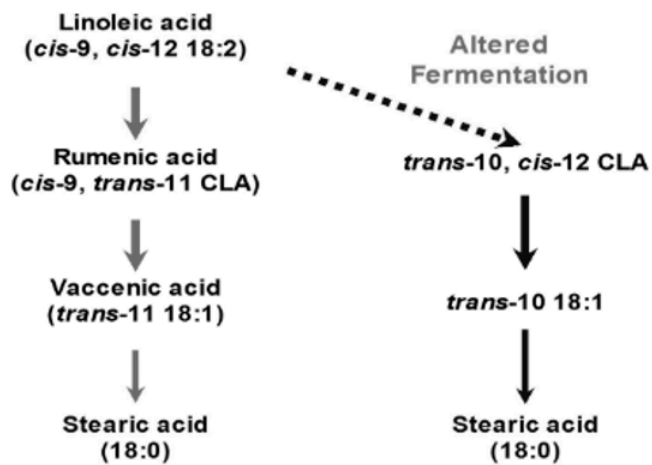


Figure 2.3 Biohydrogenation pathways of dietary lipids in the rumen. Adapted from Bauman et al., 2003.

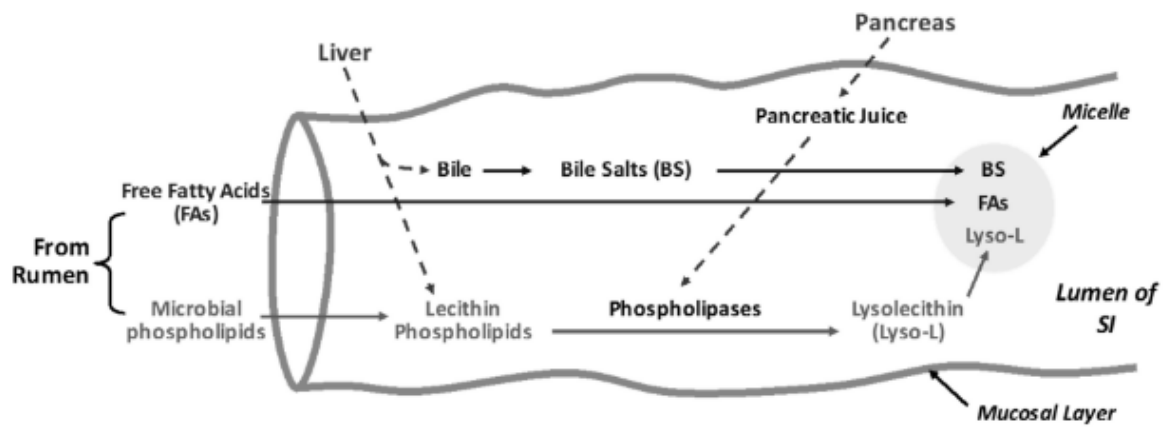


Figure 2.4 Fat digestion in the small intestine of ruminants. Adapted from Lock et al., 2006.

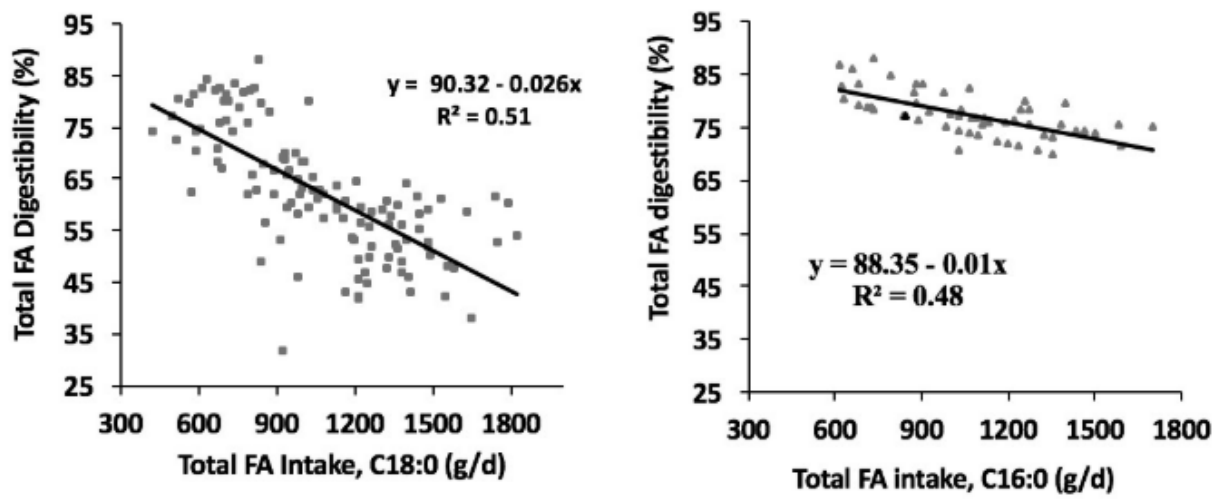


Figure 2.5 Total FA digestibility of C16:0 and C18:0. Adapted from Boerman et al., 2017 and Rico et al., 2017.

CHAPTER 3

Effect of palmitic acid-enriched supplements containing stearic or oleic acid on nutrient digestibility and milk production of low and high producing dairy cows

Abstract

We evaluated the effects of fatty acid (FA) supplement blends containing 60% palmitic acid (C16:0) and either 30% stearic acid (C18:0) or 30% oleic acid (C18:1) on nutrient digestibility and milk production of low and high producing dairy cows. Twenty-four multiparous Holstein cows (118 ± 44 DIM) were randomly assigned to treatment sequences in replicated 3 X 3 Latin squares balanced for carryover effects in 3 consecutive 21-d periods. Cows were blocked by milk yield and assigned to one of two groups ($n=12/\text{group}$): a) low group (42.5 ± 3.54 kg/day) and b) high group (55.8 ± 3.04 kg/day). Treatments consisted of: 1) control (CON; diet with no supplemental FA), 2) FA supplement blend containing 60% C16:0 and 30% C18:0 (PA+SA), and 3) FA supplement blend containing 60% C16:0 and 30% C18:1 (PA+OA). The FA blends were fed at 1.5% DM and replaced soyhulls in CON. The statistical model included the random effect of cow within production group, and the fixed effect of treatment, production group, period, and their interactions. Regardless of production level, PA+OA reduced DMI. Compared with CON, PA+SA decreased and PA+OA increased total tract FA digestibility. Treatment by production group interactions were observed for NDF digestibility, total FA intake, and the yields of FCM, ECM, and milk fat. FA treatments increased NDF digestibility in low-producing cows while PA+SA increased it in high-producing cows. PA+OA increased total FA intake in low- and high-producing cows. In low-producing cows, FA supplementation was similar to CON for milk yield, but PA+OA decreased FCM, ECM, and milk fat yield compared with PA+SA. However, in high-producing cows PA+OA increased FCM, ECM, and milk fat

yield compared with CON. In conclusion, high-producing dairy cows responded more favorably to a FA blend containing 60% C16:0 and 30% C18:1, whereas lower producing cows responded better to a FA blend containing 60% C16:0 and 30% C18:0.

Introduction

Fat supplements are commonly added to dairy cow diets to help increase the yields of milk and milk fat (Rabiee et al., 2012). The most abundant fatty acids (FA) in commercially available products fed to dairy cows are palmitic (C16:0), stearic (C18:0), and oleic (*cis*-9 C18:1) acids. These three FA are also the predominant FA found in milk fat and adipose tissue (Palmquist, 2006). Attention has been given to these FA in order to understand their effects on nutrient digestibility, milk production, and energy partitioning, and research suggests that dairy cows have different metabolic and production responses when fed combinations of these FA. For example, de Souza et al. (2018) observed that feeding a FA blend containing 80% C16:0 increased milk energy output while a FA blend of 45% C16:0 and 35% *cis*-9 C18:1 increased energy storage as body weight (BW); a combination of 40% C16:0 and 40% C18:0 decreased nutrient digestibility and did not increase energy intake.

Previous research has also observed that cows at different levels of milk production have different responses to dietary treatments. C16:0 supplementation increased milk yield, ECM, and milk fat yield independent of production compared with no FA supplementation (Piantoni et al., 2013) or compared with C18:0 supplementation (Rico et al., 2014). However, Piantoni et al. (2014) observed in one treatment period that C18:0 supplementation increased yield of milk and milk fat in high-producing cows but not in low-producing cows. Recently, de Souza et al. (2019) varied the ratio of supplemented C16:0 to *cis*-9 C18:1 and observed that increasing the level of

cis-9 C18:1 in a FA supplement blend increased FCM and ECM in high producing cows but reduced FCM and ECM in low producing cows. Western et al. (2018) observed similar results across a wide range of milk production with high-producing cows having increased FCM and ECM with a supplement blend of 60% C16:0 + 30% *cis*-9 C18:1 compared with a blend of 80% C16:0 + 10% *cis*-9 C18:1.

Rumen biohydrogenation (BH) converts unsaturated FA (UFA) to saturated FA (SFA) via ruminal microorganisms by the addition of hydrogen with the main end product being C18:0 (Palmquist et al., 2005; Jenkins and McGuire, 2006). Rumen inert fats were developed to minimize the negative effects that UFA may have on rumen microbes (Palmquist and Jenkins, 2017). Ca-salts are a class of rumen inert fats that has been reported to be effective in increasing milk production and milk fat percentage (Rabiee et al., 2012). It is well established that Ca-salts can dissociate in the rumen (Chalupa et al., 1986; Sukhija and Palmquist, 1990) with free UFA released from the salts undergoing BH to C18:0 (Hobson and Stewart, 1997).

In our recent research, higher-producing dairy cows increased production when supplemented with Ca-salts containing *cis*-9 C18:1 (Western et al., 2018; de Souza et al., 2019). Because *cis*-9 C18:1 is biohydrogenated to 18:0, we wondered whether the effect of *cis*-9 C18:1 was due to C18 FA in general or specifically to *cis*-9 C18:1. Therefore, the objective of this study was to determine the effects of C16:0 enriched supplements containing C18:0 or *cis*-9 C18:1 on nutrient digestibility and production in low- and high-producing cows. We hypothesized that compared with C18:0, *cis*-9 C18:1 would increase nutrient digestibility, yield of milk components, and body reserve gain of high producing cows.

Material and Methods

Design and Treatments

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University, East Lansing. Twenty-four mid-lactation, multiparous Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center were randomly assigned to a treatment sequence in a replicated split-plot Latin square design balanced for carryover effects in three 21-d periods. Animals were blocked by production level into two groups (n= 12/group): a) low group (40.0 ± 2.14 kg/d) and b) high group (54.3 ± 1.87 kg/d). The starting average for all animals, with mean $\pm$ standard deviation, were 118 ± 44 d DIM and 703 ± 63 kg of BW. Baseline values for both production groups are provided in Table 1. All animals received a common diet with no fat supplementation during a 7-d preliminary period to obtain baseline values

Within each production level group, a 3×3 balanced Latin square design was used to assign treatments. Treatments consisted of: 1) control (CON; diet with no supplemental FA), 2) FA supplement blend containing 60% C16:0 and 30% C18:0 (PA+SA), and 3) FA supplement blend containing 60% C16:0 and 30% *cis*-9 C18:1 (PA+OA). Both FA supplement blends were fed at 1.5% FA (DM basis) of the diet and replaced soyhulls from the control diet. The FA blends consisted of different proportions of 3 commercially available products that differed in FA profile (Table 2). These FA supplements were blended to achieve our desired ratios of C16:0, C18:0, and *cis*-9 C18:1 in the FA supplement blends (Table 2). Experimental diets were formulated to meet the nutrient requirements of the average cow (Table 2). The DM concentration of forages were determined twice weekly and diets adjusted when necessary. A base diet mix (containing forages, dry ground corn, high-moisture corn, soybean meal, and

mineral mix) was mixed in a wagon daily. Then, soyhulls, FA supplement blends, and base diet were mixed in the mixer wagon for each experimental diet. Cows were fed 115% expected intake at 1000 h daily. Feed access was blocked from 0800 to 1000 h for orts collection and offering of new feed. Cows were housed in individual tie-stalls throughout the experiment with water available *ab libitum* in each stall which were bedded with sawdust and cleaned twice daily.

Data and Sample Collection

Samples and production data were collected during the last 5 d of each treatment period (d 17-21). Samples of all diet ingredients (0.5 kg) and orts (12.5%) were collected daily and composited by cow/period for analysis. Milk yield was recorded, and two milk samples were collected at each milking. One aliquot was collected in a sealed tube with preservative and stored at 4°C for milk component analysis. The second aliquot was stored without preservative at -20°C until analyzed for FA composition. Fecal samples were taken every 15 h resulting in 8 samples/cow/period and stored in a sealed plastic cup at -20°C. Fecal samples were later dried and composited by cow/period for analysis. Blood was also taken every 15 h and was stored on ice until centrifugation at 2,000 X g for 15 min at 4°C. Plasma was transferred into microcentrifuge tubes and stored at -20°C until composited by cow/period. BW were measured 3 times per week and three trained investigators determined BCS on a 5-point scale in 0.25 increments on the last day of each period (Wildman et al., 1982).

Sample Analysis

Dietary 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 in a Wiley mill (1 mm screen; Arthur H. Thomas, Philadelphia, PA). Samples of feed ingredients, orts and feces were analyzed for neutral detergent fiber (NDF), indigestible NDF (iNDF), starch, CP, and FA according to Boerman et al.

(2017). iNDF was used as an internal marker to estimate fecal output to determine apparent total-tract digestibility of nutrients (Cochran et al., 1986). Milk samples were analyzed for fat, true protein, and lactose concentrations by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan Dairy Herd Improvement Association (Central Star DHI, Grand Ledge, MI). Yields of FCM, ECM, and milk components were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each period. Milk samples used for analysis of FA composition were composited based on milk fat yield (d 17-21 per period). Milk lipids were extracted, fatty acid methyl esters prepared, and analyzed by gas chromatography as described previously (Lock et al., 2013). Yields of individual FA (g/d) in milk fat were calculated using milk fat yield and FA concentration to determine yield on a mass basis using the molecular weight of each FA while correcting for glycerol content and other milk lipid classes (Piantoni et al., 2013).

Statistical Analysis

All data were analyzed using the GLIMMIX model procedure of SAS (version 9.4, SAS Institute, Cary, NC). Data was analyzed using the following model:

$$Y_{ijkl} = \mu + C(G)_{i(j)} + G_j + P_k + T_l + P_k \times T_l + G_j \times T_l + e_{ijkl},$$

where Y_{ijkl} = the dependent variable, μ = the overall mean, $C(G)_{i(j)}$ = random effect of cow nested in production group ($i = 1-12$), G_j = fixed effect of production group ($j = 2$), P_k = fixed effect of period ($k = 1$ to 3), T_l = fixed effect of treatment ($l = 1$ to 3), $P_k \times T_l$ = the interaction of period and treatment, $G_j \times T_l$ = the interaction of production group and treatment, and e_{ijkl} = residual error. The interaction of period and treatment and the 3-way interaction of treatment,

production level, and period were all tested but results were not significant and were removed from the model ($P > 0.20$). Main effects were declared significant at $P \leq 0.05$ and tendencies $P \leq 0.10$ and interactions were declared significant at $P \leq 0.10$ and tendencies at $P \leq 0.15$.

Results

Nutrient Intake and Total-Tract Digestibility

As expected, high-producing cows had higher intakes of DM, NDF, 16-carbon, 18-carbon, and total FA compared with low-producing cows ($P < 0.05$; Table 3.4). There was no effect of production group on the digestibility of DM, NDF, 16-carbon, 18-carbon, and total FA or on amounts of 16-carbon, 18-carbon, and total FA absorbed ($P > 0.11$).

Overall, the PA+OA treatment decreased DMI compared with CON ($P < 0.05$; Table 3.4) and decreased NDF intake compared with CON and PA+SA. PA+OA increased 16-carbon, 18-carbon, and total FA intake compared with CON and PA+SA ($P < 0.05$). Compared with the other treatments, PA+SA increased DM and NDF digestibility ($P < 0.05$). The PA+SA treatment decreased 16-carbon digestibility ($P < 0.05$) and PA+OA increased digestibility of 18-carbon and total FA (all $P < 0.05$) compared with CON and PA+SA. The PA+OA treatment increased absorbed 16-carbon, 18-carbon, and total FA (all $P < 0.05$) compared with the other treatments. PA+SA increased absorbed 16-carbon, 18-carbon, and total FA compared with CON (all $P < 0.05$).

We observed interactions between treatment and production level for 16-carbon FA intake, total FA intake, and NDF digestibility ($P < 0.10$), and a tendency for DM digestibility ($P < 0.15$; Table 3.5). The PA+OA treatment increased 16-carbon FA intake ($P < 0.05$) and total FA intake ($P < 0.10$) in both low- and high- producing cows compared with CON, with this increase

being greater in high-producing cows compared with low-producing cows. Compared with CON, the PA+SA treatment increased NDF digestibility in high-producing cows ($P < 0.10$) whereas the PA+OA treatment increased NDF digestibility in low-producing cows ($P < 0.10$). The PA+OA treatment tended to increase DM digestibility in high producing cows while the PA+SA treatment tended to increase DM digestibility in low producing cows ($P < 0.15$).

Production Responses

As expected, high-producing cows had higher yields of milk, milk components, and improved feed efficiency (ECM/DMI) compared with low-producing cows ($P < 0.05$; Table 3.6). Low-producing cows had higher milk fat and protein contents, and BCS ($P < 0.05$).

Overall, FA treatments increased FCM, milk fat yield, and feed efficiency (ECM/DMI) ($P < 0.05$; Table 3.6) and tended to increase milk yield ($P < 0.10$) compared with CON. Compared with CON, PA+SA increased ECM whereas PA+OA was not different from the other treatments. FA treatments decreased milk protein content ($P < 0.05$) compared with CON, with this decrease being greatest for PA+OA. Compared with CON, PA+OA reduced BW change ($P < 0.05$) and PA+SA was not different from the other treatments. We observed no effect of treatments for milk protein yield, milk fat and lactose contents, BW, BCS, or BCS change ($P > 0.35$).

We observed interactions between treatment and production level for yields of milk and milk components. There was no difference between treatments for milk yield in low-producing cows but PA+OA tended to increase milk yield in high-producing cows compared with CON ($P < 0.15$; Table 3.7). For low-producing cows, PA+SA increased FCM, ECM, and fat yield compared with PA+OA but was not different compared with CON (all $P < 0.05$). In contrast, PA+OA increased FCM, ECM, and fat yield in high-producing cows compared with CON (all $P < 0.05$). The CON and PA+SA treatments tended to increase protein and lactose yields in low-

producing cows compared with PA+OA (all $P < 0.15$), whereas PA+OA tended to increased yields of protein and lactose in high-producing cows compared with CON (all $P < 0.15$). Additionally, the PA+SA treatment increased feed efficiency (ECM/DMI; $P < 0.05$) in low-producing cows compared with CON and PA+OA whereas PA+OA increased feed efficiency (ECM/DMI; $P < 0.05$) in high-producing cows compared with CON and PA+SA.

Milk FA Concentrations and Yields

Milk FA are derived from two sources: de novo (<16 carbon FA) synthesis in the mammary gland and preformed (>16 carbon FA) originating from extraction from plasma. Mixed source 16-carbon FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis in the mammary gland and extraction from plasma. Compared with low-producing cows, high-producing cows had increased yields of de novo, mixed, and preformed milk FA ($P < 0.05$; Table 3.8). There were no differences in the concentrations of de novo, mixed, and performed FA between low- and high-producing cows ($P > 0.23$).

FA treatments decreased de novo FA concentration compared with CON, with this decrease being greater for PA+OA ($P < 0.05$; Table 3.8). PA+SA increased mixed source FA concentration compared with the other treatments ($P < 0.05$) and PA+OA increased mixed source FA concentration compared with CON ($P < 0.05$). PA+OA increased preformed FA concentration compared with both CON and PA+SA ($P < 0.05$) and PA+SA increased preformed FA concentration compared with CON ($P < 0.05$).

Fa treatments decreased de novo FA yield compared with CON, with this decrease being greater for PA+OA ($P < 0.05$; Table 3.8). FA treatments increased mixed source FA yield compared with CON ($P < 0.05$). PA+OA increased preformed FA yield with other treatments and PA+SA increased preformed milk FA compared with CON ($P < 0.05$).

We observed interactions between treatment and production level for the yields of all milk FA sources and for mixed milk FA concentration. Compared with CON, PA+OA reduced de novo FA yield in low-producing cows whereas both FA treatments decreased de novo FA yield in high-producing cows ($P < 0.10$; Table 3.9). The PA+SA treatment increased the yield of mixed FA in low-producing cows compared with CON ($P < 0.10$) whereas both FA treatments increased the yield of mixed FA in high-producing cows compared with CON ($P < 0.10$). PA+SA increased performed FA yield in low-producing cows compared with CON ($P < 0.10$) and PA+OA increased performed FA yield in high-producing cows compared with CON and PA+SA ($P < 0.10$). The PA+SA treatment tended to increase mixed milk FA concentration compared with CON and PA+OA in low-producing cows and tended to increase mixed milk FA concentration compared with CON and PA+OA in high-producing cows ($P < 0.15$), with the rate of increase being greatest for the high-producing cows.

Plasma Insulin

There was no effect of FA treatments for plasma insulin ($P > 0.22$; Table 3.10). Low producing cows increased plasma insulin compared with high producing cows ($P < 0.05$) and there were no detected interactions between FA treatments and production level for plasma insulin ($P > 0.44$).

Discussion

Most commercially available fat supplements fed to dairy cows contain different proportions of C16:0, C18:0, and *cis*-9 C18:1 FA and previous research has highlighted different effects of these FA on nutrient digestibility and production of dairy cows. Also, recent research results suggest that high-producing cows may benefit from increased supplementation of *cis*-9

C18:1; however these studies were not able to determine if responses were due to dietary *cis*-9 C18:1 or 18-carbon FA. In our current study, production level was used as a blocking factor to evaluate if cows with differing levels of milk yield would respond differently to the same diet. We designed the FA treatments to contain 60% C16:0 and either 30% C18:0 or 30% *cis*-9 C18:1 in order to evaluate if production responses are due to a specific effect of an individual FA or 18-carbon FA overall. We hypothesized that supplemental *cis*-9 C18:1 would increase nutrient digestibility, yield of milk components, and body reserve gain compared with a treatment containing C18:0 in high-producing cows.

C16:0 has consistently been shown to increase yields of milk and milk fat across a wide range of production levels (Piantoni et al., 2013; Rico et al., 2014). Previously observed in one treatment period, high-producing cows increased production with supplementation of C18:0 (Piantoni et al., 2014) while Boerman et al (2017) did not observe production differences with an increase in C18:0 supplementation across a wide range of milk production. In our current study, the PA+OA treatment increased yields of milk and milk components in high-producing cows. Our results are in agreement with de Souza et al. (2019) as they reported higher-producing cows (averaging 60.0 ± 1.9 kg/d) responded more favorably to increasing levels of *cis*-9 C18:1. In addition, Western et al. (2018 ADSA Abstract) also reported that different combinations of C16:0 + *cis*-9 C18:1 interacted with preliminary milk yield (PMY) where high-producing cows (greater than 55 kg/d) increased ECM and milk yield with a FA blend of 60% C16:0 + 30% *cis*-9 C18:1. Although both de Souza et al. (2019) and Western et al. (2018) found that supplementation of 60% C16:0 + 30% *cis*-9 C18:1 increased production responses of higher-yielding cows, neither of these studies had a treatment containing C18:0 to compare if a specific 18-carbon FA was a contributor to their results. Our FA treatments were designed to evaluate if

increased production previously reported were due to *cis*-9 C18:1 or 18-carbon FA due to BH. Between our current study and previous research, these results indicate that higher-producing cows increase production due to *cis*-9 C18:1 and not 18-carbon FA per se.

At the start of our study, cows in our low-production group were averaging 42.5 kg/d and we observed the PA+SA treatment increased yields of FCM, ECM, and milk fat compared with our PA+OA treatment. Piantoni et al. (2014) observed in cows producing an average of 46.1 kg/d increased responses in milk yield, milk fat yield, and FCM with C18:0 supplementation compared with no fat supplementation. Meanwhile, the lower-yielding cows, averaging 32.2 kg/d, did not respond to the C18:0 supplementation. In our current study, low-producing cows responded similarly across all treatments for milk yield but the PA+SA treatment increased yield of milk components in these cows compared with PA+OA, potentially indicating that cows in this range could have positive responses to a blend of 60% C16:0 + 30% C18:0. Supplementation of a $\geq 80\%$ C16:0 and a blend of 80% C16:0 + 10% *cis*-9 C18:1 have been shown to increase yields of ECM and FCM for lower-producing cows (Rico et al., 2014; Western et al., 2018 ADSA Abstract; de Souza et al., 2019). Without evaluating differences in production level, a FA blend of 40% C16:0 + 40% C18:0 had similar ECM and FCM compared with treatments consisting of 80% C16:0 + 10% *cis*-9 C18:1 and a blend of 45% C16:0 + 35% *cis*-9 C18:1 for cows averaging 46.5 kg/d of milk yield (de Souza et al., 2018). Similarly, Western et al. (2020) observed tendencies for a FA treatment consisting of 80% C16:0 to increase yields of FCM and ECM compared with a control diet (no FA supplementation) and a FA treatment of 30% C16:0 + 50% C18:0 to but did not evaluate production level differences. The average milk yield for cows given FA treatments from these studies (46.5 kg/d and 45.2 kg/d, de Souza et al., (2018) and Western et al., (2020) respectively) had a range in between the

average for the low- and high-producing cows in our study. Potentially a treatment higher in C16:0 would increase the yields of milk and milk components more compared with a treatment of C16:0 + C18:0, and it is necessary to evaluate different ratios of C16:0 and C18:0 to assess if these two FA have a synergistic effect in lower-producing cows.

One way to deliver *cis*-9 C18:1 is to utilize a rumen-inert fat supplement, such as a Ca-salt. These salts are not completely rumen-inert as there is some degree of dissociation of the Ca-salt in the rumen (Chalupa et al, 1986) but this dissociation does not surpass $\geq 50\%$ of the Ca-salt at normal rumen pH levels (Sukhija and Palmquist, 1990). Therefore, we acknowledge that a portion of *cis*-9 C18:1 in the Ca-salt used in our study will undergo rumen BH to C18:0 (Carriquiry et al., 2008). In order to approximate how much *cis*-9 C18:1 is surpassing the rumen, using calculations for our desired feeding rate we can estimate ~100 g of *cis*-9 C18:1 in the Ca-salt entering the rumen and utilizing a 62% rumen loss for *cis*-9 C18:1 in a protected fat supplement (Jenkins and Bridges, 2007) we can estimate that ~40 g/d of *cis*-9 C18:1 is reaching the duodenum of the cows in our study, but if we use a range of 40 to 80% rumen loss, we can estimate a range of ~20 to 60 g/d of *cis*-9 C18:1 will reach the duodenum.

We observed that the PA+OA treatment increased milk fat yield while decreasing de novo FA yield and increasing mixed and preformed FA yield in high-producing cows which are similar to results observed by de Souza et al. (2018). In contrast, de Souza et al. (2019) observed that high-producing cows increased milk fat yield due to increases in both de novo FA and preformed FA with a FA blend of 60% C16:0 + 30% *cis*-9 C18:1 and Piantoni et al. (2015) found similar results with C18:0 supplementation. Our low-producing cows also had a reduction in de novo milk FA yield and increases in mixed and preformed milk FA yield with the PA+SA treatment. Similar to our low-producing cows, de Souza et al. (2019) observed in low-producing

cows a reduction in de novo and mixed milk FA without changes to preformed milk FA yield when dietary *cis*-9 C18:1 was increased. These results are likely attributed to a substitution effect, which often occurs when FA are supplemented to the diet and there is a decrease in the yield of de novo milk FA which are compensated for by an increase in preformed milk FA (Glasser et al., 2008; He et al., 2012). Milk fat depression is a concern when feeding UFA due to BH intermediates being produced that reduce milk fat synthesis in the mammary gland (Bauman et al., 2011), and although the PA+OA treatment increased production of several *trans* FA in milk fat, milk fat yield and milk fat concentrations were not reduced indicating that this treatment did not impact BH-induced milk fat depression.

The effect of fat supplements on DMI is variable and the extent that dietary fat reduces DMI could depend on the degree of saturation (Relling and Reynolds, 2007) and the type of fat supplement being fed (Rabiee et al., 2012). In general, diets higher in UFA result in a greater decrease in DMI compared with a non-FA control and SFA treatments (Harvatine and Allen, 2005; Relling and Reynolds, 2007; de Souza et al., 2018) which could be attributed to increased secretion of gut peptides associated with satiety, e.g. CCK and glucagon-like peptide 1 (Relling and Reynolds, 2007; Bradford et al., 2008). Our study observed similar findings as the PA+OA treatment depressed DMI, potentially due to the increased level of UFA from supplementation of *cis*-9 C18:1. The addition of cottonseed in the basal diet could contribute to this decrease as well, as de Souza et al. (2018) observed that a FA blend of 45% C16:0 + 35% *cis*-9 C18:1 in a cottonseed basal diet decreased DMI whereas it did not decrease DMI in a diet in which cottonseed was replaced by soyhulls. In contrast, varying the level of *cis*-9 C18:1 in the diet did not affect DMI (He et al., 2012) and other studies with FA blends of 60% C16:0 + 30% *cis*-9 C18:1 either had no effect on DMI (de Souza et al., 2019) or increased DMI in high-producing

cows (Western et al, 2018 ADSA Abstract). As expected, higher-producing cows had an increased DMI but there was no interaction of production level and FA treatment on DMI, similar to results observed by de Souza et al., (2019). Additional research is needed to determine whether a depression in feed intake is due to a higher intake of specific FA or UFA, and if there are potential interactions with other dietary components.

Total flow of FA reaching the duodenum affects FA digestibility (Boerman et al., 2015) but there are differences among individual FA. It had been reported that *cis*-9 C18:1 has greater digestibility than C16:0 and C18:0, indicating that UFA have higher digestibility than SFA (Boerman et al., 2015). Overall, the PA+OA treatment increased 16-carbon, 18-carbon, and total FA digestibility and these results are similar to de Souza et al. (2018 and 2019). In contrast, the PA+SA treatment markedly decreased 16- and 18-carbon FA digestibility. These results are supported by other experiments that have reported C18:0 supplementation decreasing 16-carbon, 18-carbon, and total FA digestibility (Boerman et al., 2017; de Souza et al., 2018; Western et al., 2020). This decrease in FA digestion is interesting because flow of C18:0 from the rumen can be several times greater than its intake, due to BH of UFA in forages and grains fed to dairy cows. A possibility for this reduction could be linked with micelle formation, due to the micelle reaching its saturation point for C18:0 and excess C18:0 will be retained in the hydrophobic phase (Freeman, 1969). The addition of *cis*-9 C18:1 extended the saturation point of C18:0, thus allowing increased amounts of C18:0 to be solubilized in the micellar phase (Freeman, 1969). These results indicate that the profile of FA reaching the intestine can affect FA digestibility and that *cis*-9 C18:1 could be a key factor in improving FA digestibility. Further research is needed to understand reasons for FA supplements containing C18:0 decreasing FA digestibility and how this might be overcome.

Across studies, results are variable for NDF and DM digestibility when FA are supplemented. SFA have been reported to decrease these variables compared with UFA (Harvatine and Allen, 2006) while a recent meta-analysis reported a tendency for an increase in total-tract NDF digestibility with feeding SFA supplements (Weld and Armentano, 2017). Supplementation of C16:0 has been observed to consistently increase NDF digestibility (Rico et al., 2017) while C18:0 did not alter NDF digestibility (Piantoni et al., 2015; Boerman et al., 2017). In our study, overall FA supplementation increased NDF and DM digestibility in low-producing cows while the PA+SA treatment increased these in high-producing cows. In contrast to our results, de Souza et al. (2018) and Western et al. (2020) both observed that a blend of C16:0 + C18:0 (40:40% and 30:50% respectively) reduced DM and NDF digestibility compared to other treatments containing 80% C16:0. Additionally, varying levels of a blend of C16:0 + *cis*-9 C18:1 did not effect NDF or DM digestibility and did not have an interaction with production level for these variables (de Souza et al., 2019). Potential causes for this increase in NDF and DM digestibility for FA supplementation found in our study could potentially be associated with our FA blends containing 60% C16:0 and an increase in retention time driven by CCK secretion.

Regardless of production level, the CON treatment had the greatest increase in BW change. This is contrary to previous studies since de Souza et al. (2018 and 2019) both observed that supplementation of C16:0 + *cis*-9 C18:1 increased BW change. There was no difference in BCS or BCS change between treatment diets, but low-producing cows had a higher BCS compared with high-producing cows.

Even though PA+OA reduced DMI, high-producing cows increased feed efficiency (ECM/DMI) due to the increase in ECM and a decrease in DMI. Additionally, we observed that low-producing cows increased plasma insulin compared with high-producing cows. Elevated insulin

concentrations could reduce lipolysis or increase lipogenesis (Vernon, 2005), therefore there is potential that the low-producing cows partitioned energy toward body reserves in response to increased insulin levels.

Conclusion

Higher-producing dairy cows responded more favorably to a FA blend containing 60% C16:0 and 30% *cis*-9 C18:1 with increases in the yields of milk, ECM, milk fat, milk protein, and mixed and preformed FA compared with a control (no FA supplementation) diet. In contrast, lower-producing dairy cows responded more favorably to a FA blend containing 60% C16:0 and 30% C18:0 with increases in the yields of ECM, FCM, milk fat and protein, and mixed FA yield. Results indicate that higher-yielding cows could have improved production responses being fed *cis*-9 C18:1 compared to C18:0.

APPENDIX

Table 3.1. Baseline data for low and high producing cows in the study¹

Variable	Production Level ²	
	Low	High
DIM	147 ± 42	101 ± 34
Milk yield, kg/d	42.5 ± 3.54	55.8 ± 3.04
Fat, kg/d	1.52 ± 0.30	1.74 ± 0.17
Protein, kg/d	1.36 ± 0.15	1.64 ± 0.11
Lactose, kg/d	2.09 ± 0.26	2.82 ± 0.21
Milk composition, %		
Fat, %	3.56 ± 0.57	3.13 ± 0.37
Protein, %	3.21 ± 0.34	2.94 ± 0.22
Lactose, %	4.90 ± 0.33	5.05 ± 0.11
BW, kg	694 ± 81	713 ± 39
BCS	3.39 ± 0.39	3.16 ± 0.14

¹Obtained during the preliminary period when cows were fed a common diet (mean ± SD)

²Low production level (n=12), high production level (n=12)

Table 3.2 Proportion of each commercial fatty acid (FA) supplement for FA blends and FA profile of each blend¹

Item	FA Treatments	
	PA+SA	PA+OA
FA supplement in treatment blends, %		
Bergafat F-100 ²	49.5	27.8
EnergyBooster 100 ³	50.0	1.00
Megalac ⁴	0.50	71.2
FA profile of each FA blend, g/100 g of FA		
C14:0	2.08	0.87
C16:0	59.3	59.3
C18:0	27.3	3.39
<i>cis</i> -9 C18:1	6.88	28.6
<i>cis</i> -9, <i>cis</i> -12 C18:2	1.29	6.41
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	0.02	0.17

¹Average (n = 3) based on samples taken during the last 5 d of the experimental period.

²Bergafat F-100 (Berg + Schmidt America LLC, Libertyville, IL). FA composition, g/100 g of total FA. C14:0 = 0.41, C16:0 = 90.8, C18:0 = 0.27, *cis*-9 C18:1 = 6.30, *cis*-9, *cis*-12 C18:2 = 1.60, *cis*-9,*cis*-12,*cis*-15 C18:3 = 0.01.

³Energy Booster 100 (Milk Specialties Global, Eden Prairie, MN). FA composition, g/100 g of total FA. C14:0 = 3.76, C16:0 = 28.4, C18:0 = 54.3, *cis*-9 C18:1 = 7.07, *cis*-9, *cis*-12 C18:2 = 0.90, *cis*-9,*cis*-12,*cis*-15 C18:3 = 0.03.

⁴Megalac (Church and Dwight Co. Inc., Princeton, NJ). FA composition, g/100 g of total FA. C14:0 = 1.02, C16:0 = 47.6, C18:0 = 3.93, *cis*-9 C18:1 = 37.3, *cis*-9, *cis*-12 C18:2 = 8.28, *cis*-9,*cis*-12,*cis*-15 C18:3 = 0.23.

Table 3.3 Ingredient and Nutrient Composition of treatment diets for trial.

	Treatment ¹		
	CON	PA+SA	PA+OA
Ingredient, % DM			
Corn Silage	38.2	38.2	38.2
Haylage	21.1	21.1	21.1
Wheat Straw	0.81	0.81	0.81
Whole Cottonseed	4.77	4.77	4.77
Blood Meal ²	0.97	0.97	0.97
Ground Corn	16.9	16.9	16.9
High Moisture Corn	12.30	12.30	12.30
Soybean Meal	11.3	11.3	11.3
Soyhulls	7.09	5.61	5.46
Vitamin and Mineral Mix ³	3.58	3.58	3.58
PA+OA FA Blend ⁴	-	-	1.55
PA+SA FA Blend ⁵	-	1.45	-
Nutrient Composition, ⁶ % DM			
NDF	29.8	28.9	28.8
Forage NDF	21.5	21.5	21.5
CP	15.7	15.5	15.5
Starch	31.9	32.0	32.0
FA	2.53	3.89	4.02
C16:0	0.45	1.29	1.36
C18:0	0.07	0.45	0.12
<i>cis</i> -9 C18:1	0.44	0.55	0.89
<i>cis</i> -9, <i>cis</i> -12 C18:2	1.31	1.35	1.43
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	0.19	0.18	0.18

¹CON = No FA supplement; PA+SA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:0; PA+OA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:1 *cis*-9.

²Spectrum Agribblue (Perdue Agribusiness, Salisbury, SD)

³Vitamin 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.

⁴Blend of Bergafat 100, Energy Booster, and Megalac to reach a 60% C16:0 and 30% *cis*-9 C18:1 blend.

⁵Blend of Bergafat 100, Energy Booster, and Megalac to reach a 60% C16:0 and 30% C18:0 blend

⁶Expressed as percent of as fed

Table 3.4 Nutrient intake and nutrient digestibility of cows fed treatment diets (n=24)¹

Variable	Treatment ²			SEM ³	Production Level		SEM ³	P-value ⁴		
	CON	PA+SA	PA+OA		Low	High		Trt	Prod.	Trt × Prod.
Intake, kg/d										
DMI	33.2 ^a	32.8 ^{ab}	32.4 ^b	0.42	31.4 ^y	34.3 ^x	0.54	0.05	<0.01	0.77
NDF	9.90 ^a	9.34 ^b	9.20 ^c	0.07	9.06 ^y	9.90 ^x	0.15	<0.01	<0.01	0.84
Intake, g/d										
16-carbon	117 ^c	347 ^b	371 ^a	4.71	265 ^y	291 ^x	5.51	<0.01	<0.01	0.01
18-carbon	549 ^c	692 ^b	722 ^a	8.78	626 ^y	684 ^x	11.30	<0.01	<0.01	0.22
Total FA	784 ^c	1153 ^b	1183 ^a	34.31	994 ^y	1087 ^x	18.30	<0.01	<0.01	0.09
Digestibility, %										
DM	66.1 ^c	68.8 ^a	68.2 ^b	0.26	67.7	67.7	0.26	<0.01	0.92	0.13
NDF	41.6 ^c	44.8 ^a	43.4 ^b	0.50	43.2	43.3	0.43	<0.01	0.91	0.10
16-carbon	66.5 ^a	58.4 ^b	69.0 ^a	1.20	66.3	63.6	1.32	<0.01	0.11	0.52
18-carbon	70.0 ^b	66.2 ^c	76.0 ^a	1.44	72.6	68.4	1.71	<0.01	0.14	0.79
Total FA	72.3 ^b	65.7 ^c	74.4 ^a	1.22	72.5	68.8	1.44	<0.01	0.14	0.88
Absorbed, g/d										
16-carbon	77.3 ^c	203 ^b	255 ^a	3.93	174	183	4.27	<0.01	0.14	0.18
18-carbon	383 ^c	457 ^b	548 ^a	10.1	454	471	11.6	<0.01	0.33	0.59
Total FA	565 ^c	757 ^b	879 ^a	14.4	717	750	16.6	<0.01	0.18	0.50

^{a-c} For treatment effect, means in a row with different superscripts differ ($P < 0.05$). Separation conducted only if treatment effect was $P < 0.10$.

^{x, y} For production level effect, means in a row with different superscripts differ ($P < 0.05$). Separation conducted only if treatment effect was $P < 0.10$.

¹ Experimental diets fed to 24 cows in replicated 3×3 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).

² CON = control; PA+SA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:0; PA+OA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:1 cis-9.

³ Greatest SEM.

⁴ P -values refer to the ANOVA results for the fixed effects of treatment and production level.

Table 3.5. Nutrient intake and nutrient digestibility of cows at different production levels fed treatment diets (n=24)¹

Variable	Production Level	Treatment ²			SEM ³	P-value ⁴		
		CON	PA+SA	PA+OA		Trt	Production	Trt × Production
Intake, g/d								
16-carbon	Low	112 ^c	330 ^b	352 ^a	6.67	<0.01	<0.01	0.01
	High	121 ^c	364 ^b	389 ^a				
Total FA	Low	753 ^c	1099 ^b	1129 ^a	20.6	<0.01	<0.01	0.09
	High	815 ^c	1208 ^b	1237 ^a				
Digestibility, %								
DM	Low	66.1 ^b	68.4 ^a	68.6 ^a	0.37	<0.01	0.92	0.13
	High	66.1 ^c	69.2 ^a	67.9 ^b				
NDF	Low	41.8 ^b	43.9 ^a	44.0 ^a	0.7	<0.01	0.91	0.10
	High	41.5 ^b	45.7 ^a	42.8 ^b				

^{a-c} For treatment effect, means in a row with different superscripts differ ($P < 0.05$). Separation conducted only if treatment effect was $P < 0.10$.

¹ Experimental diets fed to 24 cows in replicated 3×3 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).

² CON = control; PA+SA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:0; PA+OA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:1 cis-9.

³ Greatest SEM.

⁴ P-values refer to the ANOVA results for the fixed effects of treatment and production level.

Table 3.6. Milk yield, milk components, BW and BCS of cows fed treatment diets (n=24)¹

Variable	Treatment ²			SEM ³	Production Level		SEM ³	P-value ⁴		
	CON	PA+SA	PA+OA		Low	High		Trt	Production	Trt × Production
Yields, kg/d										
Milk	44.7 ^b	45.8 ^a	46.0 ^a	0.97	37.8 ^y	53.2 ^x	1.33	0.06	<0.01	0.12
FCM ⁵	46.4 ^b	47.9 ^a	47.8 ^a	1.00	41.6 ^y	53.1 ^x	1.33	0.02	<0.01	0.01
ECM ⁶	46.9 ^b	48.2 ^a	47.8 ^{ab}	0.94	42.0 ^y	53.3 ^x	1.24	0.09	<0.01	0.02
Fat	1.67 ^b	1.73 ^a	1.72 ^a	0.04	1.55 ^y	1.86 ^x	0.06	0.02	<0.01	<0.01
Protein	1.50	1.51	1.48	0.03	1.33 ^y	1.66 ^x	0.04	0.43	<0.01	0.11
Lactose	2.15	2.20	2.21	0.06	1.77 ^y	2.61 ^x	0.08	0.15	<0.01	0.11
Milk Composition, %										
Fat	3.78	3.86	3.80	0.09	4.10 ^x	3.53 ^y	0.13	0.37	<0.01	0.24
Protein	3.41 ^a	3.35 ^b	3.27 ^c	0.05	3.53 ^x	3.15 ^y	0.06	<0.01	<0.01	0.46
Lactose	4.79	4.78	4.78	0.07	4.66 ^y	4.91 ^x	0.1	0.52	0.10	0.33
ECM/DMI	1.41 ^b	1.46 ^a	1.47 ^a	0.03	1.34 ^y	1.56 ^x	0.04	<0.01	<0.01	0.02
BW, kg	727	727	726	14.2	737	716	20.1	0.82	0.48	0.46
BW change, kg/d	0.77 ^a	0.48 ^{ab}	0.30 ^b	0.13	0.59	0.45	0.11	0.03	0.35	0.78
BCS	3.33	3.35	3.32	0.07	3.48 ^x	3.18 ^y	0.09	0.67	0.03	0.81
BCS change	0.06	0.07	0.04	0.03	0.08	0.03	0.02	0.82	0.18	0.76

^{a-c} For treatment effect, means in a row with different superscripts differ ($P < 0.05$). Separation conducted only if treatment effect was $P < 0.10$.

^{x, y} For production level effect, means in a row with different superscripts differ ($P < 0.05$). Separation conducted only if treatment effect was $P < 0.10$.

¹ Experimental diets fed to 24 cows in replicated 3×3 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).

² CON = control; PA+SA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:0; PA+OA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:1 cis-9.

Table 3.6. (cont'd)

³ Greatest SEM.

⁴ *P*-values refer to the ANOVA results for the fixed effects of treatment and production level.

⁵ Fat-corrected milk; 3.5 % FCM = $[(0.4324 \times \text{kg milk}) + (16.216 \times \text{kg milk fat})]$.

⁶ Energy-corrected milk; ECM = $[(0.327 \times \text{kg milk}) + (12.95 \times \text{kg milk fat}) + (7.20 \times \text{kg milk protein})]$.

Table 3.7. Milk yield and milk composition of cows at different production levels fed treatment diets (n = 24)¹

Variable	Production Level	Treatment ²			SEM ³	P-value ⁴		
		CON	PA+SA	PA+OA		Trt	Production	Trt × Production
Milk yield, kg/d	Low	37.4 ^a	38.5 ^a	37.6 ^a	1.41	0.06	<0.01	0.12
	High	52.0 ^b	53.1 ^{ab}	54.3 ^a				
FCM ⁵	Low	41.2 ^{ab}	42.6 ^a	40.9 ^b	1.41	0.02	<0.01	0.01
	High	51.6 ^c	53.1 ^b	54.6 ^a				
ECM ⁶	Low	41.7 ^{ab}	43.0 ^a	41.2 ^b	1.33	0.09	<0.01	0.02
	High	52.1 ^b	53.3 ^{ab}	54.5 ^a				
Fat, kg/d	Low	1.54 ^{ab}	1.60 ^a	1.52 ^b	0.06	0.02	<0.01	<0.01
	High	1.80 ^b	1.86 ^{ab}	1.92 ^a				
Protein, kg/d	Low	1.34 ^{ab}	1.37 ^a	1.29 ^b	0.04	0.43	<0.01	0.11
	High	1.66 ^a	1.66 ^a	1.68 ^a				
Lactose, kg/d	Low	1.76 ^a	1.79 ^a	1.75 ^a	0.09	0.15	<0.01	0.11
	High	2.56 ^b	2.61 ^{ab}	2.67 ^a				
ECM/DMI	Low	1.30 ^b	1.38 ^a	1.33 ^b	0.04	<0.01	<0.01	0.02
	High	1.52 ^b	1.54 ^b	1.61 ^a				

^{a-c} For treatment effect, means in a row with different superscripts differ (P < 0.05). Separation conducted only if treatment effect was P < 0.10.

¹ Experimental diets fed to 24 cows in replicated 3 × 3 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).

² CON = control; PA+SA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:0; PA+OA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:1 cis-9.

Table 3.7. (cont'd)

³ Greatest SEM.

⁴ *P*-values refer to the ANOVA results for the fixed effects of treatment and production level.

⁵ Fat-corrected milk; 3.5 % FCM = $[(0.4324 \times \text{kg milk}) + (16.216 \times \text{kg milk fat})]$.

⁶ Energy-corrected milk; ECM = $[(0.327 \times \text{kg milk}) + (12.95 \times \text{kg milk fat}) + (7.20 \times \text{kg milk protein})]$.

Table 3.8. Milk fatty acid concentration and yield for cows fed treatment diets (n=24)¹

Summation by source	Treatment ²			SEM ³	Production Level		SEM ³	P-value ⁴		
	CON	PA+SA	PA+OA		Low	High		Trt	Production	Trt × Production
g/100 g FA										
De Novo	27.9 ^a	25.6 ^b	24.5 ^c	0.24	26.2	25.9	0.3	<0.01	0.69	0.42
Mixed	39.3 ^c	41.5 ^a	40.9 ^b	0.47	40.1	41.2	0.63	<0.01	0.23	0.11
Preformed	32.8 ^b	32.9 ^b	34.6 ^a	0.43	33.9	33.0	0.59	<0.01	0.27	0.32
g/d										
De Novo	436 ^a	416 ^b	396 ^c	11.8	381 ^y	451 ^x	15.57	<0.01	<0.01	0.06
Mixed	618 ^b	676 ^a	662 ^a	20.8	586 ^y	718 ^x	28.5	<0.01	<0.01	0.03
Preformed	510 ^c	532 ^b	555 ^a	16.5	490 ^y	574 ^x	15.1	<0.01	<0.01	<0.01

^{a-c} For treatment effect, means in a row with different superscripts differ ($P < 0.05$). Separation conducted only if treatment effect was $P < 0.10$.

^{x, y} For production level effect, means in a row with different superscripts differ ($P < 0.05$). Separation conducted only if treatment effect was $P < 0.10$.

¹ Experimental diets fed to 24 cows in replicated 3×3 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).

² CON = control; PA+SA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:0; PA+OA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:1 cis-9.

³ Greatest SEM.

⁴ P-values refer to the ANOVA results for the fixed effects of treatment and period.

Table 3.9. Milk fatty acid yields for cows at different production levels fed treatment diets (n = 24)¹

Variable	Production Level	Treatment ²			SEM ³	P-value ⁴		
		CON	PA+SA	PA+OA		Trt	Production	Trt × Production
Summation by source, g/100 g								
Mixed	Low	38.4 ^c	41.0 ^a	40.6 ^b	0.7	<0.01	<0.01	0.11
	High	40.1 ^c	42.0 ^a	41.2 ^b				
Summation by source, g/d								
De Novo	Low	407 ^a	387 ^b	351 ^c	16.6	<0.01	<0.01	0.06
	High	466 ^a	446 ^b	441 ^b				
Mixed	Low	559 ^c	617 ^a	582 ^b	29.4	<0.01	<0.01	0.03
	High	678 ^b	735 ^a	742 ^a				
Preformed	Low	478 ^b	498 ^a	494 ^{ab}	16.5	<0.01	<0.01	<0.01
	High	542 ^c	565 ^b	615 ^a				

^{a-c} For treatment effect, means in a row with different superscripts differ ($P < 0.05$). Separation conducted only if treatment effect was $P < 0.10$.

¹ Experimental diets fed to 24 cows in replicated 3×3 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).

² CON = control; PA+SA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:0; PA+OA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:1 cis-9.

³ Greatest SEM.

⁴ P-values refer to the ANOVA results for the fixed effects of treatment and period.

Table 3.10. Insulin concentrations for cows fed treatment diets (n=24)¹

Variable	Treatment			SEM ³	Production Level		SEM ³	P-value ⁴		
	CON	PA+SA	PA+OA		Low	High		Trt	Production	Trt × Production
Insulin, ug/L	0.57	0.52	0.54	0.03	0.62 ^x	0.47 ^y	0.55	0.22	0.02	0.44

^{x, y} For production level effect, means in a row with different superscripts differ ($P < 0.05$). Separation conducted only if treatment effect was $P < 0.10$.

¹ Experimental diets fed to 24 cows in replicated 3×3 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).

² CON = control; PA+SA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:0; PA+OA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:1 cis-9.

³ Greatest SEM.

⁴ P-values refer to the ANOVA results for the fixed effects of treatment and period.

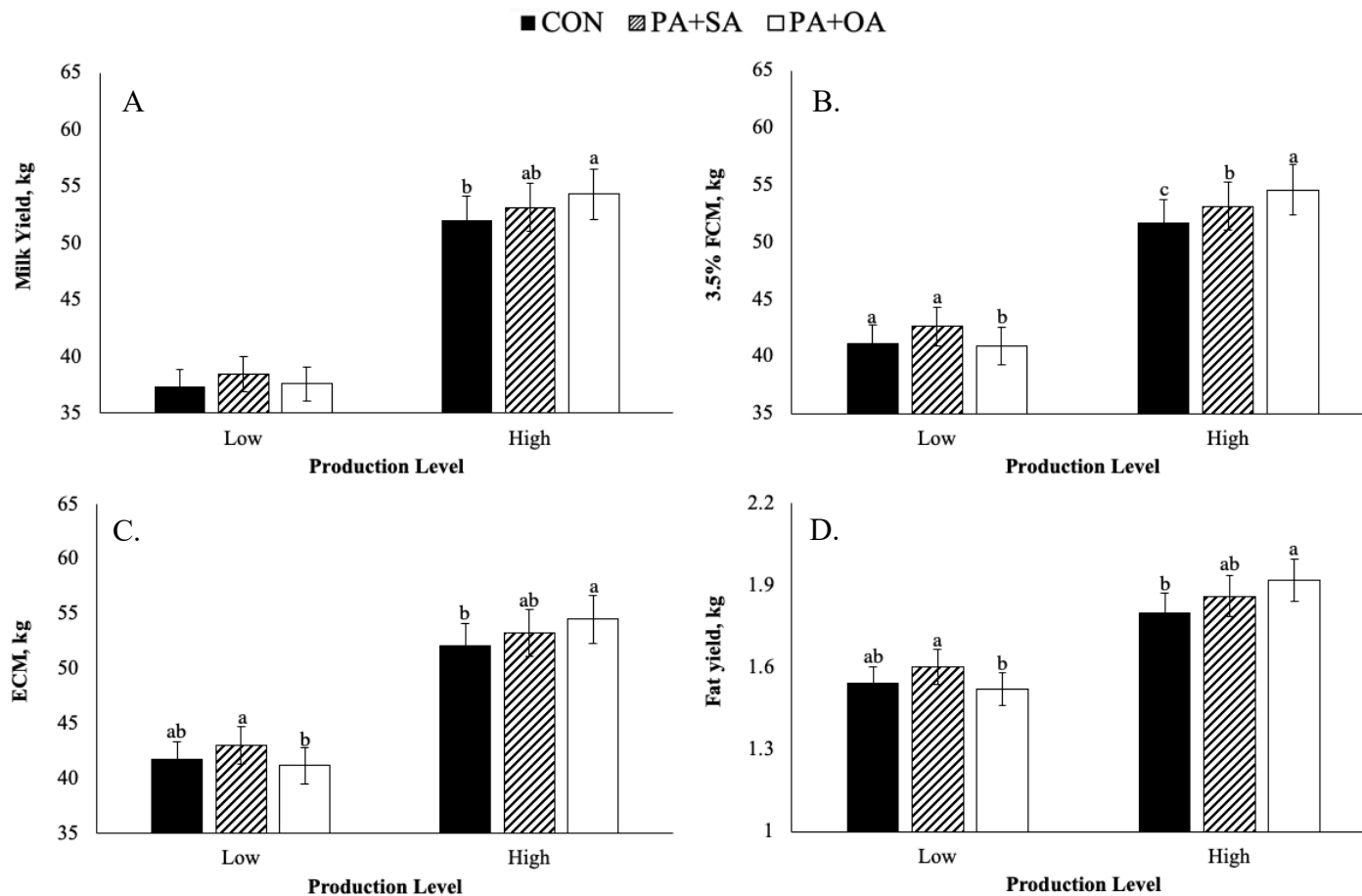


Figure 3.1. Effects of treatments on (A) milk yield, (B) 3.5% FCM, (C) ECM, and (D) milk fat yield between low-producing (42.5 ± 3.54 kg/d) and high-producing (55.8 ± 3.04 kg/d) dairy cows. Treatments were as follows: CON = no fatty acid (FA) supplementation, PA+SA = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% C18:0, and PA+OA = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1. Significant interactions between treatments and production level were detected for FCM (P = 0.01), ECM (P = 0.02), and milk fat yield (P < 0.01) and a tendency for an interaction between treatments and production level was detected for milk yield (P = 0.12). Error bars represent SEM. Means in a production group that do not share a letter (a,b,c) differ (P < 0.15).

CHAPTER 4

Milk production responses of dairy cows to supplementation of different ratios of palmitic and oleic acid in low- and high-fat basal diets

Abstract

We evaluated the effects of fatty acid (FA) supplements with different ratios of palmitic (C16:0) and oleic (C18:1) acid in low and high-fat basal diets on production responses of lactating dairy cows. Thirty-six multiparous Holstein cows (50.2 ± 5.8 kg of milk/d; 160 ± 36 d DIM) were used in a split-plot Latin square design balanced for carryover effects. Cows were blocked by milk yield and allocated to a main plot receiving either a low-fat (LF; average 2.40% FA content) basal diet (n=18) containing cottonseed meal and cottonseed hulls or a high-fat (HF; average 3.28% FA content) basal diet (n=18) containing whole cottonseed. The LF and HF diets were balanced for similar CP (17.5%), NDF (30%), and starch (28.5%). Within each plot a 3x3 Latin square arrangement of treatments was used in 3 consecutive 21 d periods. Treatments were: 1) control (CON; no FA supplementation), 2) FA supplement containing 80% C16:0 + 10% C18:1 (PA), and 3) FA supplement containing 60% C16:0 + 30% C18:1 (PA+OA). The FA supplements were fed at 1.5% DM and replaced soyhulls in CON. Treatment by basal diet interactions were observed for lactose and milk yield where FA treatments increased lactose yield and tended to increase milk yield in LF but not in HF. Basal diet had no effect on DMI or milk yield. Compared with LF, HF increased milk fat yield and FCM and tended to increase milk fat content and ECM yield. HF increased the yields of mixed milk FA and preformed milk FA compared with LF. FA treatments increased the yields of milk fat, FCM, and ECM, and milk fat content, compared with CON but there was no difference between FA treatments. Compared with CON, FA treatments decreased de novo milk FA yield. Compared with the other treatments,

PA increased mixed milk FA yield and PA+OA increased preformed milk FA yield. In conclusion, addition of FA supplements increased milk fat yield, FCM, and ECM regardless of basal diet fat level. A high-fat basal diet increased production of milk components while the addition of FA supplements to a low-fat basal diet increased the yields of milk and lactose.

Introduction

As cows increase milk production their energy demands become greater. One way to increase the energy density of the diet is the addition of FA supplements. Feeding FA supplements has become a common practice in dairy cattle nutrition to help support milk production and milk fat yield (Rabiee et al., 2012). C16:0 and *cis*-9 C18:1 acid are common FA found in commercially available fat supplements. Recent studies have shown positive effects of these two FA, when supplemented to mid-lactation dairy cows. C16:0-enriched supplements have been observed to increase milk fat yield and FCM (Lock et al., 2013; Rico et al., 2014). Evaluating the long-term effects of C16:0 supplementation, de Souza et al. (2017) observed that compared with a non-FA control diet, C16:0 supplementation increased DMI and yields of milk, milk fat, and ECM. When studying blends of different FA, de Souza et al. (2018) reported that, compared with a non-FA supplemented control diet, a FA blend containing ~80% C16:0 increased energy partitioning towards milk while a FA blend containing 45% C16:0 and 35% *cis*-9 C18:1 increased energy partitioning towards body tissues. Additionally, when comparing blends of C16:0 + *cis*-9 C18:1, de Souza et al. (2019) found that increasing the proportion of *cis*-9 C18:1 in a FA supplement increased milk yield of higher-producing cows while increasing the proportion of C16:0 increased milk production of lower-producing cows.

Additionally, the basal diet can impact the amount of FA supplied to the dairy cow. Banks et al. (1976) concluded that low-fat diets could limit the yields of milk and milk fat and increasing dietary FA supply increased these yields when different oils and oilseeds were fed (Virtanen, 1966; Banks et al., 1976). Low-fat diets have been observed to decrease in milk fat yield which could be due to the low supply of preformed FA available for milk fat synthesis (Palmquist, 2006). These low-fat diets potentially restricted energy supply to the cow and increasing the dietary FA content resulted in improved energy balance. WSC is a common by-product ingredient added to dairy cow diets. WCS is a unique ingredient because of its high content of FA, high quality fiber and moderate level of crude protein (Coppock et al., 1987; Moreira et al., 2004). Inclusion of WCS has increased milk fat percentage and the yields of milk fat and FCM compared with other ingredients such as soybean hulls, alfalfa hay, and concentrate mixtures with cottonseed meal (Smith et al., 1981; Clark and Armentano, 1993; de Souza et al., 2018). Increases in milk fat yield when feeding WCS could be attributed to the greater amount of long-chain FA in WCS and the potential for greater incorporation of preformed FA into milk fat (Harrison et al., 1995).

When considering FA supplementation and the interactions between basal diets differing in FA content, Rico et al. (2017) and de Souza et al. (2018) compared a WCS basal diet with a soybean hull basal diet. Both studies reported an increase in milk fat yield with a WCS basal diet compared with a soybean hull diet. Rico et al. (2017) reported interactions between the WCS basal diet and C16:0 supplementation with increased yields of milk and milk fat. In contrast, de Souza et al. (2018) observed that in a WCS basal diet, FA supplementation had no effect on milk yield whereas milk fat content was increased with a FA treatment of ~80% C16:0 compared with blends of C16:0 + *cis*-9 C18:1 and C16:0 + C18:0. These studies (Rico et al., 2017; de Souza et

al., 2018) compared a WCS diet with a soybean hull diet and thus not only differed in FA content but also the amount and fermentability of other nutrients. Therefore, the objective of our study was to evaluate low- and high-fat basal diets and their interactions with different ratios of C16:0 + *cis*-9 C18:1 on production of lactating dairy cows. In order to keep the basal diets as similar as possible, we utilized WCS or a combination of cottonseed hulls (CSH) + cottonseed meal (CSM) to manipulate FA content in the basal diet while keeping the rest of the ingredients the same.

Material and Methods

Design and Treatments

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University, East Lansing. Thirty-six mid-lactation, multiparous Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center were randomly assigned to a treatment sequence in a replicated split-plot 3 X 3 Latin square design balanced for carryover effects in three 21-d periods. The starting average for all animals, with mean $\pm$ standard deviation, were 50.2 ± 5.8 kg of milk yield, 160 ± 36 d DIM, and 747 ± 61 kg of BW. All animals received a common diet with no fat supplementation during a 7-d preliminary period to obtain baseline values.

This trial was designed to test the interaction between basal fat level and supplementation of different ratios of C16:0 and *cis*-9 C18:1. Cows were assigned to a main plot, with 18 cows receiving a low-fat (LF; average 2.40% FA content) basal diet containing CSH and CSM and 18 cows receiving a high-fat (HF; average 3.28% FA content) basal diet containing WCS throughout the study. Within each basal diet split-plot, replicated 3 x 3 Latin squares were used to assign FA treatments so that each animal received each of the FA treatments but only 1 basal

diet. The design of the experiment lessens the statistical power of the main plot factor (LF vs. HF: basal diet) but gives more power to test the split-plot factors (FA treatments and interaction between basal diet and FA treatments; Kutner et al., 2005; Rico et al., 2017). The FA treatments consisted of 1) control (CON; diet with no supplemental FA), 2) FA supplement containing 80% C16:0 and 10% *cis*-9 C18:1 (PA) and 3) FA supplement containing 60% C16:0 and 30% *cis*-9 C18:1 (PA+OA). Both FA supplements were fed at 1.5% FA (DM basis) of the diet and the supplements replaced soyhulls from the control diet. The FA supplements used are commercially available and their total FA content and profile are presented in Table 1. All experimental diets were formulated to meet the nutrient requirements of the average cow (Table 2). The DM concentration of forages were determined twice weekly and diets adjusted when necessary. Base diets were mixed in a wagon daily, with forages mixed in one base mix that was then split. Half of the forage base mix was added to a mixer wagon for the HF base diet (containing corn grain, high-moisture corn, soybean meal, mineral mix, and whole cottonseeds) and then the other half added back into the mixer wagon for the LF base diet (containing corn grain, high-moisture corn, soybean meal, mineral mix, cottonseed hulls and cottonseed meal). Then, soyhulls, FA supplements, and basal diet were mixed in a tumble-mixer for each experimental diet. Cows were fed 115% expected intake at 1000 h daily. Feed access was blocked from 0800 to 1000 h for orts collection and offering of new feed. Cows were housed in individual tie-stalls throughout the experiment with water available *ab libitum* in each stall which were bedded with sawdust and cleaned twice daily.

Data and Sample Collection

Samples and production data were collected during the last 5 d of each treatment period (d 17-21). Samples of all diet ingredients (0.5 kg) and orts (12.5%) were collected daily and

composited by cow/period for analysis. Milk yield was recorded, and two milk samples were collected at each milking. One aliquot was collected in a sealed tube with preservative and stored at 4°C for milk component analysis. The second aliquot was stored without preservative at -20°C until analyzed for FA composition. Blood (~15 mL) samples were collected every 15 h resulting in 8 samples/cow/period and stored on ice until centrifugation at 2,000 X g for 15 min at 4°C. Plasma was transferred into microcentrifuge tubes and stored at -20°C until composited by cow/period. Body weight (BW) was measured 3 times per week following the afternoon milking with changes in BW determined according to Boerman et al. (2015). On the last day of each period, BCS was determined by three trained investigators on a 5-point scale in 0.25 increments (Wildman, et al.,1982).

Sample Analysis

Dietary ingredients and orts were dried at 55°C in a forced-air oven for 72 h for DM determination. Dried samples were ground in a Wiley mill (1 mm screen; Arthur H. Thomas, Philadelphia, PA). Samples of feed ingredients and orts were analyzed for neutral detergent fiber (NDF), indigestible NDF (iNDF), starch, CP, and FA according to Boerman et al. (2017). Milk samples were analyzed for fat, true protein, and lactose concentrations by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan Dairy Herd Improvement Association (Central Star DHI, Grand Ledge, MI). Yields of milk components, FCM, and ECM were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each period. Milk samples used for analysis of FA composition were composited based on milk fat yield (d 17-21 per period). Milk lipids were extracted, FAME prepared, and analyzed by gas chromatography as described previously (Lock et al., 2013). Yields of individual FA (g/d) in milk fat was calculated using milk fat yield and FA

concentration to determine yield on a mass basis using the molecular weight of each FA while correcting for glycerol content and other milk lipid classes (Piantoni et al., 2013).

Statistical Analysis

All data were analyzed using the GLIMMIX model procedure of SAS (version 9.4, SAS Institute, Cary, NC). Data was analyzed using the following model:

$$Y_{ijkl} = \mu + C(B)_{i(j)} + B_j + P_k + T_l + P_k \times T_l + B_j \times T_l + B_j \times P_k + P_k \times B_j \times T_l + e_{ijkl},$$

Where Y_{ijkl} = the dependent variable, μ = the overall mean, $C(B)_{i(j)}$ = random effect of cow nested in basal diet ($i = 1$ to 18), B_j = fixed effect of basal diet ($j = 1$ to 2), P_k = fixed effect of period ($k = 1$ to 3), T_l = fixed effect of treatment ($l = 1$ to 3), $P_k \times T_l$ = the interaction of period and treatment, $B_j \times T_l$ = the interaction of basal diet and treatment, $B_j \times P_k$ = the interaction of basal diet and period, $P_k \times B_j \times T_l$ = the three-way interaction of period, treatment, and basal diet, and e_{ijkl} = residual error. $P_k \times B_j \times T_l$ was not significant for all variables and was removed from the model. Main effects were declared significant at $P \leq 0.05$ and tendencies $P \leq 0.10$ and interactions were declared significant at $P \leq 0.10$ and tendencies at $P \leq 0.15$.

Results

Production Responses and DMI

Compared with LF, HF increased milk fat yield and FCM ($P < 0.05$; Table 4.3) and tended to increase milk fat content and ECM yield ($P < 0.10$). The HF basal diet tended to decrease lactose content compared with LF ($P < 0.10$). There was no effect of basal diet on milk

yield, protein content, protein yield, lactose yield, feed efficiency (FCM/DMI), BW, BWC, or BCS ($P > 0.16$).

Both FA treatments increased milk fat content and the yields of milk fat, lactose, FCM, and ECM compared with CON ($P < 0.05$). Compared with CON, both FA treatments tended to decrease protein content ($P < 0.10$; Table 4.3) and increase feed efficiency ($P < 0.10$). PA+OA decreased DMI compared with CON and PA ($P < 0.05$) and PA+OA tended to increase feed efficiency compared with PA ($P < 0.10$). However, there was no effect of FA treatments on protein yield, lactose content, BW, BWC, or BCS ($P > 0.10$).

We observed 2 trends for interactions between basal diet and FA treatments for production responses. Compared with CON, the FA treatments tended to increase yields of milk and lactose in the LF basal diets but not in the HF basal diets ($P < 0.15$; Supplement Table S1).

Milk FA Concentration and Yield

Milk FA are derived from two sources: <16 carbon FA (de novo) from de novo synthesis in the mammary gland and >16 carbon FA (preformed) originating from extraction from plasma. Mixed source 16-carbon FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis in the mammary gland and extraction from plasma. Compared with LF, the HF basal diet increased preformed FA yield ($P < 0.05$; Table 4.5) and tended to increase mixed FA yield ($P < 0.10$). There was no difference in the yield of de novo milk FA between basal diets ($P > 0.10$). There was no difference between the LF and HF basal diets for the concentrations of de novo, mixed, and preformed milk FA sources ($P > 0.20$).

Compared with CON, both FA treatments decreased the yield of de novo milk FA ($P < 0.05$, Figure 4.3). Both FA treatments increased the yields of mixed and preformed milk FA compared with CON ($P < 0.05$). The PA treatment increased mixed milk FA yield compared with

PA+OA ($P < 0.05$) and the PA+OA treatment increased the yield of preformed milk FA compared with PA ($P < 0.05$). Both FA treatments decreased the concentration of de novo milk FA compared with CON ($P < 0.05$; Table 4.5). Compared with CON, both FA treatments increased mixed milk FA concentration ($P < 0.05$) and PA increased mixed milk FA concentration compared with PA+OA ($P < 0.05$). The PA+OA treatment increased the concentration of preformed milk FA compared with CON and PA ($P < 0.05$) and PA decreased preformed milk FA concentration compared with CON ($P < 0.05$).

No interactions between basal diet and FA treatments were observed for the yields of de novo, mixed, or preformed milk FA ($P > 0.15$; Table 4.4). In contrast, we observed an interaction between basal diet and FA treatments for de novo and preformed milk FA concentrations. Compared with CON, both FA treatments decreased de novo milk FA concentration in LF and HF but the magnitude of change was greater in LF compared with HF ($P < 0.05$, Figure 4.2). The PA+OA treatment increased preformed milk FA concentration in LF compared with the CON and PA ($P < 0.05$). In HF, PA+OA increased preformed milk FA concentration compared with CON and PA, and PA decreased preformed milk FA concentration compared with CON ($P < 0.05$).

Plasma Insulin

There were no main effects of basal diet or FA treatments on plasma insulin ($P > 0.41$; Table 4.5). We observed an interaction between basal diet and FA treatments for plasma insulin. There was no difference among treatments for the LF basal diet; however, PA+OA decreased plasma insulin in the HF basal diet compared with CON and PA ($P = 0.05$; Figure 4.4).

Discussion

There is increasing interest in the effects that individual FA have on digestibility, metabolism and production responses of dairy cows. C16:0 and *cis*-9 C18:1 are two of the most common FA found in commercially available FA supplements and blends of differing ratios of C16:0 and *cis*-9 C18:1 have been shown to increase milk yield compared with a non-FA supplemented control diet (de Souza et al., 2018) and have different production responses across production levels (de Souza et al., 2019). In our study we evaluated if differing basal FA content interacted with supplementation of C16:0 and *cis*-9 C18:1. Our results show that production can be affected by FA supplementation and basal FA content.

Altering the FA content of the basal diet in the current study had no effect on DMI. The inclusion rate of WCS in our study likely impacted our results. In our current study, WCS was included at 8.95% DM, similar to de Souza et al. (2019) at 8.60% DM while Rico et al (2017) inclusion rate was 16.7% DM which could explain the difference in results de Souza et al. (2018) observed no difference in DMI between a soyhull basal diet and a WCS basal diet while Rico et al. (2017) observed a tendency for the WCS basal diet to decrease DMI compared with the soyhull basal diet. Our results are also similar to Sklan et al. (1992) who also observed no difference in DMI between cows fed a control diet with no cottonseed products, a WCS diet, and a diet containing CSM and CSH with added FA. We also did not observe interactions between FA treatments and basal diet for DMI. In contrast, de Souza et al. (2018) reported interactions between basal diet and FA supplementation with a 45% C16:0 + 35% *cis*-9 C18:1 FA blend decreasing DMI in the WCS basal diet compared with a non-FA supplemented control and decreasing DMI compared with other FA treatments in the soyhull basal diet. Potential differences in results could be attributed to a higher level of *cis*-9 C18:1 in diets from de Souza et

al (2018) as their WCS basal diets had a higher total FA content and they included 5% more *cis*-9 C18:1 in their FA blend compared to our 30% *cis*-9 C18:1. Additionally, DMI is variable for cows supplemented with dietary fat, due to degree of saturation (Relling and Reynolds, 2007) and the type of supplement being fed (Rabiee et al., 2012). Ca-salts, for example, have been reported to decrease in DMI compared with other supplements (Rabiee et al., 2012). In general, diets higher in UFA have been observed to decrease DMI compared with cows supplemented with SFA and diets with no FA supplementation (Harvatine and Allen, 2005; Relling and Reynolds, 2007; de Souza et al., 2018). Effects of UFA could be attributed to increased secretion of gut peptides associated with satiety, e.g. CCK and glucagon-like peptide 1 (Relling and Reynolds, 2007; Bradford et al., 2008). Our results support this as the PA+OA treatment decreased DMI compared with CON and PA, suggesting that the PA+OA treatment increased the supply of UFA past the rumen.

We observed an interaction between basal diet and FA treatments with FA treatments increasing milk yield in LF but not in HF diets. Similarly, when evaluating basal fat levels, de Souza et al. (2018) observed that FA treatments increased milk yield in a soyhull basal diet but had no effect in a WCS basal diet. Overall, basal FA level in our study had no effect on milk yield. Similarly, Smith et al. (1981) observed that increasing the FA content of the diet, by increasing the percentage of WCS included in the diet, did not effect milk production. When evaluating basal diets containing WCS or soyhulls, Rico et al. (2017) observed no difference in milk yield whereas de Souza et al. (2018) observed that the WCS basal diet tended to decrease milk yield compared with the soyhull basal diet. Although we did not observe a milk production response between basal diets, HF increased milk fat yield, FCM, and ECM compared with LF.

Increasing the FA content of a diet utilizing WCS has been observed to increase the yields of milk fat and FCM (Smith et al., 1981; Harrison et al., 1995).

de Souza et al. (2018) evaluated different blends of supplemented FA and observed increases in milk yield and milk fat yield for treatments containing 80% C16:0 and 45 % C16:0 + 35% *cis*-9 C18:1. Our results were similar as both FA treatments increased yields of milk, FCM, and ECM. Previously, altering the ratios of C16:0 and *cis*-9 C18:1 has been observed to increase yields of milk, milk fat, and ECM but production responses were dependent on production level (de Souza et al., 2019). The cows at the start of our current study averaged 50 kg/d of milk yield and we observed no difference between the PA and PA+OA treatments for production responses. de Souza et al. (2019) observed no production differences between differing ratios of C16:0 + *cis*-9 C18:1 in cows averaging 53 kg/d of milk yield. A treatment with a FA ratio of 60% C16:0 + 30% *cis*-9 C18:1 increased milk in cows greater than 60 kg/d whereas cows averaging 45 kg/d responded better to 80% C16:0 + 10% *cis*-9 C18:1 supplementation (de Souza et al., 2019). The cows in our study were in between the low- and medium-producing cows in de Souza et al. (2019) and this could be the reason we did not observe a difference between FA treatments. Therefore it is likely we would have observed production response differences between PA and PA+OA if the cows in our study were producing greater than 55 kg/d of milk or less than 45 kg/d of milk. Reasons for differences due to FA profile at different production levels could be due to other metabolic responses and needs to be further investigated.

The increase in milk fat yield in the HF diet was due to an increase in preformed milk FA, which would be expected due to the greater intake of long-chain FA from the WCS. These results are similar to de Souza et al. (2018) where a basal diet with WCS increased preformed milk FA yield compared with a basal diet containing soyhulls. The LF basal diet potentially

limited milk fat synthesis due to the lower yields of preformed milk FA compared with HF, although there was no difference in de novo milk FA between LF and HF. Both PA and PA+OA increased milk fat yield, and although both FA treatments increased mixed and preformed milk FA, the increase for the PA treatment was due to a greater increase in mixed milk FA yield while the increase in the PA+OA treatment was due to a greater increase in preformed milk FA yield. Our results support de Souza et al. (2019), as they reported that increasing the level of *cis*-9 C18:1 in a FA blend linearly increased preformed milk FA yield while a blend with a higher level of C16:0 increased mixed milk FA. The decrease in de novo milk FA in the PA+OA treatment could be attributed to a substitution effect, where de novo milk FA are compensated for by an increase in preformed milk FA, which often occurs when FA, especially 18-carbon FA, are supplemented in the diet (Glasser et al., 2008; He et al., 2012). Interestingly, the yield of C4:0 in milk fat was increased by the HF diet and both FA treatments, with the increase being greatest for PA+OA. Increases in the yield of C4:0 have also been observed with supplementation of C16:0 compared with a non-FA control diet (Lock et al., 2013; de Souza et al., 2016). This increase likely occurs as part of the mechanism to maintain milk fluidity when more long chain FA enter the mammary gland (Barbano et al., 1980).

Supplementation of *cis*-9 C18:1 has been observed to increase plasma insulin (de Souza et al. 2018 and 2019) but we did not observe an effect of treatment on plasma insulin in the current study. Furthermore, the PA+OA treatment in our study decreased plasma insulin in the HF basal diet but not in the LF basal diet. Our results differ from de Souza et al. (2018), who did not observe an interaction between basal diet and FA treatments containing C16:0 and C16:0 + *cis*-9 C18:1, although de Souza et al. (2019) observed an increase in plasma insulin when increasing the amount of dietary *cis*-9 C18:1. Previously, high-fat diets decreased plasma insulin

which could be attributed to a decrease in DMI (Choi and Palmquist, 1996) but the increase in plasma insulin from other studies could also be due to specific effects of *cis*-9 C18:1 on insulin (de Souza et al. 2018 and 2019). We also did not observe a change in BW or BCS due to FA treatments or basal diet. In contrast to our results, a blend of 45% C16:0 + 35% *cis*-9 C18:1 and increasing the level of *cis*-9 C18:1 in a FA blend increased energy partitioning towards body reserves (de Souza et al., 2018 and 2019). Although we observed an increase in the content of *trans* FA in milk fat, there was also an increase in milk fat yield and milk fat content for the PA+OA treatment, suggesting that this treatment did not impact BH induced milk fat depression. Even though the PA+OA treatment provided a higher load of UFA, which could overcome normal BH capacity and change BH pathways, there was no indication of energy being repartitioned towards body reserves. Reasons for no differences in change in BW in our study could be due to the lack of response in plasma insulin and/or insufficient production of BH intermediates towards energy partitioning.

Conclusion

A high FA basal diet (average 3.28% FA content) increased milk fat yield (0.18 kg/d) and FCM (3.5 kg/d) and tended to increase milk fat content (0.30 %/d) and ECM (2.9 kg/d) compared with a low FA basal diet (average 2.40% FA content). The increase in milk fat yield and FCM was due to an increase in preformed milk FA with the HF diet. Both the FA treatments tended to increase milk yield (average of 1.35 kg/d) and lactose yield (average of 0.07 kg/d) compared with a non-FA control in the LF diet but did not affect these yields in the HF diet. Regardless of basal FA content, both FA treatments increased yield of milk fat, ECM, and FCM (average increase of 0.07 kg/d, 1.20 kg/d, and 1.60 kg/d respectively) compared with a non-FA

supplemented control but were not different from each other. Overall, a high FA basal diet increased production compared with a low-fat diet, and addition of FA supplements to a low-fat diet increased milk yield.

APPENDIX

Table 4.1. FA profile of FA supplements¹

Item	Fat Supplement ²	
	C16:0 enriched FA Supplement	Ca-salt of palm FA Supplement
Total FA content, %DM	92.6	79.1
FA profile of each FA supplement ³ , g/100 g of FA		
C14:0	0.78	1.02
C16:0	81.1	61.6
C18:0	1.32	4.59
<i>cis</i> -9 C18:1	13.0	26.7
<i>cis</i> -9, <i>cis</i> -12 C18:2	2.92	4.71
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	0.10	0.03

¹Average (n = 3) based on samples taken during the last 5 d of the experimental period.

² Spectrum Fusion (Perdue Agribusiness, Salisbury, MD) and MegaMax (Perdue Agribusiness, Salisbury, MD)

Table 4.2. Ingredient and nutrient composition of treatment diets¹

Item	Basal Diet ²					
	LF			HF		
	CON	PA	PA+OA	CON	PA	PA+OA
Ingredient, % DM						
Corn Silage	39.1	39.1	39.1	39.1	39.1	39.1
Whole Cottonseed	-	-	-	8.95	8.95	8.95
Cottonseed Hulls	4.27	4.27	4.27	-	-	-
Cottonseed Meal	3.84	3.84	3.84	-	-	-
Ground Corn	15.47	15.47	15.47	15.47	15.47	15.47
Haylage	3.84	3.84	3.84	8.71	8.71	8.71
High Moisture Corn	9.18	9.18	9.18	8.71	8.71	8.71
Soybean Meal	7.96	7.96	7.96	7.81	7.81	7.81
Soyhulls	4.74	3.31	3.09	4.74	3.33	3.09
Dairy Base ³	1.92	1.92	1.92	1.92	1.92	1.92
Vitamin and Mineral Mix ⁴	6.44	6.44	6.44	6.44	6.44	6.44
C16:0-enriched FA ⁵	-	1.37	-	-	1.37	-
Ca-salt of palm FA ⁶	-	-	1.59	-	-	1.60
Nutrient Composition, % of DM ⁷						
NDF	29.1	28.2	28.1	29.4	28.5	28.4
Forage NDF	15.8	15.7	15.8	16.4	16.4	16.4
CP	17.4	17.2	17.2	17.0	16.8	16.8
Starch	31.2	31.2	31.2	30.7	30.7	30.7
FA	1.77	3.02	3.01	2.65	3.91	3.90
16:0	0.32	1.36	1.09	0.45	1.50	1.23
18:0	0.06	0.07	0.12	0.07	0.09	0.13
<i>cis</i> -9 18:1	0.33	0.49	0.67	0.40	0.57	0.76
<i>cis</i> -9, <i>cis</i> -12 18:2	0.90	0.93	0.96	1.22	1.25	1.28
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.10	0.10	0.10	0.10	0.10	0.10

¹CON = no FA supplementation, PA = 1.5% of DM to provide approximately 80% C16:0 + 10% *cis*-9 C18:1, and PA+OA = 1.5% of DM to provide approximately 60% C16:0 + 30% *cis*-9 C18:1.

²Basal diets containing either whole cottonseed (HF) or cottonseed hulls and meal (LF).

³Dairy base contained

⁴Vitamin and mineral mix contained

⁵Fusion (Purdue Agribusiness, Salisbury, MD)

⁶MegaMax (Purdue Agribusiness, Salisbury, MD).

⁷Expressed as a percent of as fed.

Table 4.3. Production responses and DMI of cows fed treatment diets (n=36) ¹

Variable	Treatment (trt) ²			SEM ³	Basal ⁴		SEM ³	P-value ⁵		
	CON	PA	PA+OA		LF	HF		Trt	Basal	Trt × Basal
DMI, kg/d	33.0 ^a	33.1 ^a	32.0 ^b	0.46	32.5	32.9	0.65	<0.01	0.66	0.43
Yields, kg/d										
Milk yield	46.1 ^b	47.3 ^a	46.9 ^a	0.89	46.4	47.2	1.25	<0.01	0.62	0.14
FCM ⁶	47.4 ^b	48.9 ^a	49.0 ^a	0.82	46.7 ^y	50.2 ^x	1.16	<0.01	0.04	0.35
ECM ⁷	47.8 ^b	49.0 ^a	48.9 ^a	0.82	47.1 ^y	50.0 ^x	1.15	<0.01	0.07	0.44
Fat	1.69 ^b	1.76 ^a	1.76 ^a	0.04	1.65 ^y	1.83 ^x	0.05	<0.01	0.02	0.40
Protein	1.51	1.52	1.50	0.03	1.49	1.54	0.04	0.11	0.40	0.60
Lactose	2.17 ^b	2.23 ^a	2.21 ^a	0.05	2.24	2.17	0.07	<0.01	0.44	0.01
Milk composition, %										
Fat	3.70 ^b	3.76 ^a	3.79 ^a	0.09	3.60 ^y	3.90 ^x	0.13	<0.01	0.09	0.34
Protein	3.30 ^a	3.24 ^b	3.21 ^b	0.03	3.23	3.26	0.04	<0.01	0.65	0.31
Lactose	4.72	4.73	4.72	0.07	4.84 ^x	4.60 ^y	0.10	0.21	0.09	0.20
FCM/DMI	1.42 ^c	1.48 ^b	1.52 ^a	0.02	1.44	1.51	0.05	<0.01	0.16	0.56
BW, kg	758	760	760	11.0	761	758	21.5	0.61	0.87	0.34
BW change, kg	0.37	0.47	0.19	0.10	0.30	0.40	0.08	0.12	0.36	0.80
BCS	3.45	3.42	3.44	0.05	3.43	3.43	0.06	0.23	0.98	0.57
BCS change	0.04	0.06	0.07	0.03	0.05	0.06	0.03	0.87	0.71	0.83

^{a-c} For FA treatment effect, means in a row with different superscripts differ ($P < 0.05$). Separation conducted only if treatment effect was $P < 0.10$.

^{x, y} For basal diet effect, means in a row with different superscripts differ ($P < 0.05$). Separation conducted only if treatment effect was $P < 0.10$.

¹ Experimental diets fed to 36 cows in replicated 3×3 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).

² CON = control; PA = 1.5% of FA supplement to provide approximately 80% of C16:0 + 10% of C18:1 cis-9; PA+OA = 1.5% of FA supplement to provide approximately 60% of C16:0 + 30% of C18:1 cis-9.

³ Greatest SEM.

Table 4.3. (cont'd)

⁴ Basal diets include diets with cottonseed hulls and cottonseed meal (LF) and whole cottonseeds (HF).

⁵ P-values refer to the ANOVA results for the fixed effects of treatment and period.

⁶ Fat-corrected milk; 3.5 % FCM = $[(0.4324 \times \text{kg milk}) + (16.216 \times \text{kg milk fat})]$.

⁷ Energy-corrected milk; ECM = $[(0.327 \times \text{kg milk}) + (12.95 \times \text{kg milk fat}) + (7.20 \times \text{kg milk protein})]$.

Table 4.4. Milk yield and milk composition of cows at different basal levels fed treatment diets (n = 36)¹

Variable	Basal Level ⁴	Treatment (trt) ²			SEM ³	P-value ⁵		
		CON	PA	PA+OA		Trt	Basal	Trt × Basal
Milk Yield, kg/d	Low-fat	45.5 ^b	47.2 ^a	46.5 ^a	1.25	<0.01	0.62	0.14
	High-fat	46.8	47.4	47.3				
Lactose, kg/d	Low-fat	2.19 ^b	2.28 ^a	2.25 ^a	0.07	<0.01	0.44	0.01
	High-fat	2.15	2.17	2.17				

^{a-c} For FA treatment effect, means in a row with different superscripts differ (P < 0.10). Separation conducted only if treatment effect was P < 0.15.

¹ Experimental diets fed to 36 cows in replicated 3 × 3 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).

² CON = control; PA = 1.5% of FA supplement to provide approximately 80% of C16:0 + 10% of C18:1 cis-9; PA+OA = 1.5% of FA supplement to provide approximately 60% of C16:0 + 30% of C18:1 cis-9.

³ Greatest SEM.

⁴ Basal diets include diets with cottonseed hulls and cottonseed meal (LF) and whole cottonseeds (HF).

⁵ P-values refer to the ANOVA results for the fixed effects of treatment and period.

Table 4.5. Milk fatty acid yield and concentration by source for cows fed treatment diets (n=36)¹

Summation by source	Treatment ²			SEM ³	Basal Diet ⁴		SEM ³	P-value ⁵		
	CON	PA	PA+OA		LF	HF		Treatment	Basal	Treatment × Basal
Yield, g/d										
De Novo	451 ^a	423 ^b	413 ^c	12.0	411	447	16.8	<0.01	0.14	0.35
Mixed	590 ^c	668 ^a	650 ^b	17.6	606 ^y	666 ^x	24.8	<0.01	0.10	0.45
Preformed	546 ^c	556 ^b	597 ^a	9.80	525 ^y	608 ^x	13.5	<0.01	<0.01	0.54
Concentration, g/100g										
De Novo	28.5 ^a	25.6 ^b	24.8 ^c	0.33	26.6	26.0	0.47	<0.01	0.33	<0.01
Mixed	37.1 ^c	40.4 ^a	39.0 ^b	0.36	39.1	38.6	0.5	<0.01	0.42	0.48
Preformed	34.5 ^b	34.1 ^c	36.1 ^a	0.43	34.4	35.4	0.6	<0.01	0.22	<0.01

^{a-c} For FA treatment effect, means in a row with different superscripts differ (P < 0.05). Separation conducted only if treatment effect was P < 0.10.

^{x,y} For basal diet effect, means in a row with different superscripts differ (P < 0.05). Separation conducted only if treatment effect was P < 0.10.

¹ Experimental diets fed to 36 cows in replicated 3 × 3 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).

² CON = control; PA = 1.5% of FA supplement to provide approximately 80% of C16:0 + 10% of C18:1 cis-9; PA+OA = 1.5% of FA supplement to provide approximately 60% of C16:0 + 30% of C18:1 cis-9.

³ Greatest SEM.

⁴ Basal diets include diets with cottonseed hulls and cottonseed meal (LF) and whole cottonseeds (HF).

⁵ P-values refer to the ANOVA results for the fixed effects of treatment and period.

Table 4.6. Individual milk fatty acid yields for cows fed treatment diets (n = 36)¹

Variable	Treatment (trt) ²			SEM ³	Basal Diet ⁴		SEM ³	P-value ⁵		
	CON	PA	PA+OA		LF	HF		Trt	Basal	Trt × Basal

Selected individual fatty acids, g/d										
C4:0	47.3 ^c	49.4 ^b	51.0 ^a	1.29	46.2 ^y	52.5 ^x	1.77	<0.01	0.02	0.27
C6:0	34.0	33.4	33.5	1.06	31.6 ^y	35.7 ^x	1.48	0.36	0.06	0.09
C8:0	21.0 ^a	19.5 ^b	19.4 ^b	0.69	19.0	20.9	0.96	<0.01	0.17	0.15
C10:0	57.9 ^a	51.8 ^b	49.8 ^c	2.01	51.1	55.3	2.83	<0.01	0.30	0.23
C12:0	68.1 ^a	60.1 ^b	57.0 ^c	2.26	60.2	63.2	3.18	<0.01	0.50	0.29
C14:0	210 ^a	197 ^b	192 ^c	5.27	192	208	7.38	<0.01	0.14	0.29
C16:0	567 ^c	644 ^a	627 ^b	17.0	583 ^y	642 ^x	23.9	<0.01	0.09	0.44
<i>cis</i> -9 C16:1	22.8 ^c	24.5 ^a	23.3 ^b	0.90	23.3	23.7	1.28	<0.01	0.83	0.36
C18:0	140 ^b	139 ^b	149 ^a	4.23	121 ^y	164 ^x	5.88	<0.01	<0.01	0.12
<i>trans</i> -6 to -8 C18:1	3.84 ^c	4.28 ^b	4.80 ^a	0.11	4.03 ^y	4.58 ^x	0.14	<0.01	0.01	0.67
<i>trans</i> -9 C18:1	2.98 ^c	3.12 ^b	3.56 ^a	0.06	2.92 ^y	3.52 ^x	0.08	<0.01	<0.01	0.77
<i>trans</i> -10 C18:1	7.05 ^c	7.72 ^b	8.22 ^a	0.55	7.38	7.95	0.78	<0.01	0.60	0.85
<i>trans</i> -11 C18:1	12.0 ^b	12.3 ^b	12.8 ^a	0.42	11.5 ^y	13.3 ^x	1.80	0.02	0.02	0.95
<i>cis</i> -9 C18:1	244 ^c	255 ^b	288 ^a	4.76	247 ^y	277 ^x	6.58	<0.01	0.002	0.07
<i>cis</i> -9, <i>cis</i> -12 C18:2	40.6 ^a	39.8 ^b	41.2 ^a	0.83	39.9	41.2	1.14	<0.01	0.43	0.28
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	4.12 ^a	3.95 ^b	3.88 ^b	0.09	3.86 ^y	4.11 ^x	0.12	<0.01	0.12	0.08

^{a-c} For FA treatment effect, means in a row with different superscripts differ (P < 0.05). Separation conducted only if treatment effect was P < 0.10.

^{x, y} For basal diet effect, means in a row with different superscripts differ (P < 0.05). Separation conducted only if treatment effect was P < 0.10.

Table 4.6. (cont'd)

¹ Experimental diets fed to 36 cows in replicated 3×3 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).

² CON = control; PA = 1.5% of FA supplement to provide approximately 80% of C16:0 + 10% of C18:1 cis-9; PA+OA = 1.5% of FA supplement to provide approximately 60% of C16:0 + 30% of C18:1 cis-9.

³ Greatest SEM.

⁴ Basal diets include diets with cottonseed hulls and cottonseed meal (LF) and whole cottonseeds (HF).

⁵ P-values refer to the ANOVA results for the fixed effects of treatment and period.

Table 4.7. Individual milk fatty acid concentration for cows fed treatment diets (n = 36)¹

Variable	Treatment(trt) ²			SEM ³	Basal ⁴		SEM ³	P-value ⁵		
	CON	PA	PA+OA		LF	HF		Trt	Basal	Trt × Basal
Selected individual fatty acids, g per 100g										
C4:0	2.99 ^b	3.00 ^b	3.05 ^a	0.05	2.99	3.03	0.08	0.05	0.64	0.35
C6:0	2.13 ^a	2.03 ^b	2.01 ^b	0.04	2.03	2.08	0.06	<0.01	0.57	0.34
C8:0	1.32 ^a	1.19 ^b	1.16 ^b	0.03	1.23	1.22	0.04	<0.01	0.85	0.40
C10:0	3.65 ^a	3.12 ^b	2.96 ^c	0.08	3.28	3.2	0.12	<0.01	0.64	0.02
C12:0	4.30 ^a	3.63 ^b	3.39 ^c	0.09	3.89	3.66	0.13	<0.01	0.20	<0.01
C14:0	13.2 ^a	12.0 ^b	11.5 ^c	0.13	12.4	12.1	0.18	<0.01	0.16	0.01
C16:0	35.6 ^c	38.9 ^a	37.7 ^b	0.36	37.6	37.2	0.5	<0.01	0.57	0.79
<i>cis</i> -9 C16:1	1.43 ^b	1.49 ^a	1.40 ^c	0.04	1.51	1.38	0.06	<0.01	0.14	0.08
C18:0	8.76 ^a	8.337 ^b	8.89 ^a	0.18	7.83 ^y	9.52 ^x	0.25	<0.01	<0.01	0.03
<i>trans</i> -6 to -8 C18:1	0.24 ^c	0.26 ^b	0.29 ^a	0.01	0.27	0.27	0.01	<0.01	0.99	0.37
<i>trans</i> -9 C18:1	0.19 ^c	0.19 ^b	0.22 ^a	0.01	0.19	0.21	0.01	<0.01	0.16	0.89
<i>trans</i> -10 C18:1	0.48 ^c	0.50 ^b	0.53 ^a	0.06	0.58	0.44	0.09	<0.01	0.27	0.69
<i>trans</i> -11 C18:1	0.77	0.75	0.79	0.03	0.76	0.78	0.04	0.11	0.73	0.79
<i>cis</i> -9 C18:1	15.4 ^c	15.6 ^b	17.4 ^a	0.25	16.1	16.2	0.36	<0.01	0.88	<0.01
<i>cis</i> -9, <i>cis</i> -12 C18:2	2.58 ^a	2.44 ^c	2.51 ^b	0.05	2.69 ^x	2.32 ^y	0.06	<0.01	<0.01	0.65
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	0.27 ^a	0.24 ^b	0.24 ^b	0.01	0.27 ^x	0.22 ^y	0.01	<0.01	<0.01	0.65

^{a-c} For FA treatment effect, means in a row with different superscripts differ (P < 0.05). Separation conducted only if treatment effect was P < 0.10.

^{x, y} For basal diet effect, means in a row with different superscripts differ (P < 0.05). Separation conducted only if treatment effect was P < 0.10.

¹ Experimental diets fed to 36 cows in replicated 3 × 3 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).

² CON = control; PA = 1.5% of FA supplement to provide approximately 80% of C16:0 + 10% of C18:1 *cis*-9; PA+OA = 1.5% of FA supplement to provide approximately 60% of C16:0 + 30% of C18:1 *cis*-9.

Table 4.7. (cont'd)

³ Greatest SEM.

⁴ Basal diets include diets with cottonseed hulls and cottonseed meal (LF) and whole cottonseeds (HF).

⁵ P-values refer to the ANOVA results for the fixed effects of treatment and period.

Table 4.8. Plasma insulin for cows fed treatment diets (n=36)¹

Variable	Treatment (trt) ²			SEM ³	Basal ⁴		SEM ³	P-value ⁵		
	CON	PA	PA+OA		LF	HF		Trt	Basal	Trt × Basal
Insulin, ug/L	0.59	0.59	0.56	0.02	0.59	0.56	0.02	0.41	0.51	0.05

¹ Experimental diets fed to 36 cows in replicated 3 × 3 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).

² CON = control; PA = 1.5% of FA supplement to provide approximately 80% of C16:0 + 10% of C18:1 cis-9; PA+OA = 1.5% of FA supplement to provide approximately 60% of C16:0 + 30% of C18:1 cis-9.

³ Greatest SEM.

⁴ Basal diets include diets with cottonseed hulls and cottonseed meal (LF) and whole cottonseeds (HF).

⁵ P-values refer to the ANOVA results for the fixed effects of treatment and period.

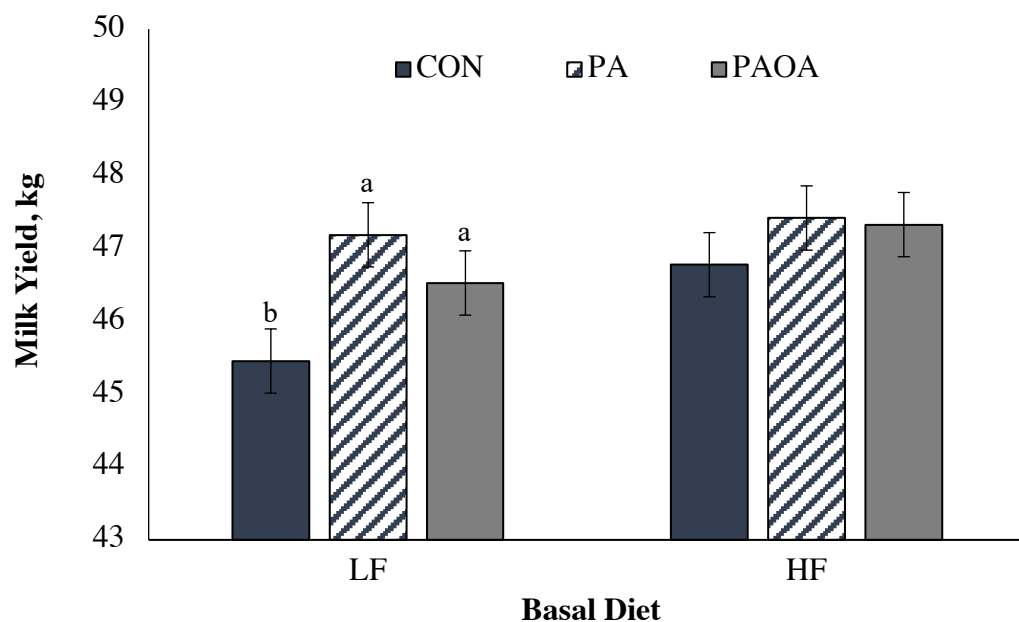


Figure 4.1. Interaction between basal diet and FA treatment for milk yield for cows fed different FA treatments in both basal diets. A tendency for an interaction between basal diet and FA treatment was detected for milk yield ($P=0.14$). Basal diet includes diets containing low-fat (LF) and high-fat (HF). CON = non-FA control diet, PA = 1.5% of FA supplement to provide 80% C16:0 + 10% *cis*-9 C18:1 and PA+OA = 1.5% of FA supplement to provide 60% C16:0 + 30% *cis*-9 C18:1. Error bars represent SEM. For FA treatment effect, means within basal fat diets with different letters (a–b) differ ($P < 0.15$)

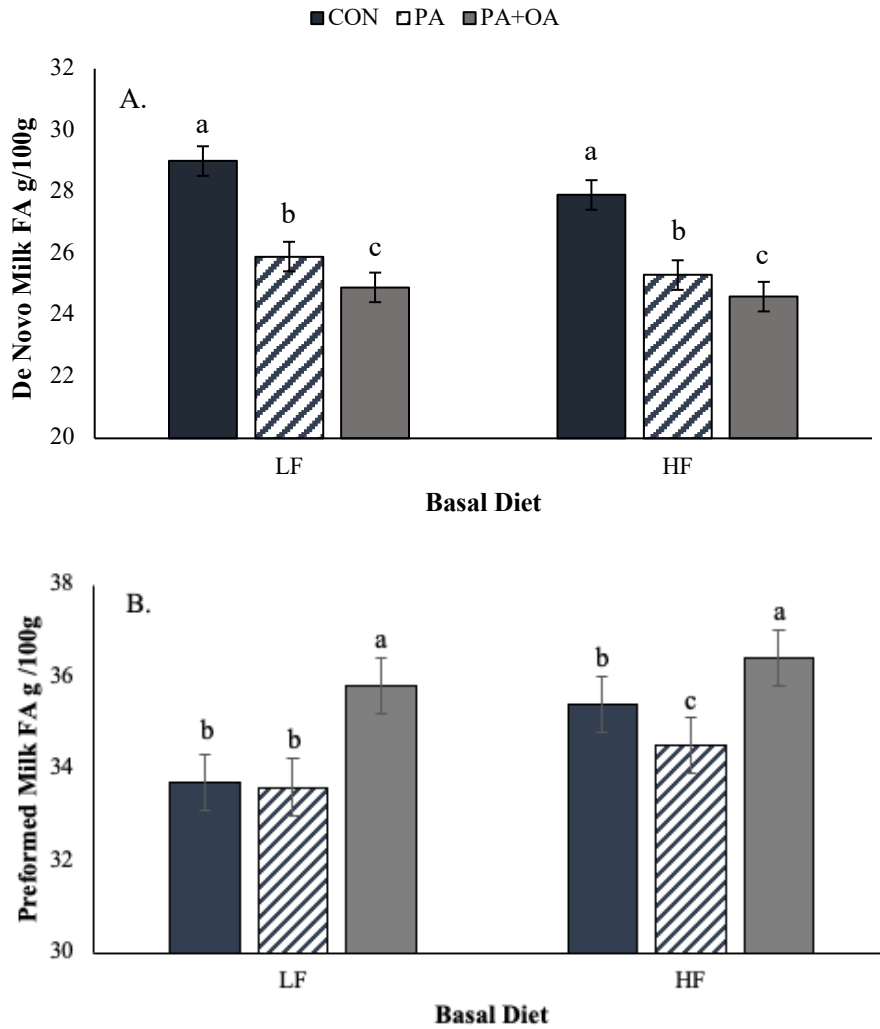


Figure 4.2. Interaction between basal diet and FA treatment for (A) de novo milk FA concentration and (B) preformed milk FA concentration for cows fed different FA treatments in both basal diets. An interaction between basal diet and FA treatment was detected for de novo milk FA concentration ($P = <0.01$) and preformed milk FA concentration ($P = <0.01$). Basal diet includes diets containing low-fat (LF) and high-fat (HF). CON = non-FA control diet, PA = 1.5% of FA supplement to provide 80% C16:0 + 10% *cis*-9 C18:1 and PA+OA = 1.5% of FA supplement to provide 60% C16:0 + 30% *cis*-9 C18:1. Error bars represent SEM. For FA treatment effect, means within basal fat diets with different letters (a–b) differ ($P < 0.15$).

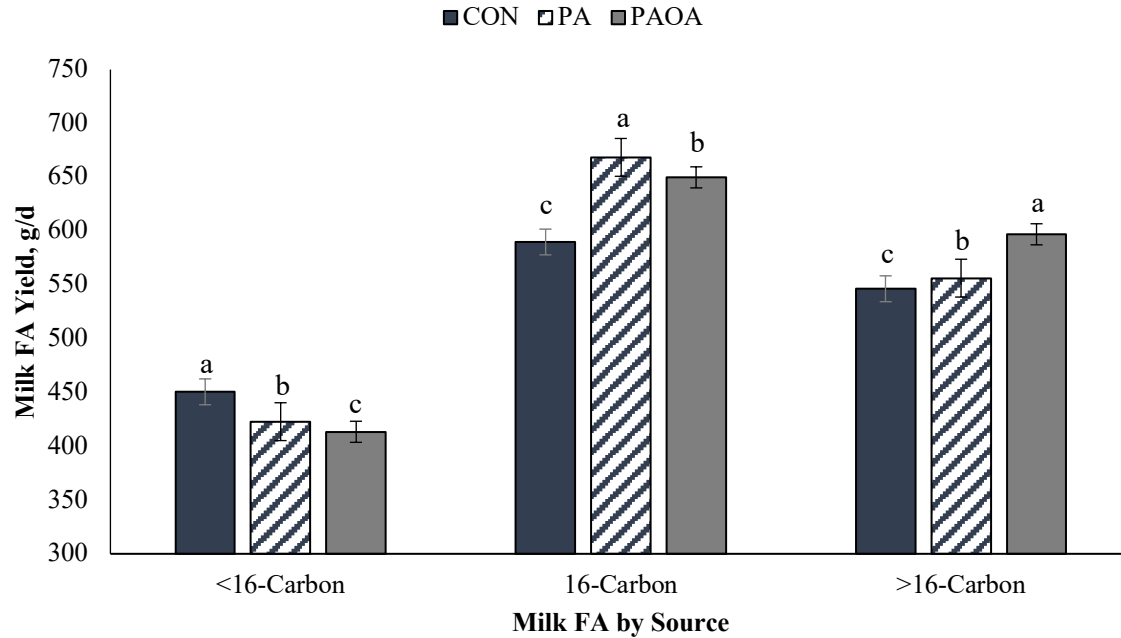


Figure 4.3. Effects of FA treatments on yield of de novo milk FA, mixed milk FA, and preformed milk FA. Basal diet includes diets containing low-fat (LF) and high-fat (HF). CON = non-FA control diet, PA = 1.5% of FA supplement to provide 80% C16:0 + 10% *cis*-9 C18:1 and PA+OA = 1.5% of FA supplement to provide 60% C16:0 + 30% *cis*-9 C18:1. Error bars represent SEM. For FA treatment effect, means within basal fat diets with different letters (a–c) differ ($P < 0.10$).

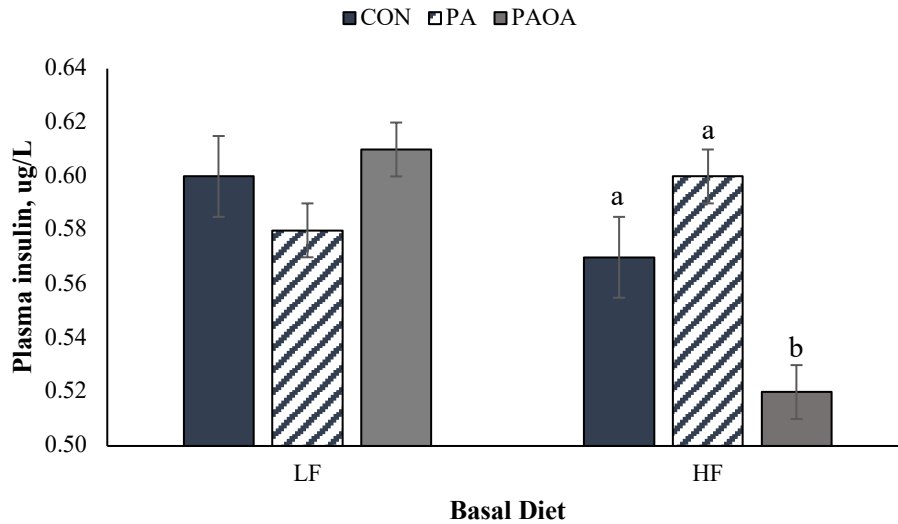


Figure 4.4. Interaction between basal diet and FA treatment for plasma insulin for cows fed different FA treatments in both basal diets. An interaction between basal diet and FA treatment was detected for plasma insulin in the HF basal diet ($P=0.05$). Basal diet includes diets containing low-fat (LF) and high-fat (HF). CON = non-FA control diet, PA = 1.5% of FA supplement to provide 80% C16:0 + 10% *cis*-9 C18:1 and PA+OA = 1.5% of FA supplement to provide 60% C16:0 + 30% *cis*-9 C18:1. Error bars represent SEM. For FA treatment effect, means within basal fat diets with different letters (a–b) differ ($P < 0.15$).

CHAPTER 5

Overall Conclusions

Supplementation of FA can increase production yields and thus increase farm profitability. The three predominant FA found in FA supplements and adipose tissue are C16:0, C18:0, and *cis*-9 C18:1. Multiple studies have reported how these three FA can increase the yield of milk and milk components, but there is different results depending on how these FA interact with production level and other dietary ingredients. The objective of our studies was to determine nutrient digestibility and production responses of low- and high-producing lactating dairy cows supplemented with ratios of C16:0 + C18:0 and C16:0 + *cis*-9 C18:1 as well as the effect of low and high fat diets supplemented with different ratios of C16:0 + *cis*-9 C18:1. Together, both of these studies evaluated the effects of the predominant FA found in commercially available supplements on performance of lactating dairy cows.

In Chapter 3, high-producing dairy cows respond more in favor to a FA blend containing 60% C16:0 and 30% *cis*-9 C18:1 with increases in the yields of milk, milk protein, ECM, milk fat, and mixed and preformed FA compared with a control (no FA supplementation) diet. In contrast, lower-producing dairy cows responded more favorably to a FA blend containing 60% C16:0 and 30% C18:0 with increases in the yields of ECM, FCM, milk fat and protein. Thus, these results indicate that high-producing cows have increased production responses being fed *cis*-9 C18:1 compared to C18:0.

In Chapter 4, the high FA basal diet increased milk fat yield and FCM and tended to increase milk fat content and ECM compared with the low FA basal diet. This increase in milk fat content and yield, and FCM was due to the increase in preformed milk FA in the high FA basal diet. Compared with a non-FA control, both FA treatments tended to increase yields of

milk and lactose in the LF basal diet, but not in the HF diet. Additionally, FA supplements increased milk fat content, and yields of milk fat, ECM, and FCM compared with a non-FA control, but were not different from each other. Overall, a high FA basal diet increased production responses in lactating dairy cows compared with a low-fat diet, while addition of FA supplements to a low FA basal diet increased milk yield.

Both chapters support recent research completed in our lab. Although the magnitude of response was less in our study, de Souza et al. (2019) and Western et al. (2018 ADSA Abstract) observed increases in production responses for high-producing cows with a higher amount of *cis*-9 C18:1 in a FA blend. This study also was able to show that production responses in high-producing cows were due to supplementation of *cis*-9 C18:1 and not C18:0. Our study in chapter 4 was similar to de Souza et al. (2018) who also observed increases in milk fat yield with a high FA basal diet. Even though there was no difference between the different treatment ratios of C16:0 + *cis*-9 C18:1, they both increased production responses. The lack of difference between these FA treatments could be due to the production level not being greater than 55 kg/d of milk, as seen in de Souza et al. (2019) and Western et al. (2018 ADSA Abstract) when supplementing a higher level of *cis*-9 C18:1. In conclusion, blends of FA can have different production responses depending on the production level of the dairy cow. High producing cows respond more in favor to a diet higher in *cis*-9 C18:1 and the basal fat content of the diet can have differing production responses. This work allows for more precise feed management and decision making to increase production yields and farm profitability. Our results support that optimal FA supplementation depends on FA profile of the supplement, the basal fat contents, and can be tailored to certain production levels.

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