EFFECTS OF AMINO ACID AND FATTY ACID SUPPLEMENTATION ON PRODUCTION RESPONSES OF LACTATING COWS

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ABSTRACT

EFFECTS OF AMINO ACID AND FATTY ACID SUPPLEMENTATION ON PRODUCTION RESPONSES OF LACTATING COWS

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Addition of fatty acids (FA) and amino acid (AA) supplements to dairy cow diets is becoming common practice due to the growing demand to increase milk fat and milk protein yields. This thesis contains two studies that evaluate the effects of supplemental palmitic (C16:0) and oleic (*cis*-9 C18:1) acids, and methionine (Met) and lysine (Lys), on lactating dairy cows. The first experiment used a product containing 80% C16:0 + 10% *cis*-9 C18:1 supplied at 1.5% diet dry matter (DM) and supplemental Met and Lys in low forage diets (LF) and a control diet with no added FA or AA at typical midwestern forage content (CON). Compared with CON, LF increased dry matter intake (DMI), milk fat yield, milk protein yield, energy-corrected milk (ECM) yield, and body condition score (BCS). In the second experiment, different ratios of palmitic (C16:0) and oleic (*cis*-9 C18:1) acid were supplemented in basal diets containing high CP without supplemental AA (HP) or low CP with supplemental AA (LP). FA treatments were products consisting of 80% C16:0 + 10% *cis*-9 C18:1 (PA) and 60% C16:0 + 30% *cis*-9 C18:1 (OA) supplemented at 1.5% diet DM and a non-FA supplemented control diet (CON). No interactions were observed between basal diet and FA treatment for the yields of milk or milk components. Compared with HP, LP decreased milk urea nitrogen and blood urea nitrogen concentrations, and did not impact milk, milk fat, or milk protein yields. FA treatments decreased DMI and increased milk yield, fat yield, ECM yield, and feed efficiency. Results from this work can provide information that can be used as a foundation for future studies and to guide feeding decision to maximize performance and farm income.

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KEY TO ABBREVIATIONS

AA	Amino acid
BCS	Body condition score
BH	Biohydrogenation
BUN	Blood urea nitrogen
BW	Body weight
CCK	Cholecystokinin
CLA	Conjugated linoleic acid
CP	Crude protein
CON	Control treatment
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 chromatograpy
GLUT	Glucose transporter

HF	High forage
HP	High crude protein basal diet
LCFA	Long-chain fatty acid
LF	Low forage
LP	Low crude protein basal diet
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
NPN	Nonprotein nitrogen
OA	Oleic acid treatment
PA	Palmitic acid treatment
PUFA	Polyunsaturated fatty acids
SEM	Standard error of the mean
SD	Standard deviation
SFA	Saturated fatty acids
TAG	Triacylglycerides
TG	Triglycerides
TMR	Total mixed ration
UFA	Unsaturated fatty acids

CHAPTER 1

INTRODUCTION

Milk income in most markets is driven by the yields of milk fat and protein. Therefore, nutritional strategies to improve the yield of milk fat and milk protein production are a particular area of interest. Both FA and AA supplementation have been studied for many years, and the benefits of each have been highlighted in separate 100-year reviews (Palmquist and Jenkins, 2017; Schwab and Broderick, 2017). FA supplementation increases the yield of milk and milk fat (Rabiee et al., 2012), improve reproductive efficiency (Rodney et al., 2015), alleviate heat stress (Wang et al., 2010), and modulate energy metabolism (Staples et al., 1998; Hutchinson et al., 2012). AA supplementation increases the yields of milk and milk protein (Schwab and Broderick, 2017; Clark et al., 1977), whole-body glucose appearance (Lemosquet et al., 2009b), lactose yield (Galindo et al., 2011), and milk fat yield (Socha et al., 2005). However, great variation in milk fat and protein responses have been observed across studies, possibly due to the type of FA or AA supplementation, level of supplementation, or basal diet. Also, limited research is available on the interaction between dietary FA and AA supplementation. Recent studies investigating the effects of fat and protein supplementation have not observed any interactions between fat and protein on milk production or composition (Nichols et al., 2018a; 2019).

We propose that the FA profile of a fat supplement and the methionine and lysine content of metabolizable protein are most likely the major factors affecting possible interactions on the yields of milk components. Inclusion of different ratios of FA, especially C16:0, C18:0, and cis-9 C18:1 have variable impacts on nutrient digestibility, energy partitioning, and milk production due to inclusion rate, production level of the cow, stage of lactation, or other dietary nutrients.

Supplementing C16:0 and *cis*-9 C18:1 increases the yield of milk and ECM (Rico et al., 2014; Western et al., 2020b; de Souza et al., 2018a). Additionally, de Souza et al. (2019) altered the ratio of C16:0 and *cis*-9 C18:1 in FA blends and observed that increasing the amount of *cis*-9 C18:1 increased the yield of milk and ECM in high producing cows, while C16:0 increased milk and ECM in low producing cows. FA supplementation often increases yields of milk and milk fat but typically does not increase milk protein yield (Rabiee et al., 2012). Interestingly, increases in milk protein yield were observed with C16:0 supplementation compared with a nonfat control and other FA supplements in studies where the basal diet contained high quality blood meal (de Souza et al., 2019; Western et al., 2020a). Importantly, C16:0 and *cis*-9 C18:1 supplementation has been observed to impact plasma insulin concentrations in various studies (de Souza et al., 2018a; Piantoni et al., 2013) which, in turn, influences milk protein synthesis (Arriola Apelo et al., 2014). Additionally, providing a more complete supply of essential amino acids (EAA) for absorption improves nitrogen efficiency and increase milk protein yield as long as protein is not oversupplied (Haque et al., 2012; Lee et al., 2012; Arriola Apelo et al., 2014). Higgs (2014) observed that cows maintained high levels of performance at lower levels of CP (~14 % diet DM) when balanced for EAA and provided an adequate supply of rumen nitrogen. However, oversupplying protein decreases nitrogen efficiency. Excess AA are catabolized and excreted as urea via ureagenesis, an energetically demanding process (Lapierre et al., 2002; Reed et al., 2017).

The metabolic flexibility of the mammary gland allows for variable responses to dietary supplementation and nutritional strategies, which support milk production under periods of nutrient deficiency, but also creates complexity in manipulating the production of milk fat and milk protein. Although considerable research has examined production responses to AA and FA

supplementation individually, responses may be impacted by interactions of individual AA and FA supply. Therefore, the main objective of this thesis was to investigate the interaction between AA (methionine and lysine) and FA (C16:0 and *cis*-9 C18:1), and their effects on the yields of milk fat and protein.

CHAPTER 2

LITERATURE REVIEW

Importance of Milk Components

When establishing milk prices, the Federal Milk Order program uses milk fat and protein yield as major price indicators. Therefore, milk component yields greatly influence dairy farm income. Feeding fat improves the yield of milk and milk fat, and different FA ratios have variable impacts on digestibility and production in mid-lactation dairy cattle (de Souza et al., 2018a; Western et al., 2020a; Western et al., 2020b). However, fat supplementation typically does not improve milk protein yield (Rabiee et al., 2012). Different (AA) supplementation strategies have demonstrated an ability to increase milk protein yields in relationship to energy supply (Vyas and Erdman, 2009; Arriola Apelo et al., 2014; Doepel and Lapierre, 2010). Therefore, it is important to investigate the relationship and interaction between protein and fat supplementation to increase yields of milk fat and protein and improve farm profitability.

Nutrient Composition of Feed Ingredients

Ingredients commonly fed to lactating dairy cattle include forages, grains, byproducts, FA supplements, and AA supplements. Inclusion rates of feed ingredients are dependent on production goals and what is available due to cropping seasons, geographic location, and purchased feed prices. Other than simply meeting nutrient requirements for maintenance and milk production, different nutrition strategies can be employed to increase feed efficiency and increase production of milk fat and milk protein, which is often done through the inclusion of FA and AA supplements.

Forage and Nonforage Fiber

Dairy cattle require enough effective fiber for optimal rumen health. During periods where forage availability is limited, nonforage fiber sources such as whole cottonseed, wheat middlings, distillers grains, soybean hulls, and beet pulp can be used to supplement fiber in the place of conventional forage fiber. Rumination increases the secretion of buffers through saliva and fermentation of organic matter (Bailey and Balch, 1961), which helps maintain a healthy ruminal pH (Allen, 1997). Although nonforage NDF can support rumen fermentation, NDF from most nonforage fiber sources does not stimulate chewing activity as effectively as forage NDF (Clark and Armentano, 1997; Grant, 1997; Pereira et al., 1999). Byproducts generally are more dense and have a smaller particle size than forages, which can also lead to increased passage rates from the rumen (Bhatti and Firkins, 1995), decreased NDF digestion in the rumen and increase digestion of fiber in the hindgut (Firkins, 1997).

Fat

Dietary FA supplied by forages, grains, and byproducts fed to lactating dairy cattle are mostly esterified FA (triglycerides, glycolipids, or phospholipids). Corn, grass, and legume forages contain approximately 2-3% total FA as a percent of DM (Drackley, 2004). Most grains contain 1-4% total FA as a percent of DM, while whole cottonseed, whole canola seed, and whole soybeans range from 15-20% (NRC, 2001). FA supplements are high in total FA, with prilled FA containing approximately 95% total FA and Ca-salts containing 80-85% total FA on a DM basis. FA supplements are often used to increase the total FA content of the diet and supply specific FA to the cow.

Most feedstuffs, including byproduct feeds, contain high levels of unsaturated FA (UFA), with corn silage and grains consist of linoleic acid (*cis*-9, *cis*-12 C18:2), while grass and legume forages contain high levels of linolenic acid (*cis*-9, *cis*-12, *cis*-15 C18:3). In forages, glycolipids are the predominant form of FA, which contain two FA tails and one or two sugars linked to a glycerol backbone. In cereal grains and oilseeds, triglycerides are the predominant form of FA, which are composed of three medium- to long-chain FA (LCFA) linked to a glycerol backbone (Khan et al., 2012). FA supplements are abundant in palmitic (C16:0), stearic (C18:0), and oleic (*cis*-9 C18:1) acids, but relative content of each FA is dependent on the product. The FA profile and total FA content of common feedstuffs are present in Table 2.1.

Protein

Ruminal degradation of feed protein is affected by microbial proteolytic activity, rumen pH, and rumen retention time of protein (NRC, 2001). Microbial protein synthesis in the rumen depends on the availability of carbohydrates and nitrogen (Hoover and Stokes, 1991; Clark et al., 1992; Dewhurst et al., 2000). Chikunya et al. (1996) observed that microbial growth was only enhanced when peptides were supplied with rapidly degraded fiber. Rumen pH, frequency of feeding, rate of passage from the rumen, and particle size can affect rate of protein degradation (Lindberg, 1985; Michalet-Doreau and Ould-Bah, 1992; Nocek and Russell, 1988).

Feeds of plant origin contain all globular proteins but at different ratios. Globular proteins include Albumins, globulins, glutelins, prolamines, and histones. Cereal grains and by-product feeds derived from cereal grains contain more prolamines and glutelins, whereas leaves and stems are rich in albumins (Blethen et al., 1990; Sniffen, 1974; Van Soest, 1994). Classic protein fractions (albumins, globulins, prolamines, and glutelins) and nonprotein nitrogen account for 65% of total nitrogen in common feedstuffs (Blethen et al., 1990). The other 35% is made up of

insoluble N, including protein bound in intact aleurone granules of cereal grains, cell-wall associated proteins, and some of the chloroplasmic and heat-denatured proteins associated with NDF (Van Soest, 1994). Forages, beet pulp, soy hulls, sorghum, dried breweres grains, dried distillers grains, and meat and bone meal are feedstuffs with the highest percentage of insoluble protein (Blethen et al., 1990).

Common feedstuffs have variable contents of nonprotein nitrogen (NPN) compounds. This includes peptides, free AA, amides, amines, ammonia, and nucleic acids (NRC, 2001). Grasses and legume forages contain the highest and most variable concentrations of nonprotein nitrogen. Silages contain higher amounts of NPN than the same feed when fresh because of the action of fermentation on proteolysis, which occurs as a result of microbial and plant proteases and peptidases (NRC, 2001).

Most feedstuffs commonly fed to dairy cattle have lower amounts of methionine and lysine (especially lysine) than what is required for lactation (NRC, 2001). As a percent of total essential AA, the concentration of methionine in forage and most byproducts (except corn grain, corn gluten meal, and corn silage) is lower than the methionine requirements of lean tissue and milk (NRC, 2001). Similarly, the concentration of lysine in forage and byproducts (except some animal protein products) is lower than the lysine requirements of lean tissue and milk (NRC, 2001). The AA profile and total AA content of common feedstuffs are present in Table 2.2.

Fatty Acid Digestion and Metabolism

Rumen Metabolism of Dietary Fatty Acids

Although most of the FA in common feedstuffs are comprised of UFA, the main FA reaching the small intestine for absorption are saturated FA due to biohydrogenation in the rumen (Harfoot and Hazlewood, 1997). The two main processes in the metabolism of dietary FA are lipolysis and biohydrogenation (Figure 2.1; Buccioni et al., 2012). Lipolysis is the first step of FA digestion, where microbial lipases hydrolyzing the ester linkages in lipids to release and expose FA for further metabolism by ruminal microbes (Jenkins et al., 2008). Rumen biohydrogenation of FA influences rumen microbial populations and the FA profile that is later absorbed and utilized throughout the body (Doreau and Ferlay, 1994).

Rumen protozoa and cellulolytic bacteria are most affected by dietary FA (Hino and Nagatake, 1993). The FA concentration and profile entering the rumen can impact rumen fermentation by altering the digestibility of nutrients, sites of digestion, and microbial cell synthesis (Jenkins and Palmquist, 1984). In contrast to SFA which are considered to be mostly rumen-inert (Grummer, 1988), UFA are toxic to rumen bacteria and can alter fermentation (Jenkins, 1993). UFA that are not rumen-protected undergo biohydrogenation, which is the reduction of double bonds on the FA carbon chain (Buccioni et al., 2012). UFA are toxic to rumen microbes, and therefore undergo biohydrogenation as a protective measure. However, certain species are more susceptible to UFA toxicity than others, and increasing supply of UFA to the rumen increases biohydrogenation and can alter the rumen environment (Maia et al., 2010). Certain factors including changes in rumen pH, UFA content, and fermentability of the diet can alter fermentation pathways, which produce intermediates, such as conjugated linoleic acid (CLA), that can cause milk fat depression (Bauman et al., 2011). Predominant

biohydrogenation pathways and intermediates produced are shown in Figure 2.2.

Although UFA are mostly biohydrogenated in the rumen, some biohydrogenation intermediates and dietary UFA escape the rumen and are available for absorption in the small intestine due to the continual passage of digesta from the rumen. FA supplements are often rumen-protected in an effort to reduce the amount of biohydrogenation of UFA. Since SFA are rumen inert, they pass through the rumen unaltered and are available for absorption in the small intestine.

Effects of Fatty Acid Supplements on Nutrient Digestibility

Fiber digestibility can be altered by fat supplementation, and different responses have been observed dependent on FA profile of the supplement. UFA have been proposed to disrupt rumen metabolism because of the double bonds in their structure, and the effects increase with increased unsaturation of FA (Maia et al., 2010). Increasing diet fermentability is negatively related to milk fat in a meta-analysis by Ferraretto et al., (2013). This could be due to the fermentation of starches lowering rumen pH, altering BH and shifting pathways to the route that produce MFD intermediates. In contrast, inclusion of SFA and calcium salts of LCFA may increase NDF digestibility (Weld and Armentano, 2017). De Souza et al. (2018a) observed increased NDF digestibility with a blend of C16:0 and C18:1 compared with supplementation of mostly C18:0 or a non-FA supplemented control diet. Most of the increases in NDF digestibility with C18:1 have been associated with a decrease in DMI. However, multiple studies have reported increases in NDF digestibility with C16:0 supplementation (Piantoni et al., 2013; de Souza et al., 2018b; Western et al., 2020a), even when there is no decrease in DMI. Piantoni et al. (2013) suggested an increase in NDF digestibility may be associated with an increase in rumen retention time driven by an increase in cholecystokinin (CCK) secretion in response to

C16:0 absorption in the small intestine. Another possible explanation is the action of C16:0 in the rumen. Typically, Butyrivibrio bacteria synthesize C16:0 de novo to produce phosphatidic acid, which is used as a precursor for the FA components in their membranes (Hackmann and Firkins, 2015). However, if dietary C16:0 can be incorporated into rumen bacteria membranes, the bacteria would not have to synthesize C16:0, and considerable ATP would be spared from this energy-demanding process that could be used to support bacterial growth (Vlaeminck et al., 2006). Vargas-Bello-Perez et al. (2016) demonstrated that hydrogenated palm oil (47% C16:0 and 43% C18:0) increased total bacteria, measured as copies of 16S ribosomal DNA, in the rumen compared with soybean oil and a non-FA supplemented control. Therefore, C16:0 supplementation can possibly increase NDF digestibility by supporting bacterial growth in the rumen.

Digestion and Absorption of Dietary Fatty Acids

Fat supplements impact FA digestibility depending on the profile and total supply of different FA. The digestibility of FA decrease as fat content of diets increase (Piantoni et al., 2015; Rico et al., 2017), suggesting that absorption of FA in the small intestine may be limited when the supply of FA increases (Bauchart, 1993). Individual FA have different intestinal digestibilities, with higher digestibility for UFA than SFA. Boerman et al., (2015a) observed FA digestibility decreased linearly as the flow of C18:0 through the duodenum increased. In contrast, the amount of C16:0 reaching the duodenum had positive effects on total FA digestibility. Overall, supplemental C16:0 and C18:0 have been observed to decrease total FA digestibility, with more pronounced effects with C18:0 (Boerman et al., 2017; Rico et al., 2017). As mentioned, UFA have been found to increase total FA digestibility, with *cis*-9 C18:1 having the most pronounced impacts (Boerman et al., 2015a; de Souza et al., 2018a; de Souza et al.,

2019). The amphiphilic properties of *cis*-9 C18:1 (Freeman, 1969) could contribute to the increase in total FA digestibility observed in these studies.

Due to the high concentration of UFA in most feedstuffs, and biohydrogenation of UFA, C18:0 is the predominant FA available for absorption by the dairy cow. The FA that leave the rumen comprise of mainly free FA attached to feed particles and microbial phospholipids (Doreau and Ferlay, 1994). Most absorption of FA takes place in the jejunum of the small intestine, and the low pH (< 2.5) in the ruminant small intestine keeps the FA in a protonated state (Drackley, 2004). However, FA must be solubilized into the aqueous environment to be absorbed. Bile salts and lecithin from bile and pancreatic secretions (Bauman and Lock, 2006) aid in the formation of micelles and are essential for the absorption of FA (Doreau and Ferlay, 1994). Pancreatic secretions provide enzymes to convert lecithin to lysolecithin, allowing for bile salts and lysolecithin to dissociate FA from feed particles (Lock et al., 2005). Lysolecithin acts as an amphiphile in ruminants, which aids the formation of micelles for FA absorption, and is the most effective amphiphile at increasing the solubility of C18:0 (Freeman, 1969). Micelles consist of water-insoluble lipids surrounded by bile salts and phospholipids that transport lipids across the intestinal epithelial cells of the small intestines (Lock et al., 2006), and formation is a requirement for FA absorption to occur (Moore and Christie, 1984). F at digestion in the small intestine of ruminants is shown in Figure 2.3. After absorption, the FA are re-esterified into triglycerides in the endoplasmic reticulum of the enterocyte, then incorporated back into triglycerides and combined into lipoprotein particles for transport in the blood (Drackley, 2004; Cifarelli and Abumrad, 2018).

Milk Fat Synthesis in the Mammary Gland

Milk fat is a major component in milk and its synthesis constitutes a major energy expense to the cow (Emery, 1973). It is the most variable component in milk, and milk fat yield can be readily manipulated through nutrition (Bauman and Griinari, 2003). Bovine milk fat concentrations typically range from 3 to 5%, and is mostly composed of triglycerides (98%), with the remaining being phospholipids, diglycerides, and cholesterol (Jensen, 2002). In addition to the overall yield of milk fat, the FA concentrations found in milk can be affected by physiological state, stage of lactation, and nutrition (Palmquist, 2006). The FA found in milk fat can exceed 400 different FA (Jensen, 2002), which are broken down into three main categories; de novo, mixed, and preformed. De novo synthesis of milk FA occurs in the mammary gland and produces short-chained FA, while preformed are LCFA extracted from plasma and incorporated into milk fat (Palmquist, 2006). Mixed source FA (C16:0 and *cis*-9 C16:1) are derived from both extraction from plasma and de novo synthesis in the mammary gland.

De Novo Fatty Acid Synthesis

Short- and medium-chain FA in the mammary gland are produced through de novo synthesis in the mammary gland. In ruminants, de novo synthesis typically accounts for approximately half of the FA in milk (Bauman and Griinari, 2003). Carbon sources, mainly acetate and beta-hydroxbutyrate, and reducing equivalents in the form of NADPH (sourced from glucose and acetate) are required for de novo milk FA synthesis (Palmquist, 2006). C4:0 is produced in the mammary gland by condensation of acetyl units or reduction of beta-hydroxy butyrate in a malonyl-CoA-independent pathway, while the other de novo FA are synthesized mainly from acetyl-CoA in the malonyl-CoA pathway (Palmquist et al., 1993). Acetate is produced in the rumen during fermentation, which is then activated in the cytosol resulting in

acetyl-CoA, the basic starting substrate for FA synthesis (Bauman and Davis, 1974; Bauman and Griinari, 2003). Beta-hydroxybutyrate is used for a carbon source at a lesser extent than acetate (Bauman and Griinari, 2003). Glucose is also required for FA synthesis, and in ruminants gluconeogenesis is used to make most of the available glucose in the mammary gland. In adipose tissue and the mammary gland, glucose is used in combination with acetate to initiate lipogenesis (Laliotis et al., 2010). Most of the reducing equivalents originate from glucose oxidation via the pentose phosphate pathway or acetate via the isocitrate pathway, and the demand for these reducing equivalents can be decreased due to a reduction in de novo FA synthesis in high-fat diets (Emery et al., 1973; Palmquist, 2006), consequently sparing glucose for mammary gland utilization and lactose synthesis (Cant et al., 1993).

Preformed Fatty Acid

Most preformed FA for milk fat synthesis are sourced from absorption of dietary FA. LCFA comprise about 50% of milk fat, with more than 90% of these FA being of plasma origin, demonstrating that minimal FA elongation occurs in the mammary gland (Glascock et al., 1966; Palmquist et al., 1969). The TAG contained within chylomicrons and VLDL in plasma are the primary source of FA taken up by the mammary gland (Palmquist 2006), whereas NEFA from body fat mobilization account for a small percentage of these FA (Bauman and Griinari, 2003). Most of the FA taken up by the mammary gland are directly related to fat absorption, with 76% of intestinal lipoprotein TAG taken up by lactating mammary glands (Palmquist et al., 2006). C16:0 in milk can come from preformed sources or de novo synthesis, and is influenced directly by the amount of FA uptake from blood TAG (Palmquist et al., 2006).

Triglyceride Synthesis

Triglyceride synthesis incorporates both de novo and preformed FA onto a glycerol-3 phosphate backbone, which primarily occurs through the *sn*-glycerol 3 phosphate pathway (Dils, 1983). Fatty-acyl CoA are added to the glycerol-3 phosphate backbone by glycerol phosphate acyl transferase (GPAT), acyl glycerol phosphate acyl transferase (AGPAT), and diglyceride acyl transferase (DGAT), at the sn-1, sn-2, and sn-3 positions, respectively (Palmquist, 2006). Individual specificity of FA dictates their location of the FA on the glycerol backbone, and the specific structure of TAG allows for secretion into lipid droplets and incorporation into milk (Jensen, 2002). LCFA are mostly esterified at sn-1, medium-chain FA at sn-2, and short-chain FA at sn-3 (Jensen, 2002). Although very little (<10%) FA are elongated in the mammary gland (Glascock et al., 1966; Palmquist et al., 1969), the mammary gland commonly desaturates FA by adding double bonds to regulate melting point of TAG and milk fluidity (Dils, 1983). Ruminant mammary gland microorganisms most commonly desaturate C18:0 to *cis*-9 C18:1 (Dils, 1983).

Effects of Fatty Acid Supplementation

Effects on DMI

Fat supplements have various effects on DMI depending on the type, degree of saturation, and FA profile of the fat being fed (Rabiee et al., 2012). Allen (2000) reported that saturated fats do not affect DMI, while Ca-salts of palm FA and unprocessed animal fats decrease DMI. It was hypothesized that fat supplementation impact DMI through its effect on rumen fermentation and fiber digestion (Allen, 2000). SFA are rumen-inert, while UFA can have negative effects on rumen microbial populations and alter fermentation (Maia et al., 2007;

Palmquist and Jenkins, 1980). Also, FA absorption in the small intestine increases plasma CCK and GLP-1 concentrations (Relling and Reynolds, 2007) which can influence feed intake (Choi et al., 2000). The hypophagic effect of FA supplementation increases as the degree of unsaturation increases (Relling and Reynolds, 2007). Furthermore, the same FA supplements can have different impacts on DMI across multiple studies, which could be due to different inclusion rates (Rabiee et al., 2012), basal diets (de Souza et al., 2018a; Burch, thesis), or cows of different production level (Western et al., 2020b).

Effects on Energy Partitioning

Insulin plays an important role in energy partitioning. Various insulin responses to fat supplementation have been observed, with production level, stage of lactation, and FA profile being influential. In multiparous post-peak dairy cows, C16:0 supplementation increased plasma insulin concentrations when compared with a non-FA supplemented control (Piantoni et al., 2013), while in other studies it had no effect on insulin (Western et al., 2020b; de Souza et al., 2018a). de Souza et al. (2016) observed a tendency for an increase in plasma insulin response when C16:0 was supplemented as a replacement for soyhulls but not when supplemented as a replacement for dry ground corn, suggesting an interaction between basal diet composition and C16:0 supplementation.

C16:0 caused mitochondrial dysfunction resulting in palmitate-induced insulin resistance in muscle cells of in vitro and non-ruminant animal models (Yuzefovyck et al., 2010). However, *cis*-9 C18:1 improved palmitate-induced mitochondrial dysfunction and prevented palmitate-induced insulin resistance (Yuzefovyck et al., 2010). In humans, *cis*-9 C18:1 increases insulin sensitivity by inhibiting endoplasmic reticulum stress, therefore preventing attenuation of the insulin signaling pathway, and improving beta cell survival (Palomer et al., 2018). Previous

studies observed that *cis*-9 C18:1 supplementation increased plasma insulin concentration compared with non-FA supplemented controls and other FA supplements (de Souza et al., 2018a; de Souza et al., 2019). de Souza et al. (2019) reported an interaction between FA treatments and production level for plasma insulin, where increasing dietary *cis*-9 C18:1 in FA treatments linearly increased plasma insulin in low producing cows while quadratically affecting insulin in high producing cows.

Insulin responses have variable impacts on milk fat production. Previously, insulin was hypothesized to cause milk fat depression in lactating dairy cows (McClymont, 1951; Van Soest, 1963). The theory was based on the action of insulin on adipose tissue, where it stimulates rates of lipid synthesis and inhibits rates of lipolysis (Bauman and Elliot, 1983; Bell et al., 1987). However, in mammary tissue, insulin has no effect on the uptake of de novo milk FA metabolites or their rate of incorporation into milk fat (Annison, 1976; Bauman et al., 1973; Laarveld et al., 1985). Therefore, the glucogenic-insulin theory postulated that the tissue specific action of insulin results in preferential channeling of nutrients to adipose tissue, and that mammary tissue will receive inadequate supply of precursors for mammary synthesis of milk fat. Insulin is known to decrease lipolysis in adipose tissue, which would reduce circulating plasma FA concentrations by partitioning circulating FA in triglycerides into adipose tissue instead of uptake by the mammary gland (Vernon, 2005). In contrast to this theory, recent research has shown no impact or increases in milk fat yield with elevated plasma insulin concentrations (McGuire et al., 1995; Corl et al., 2006; Boerman et al., 2015b). Cows receiving injections of long-acting insulin for 10 d did not reduce milk fat yield, but changed the FA profile of milk, increasing de novo milk FA and reducing preformed milk FA (Winkelman and Overton, 2013). Insulin stimulates the activation of acetyl CoA carboxylate (Witters et al., 1988), which shares control of medium- and

short-chain FA synthesis in bovine mammary tissue (Wright et al., 2006; Bionaz and Loor, 2008). Therefore, insulin may stimulate de novo FA synthesis, subsequently increasing milk fat production through this mechanism.

Effects on Mammary Synthesis of Milk Components

FA supplementation typically does not impact milk protein yields, and reduces milk protein content due to a dilution effect (Rabiee et al., 2012). Wu and Huber (1994) speculated that fat supplementation negatively effects protein percentage due to a decrease in glucose availability, reduced plasma somatotropin, or development of insulin resistance. Cant et al., (1993) found evidence of a decrease in AA supply to the mammary gland when fats were fed. They hypothesized that energy provided by dietary fat improve energy availability to mammary cells, decreasing the production and release of adenosine. As a result, vasodilation closes precapillary sphincters and reduces mammary blood flow, therefore reducing availability of AA to the mammary gland. Consequently, AA uptake cannot increase with dietary fat supplementation to the same extent as energy uptake and milk yield, resulting in no effect on milk protein yield. However, this is based on the assumption that mammary blood flow functions independently of AA availability, and has an upper limit influenced by energy metabolism in the mammary gland (Cant et al., 1993). In contradiction to this theory, some studies have observed an increase in milk protein yield with supplemental C16:0 (de Souza et al., 2019; Western et al., 2020b). Nichols et al. (2018b) found no significant differences in AA uptake by the mammary gland with supplemental fat. UFA may also impact protein supply to the mammary gland by altering rumen function. Rabiee et al., (2012) reported that calcium salts had a significant negative effect on milk protein, while prilled SFA had minimal impacts. UFA are toxic to rumen microbes, and can inhibit their growth (Palmquist and Jenkins, 1980). Differences in FA could

affect microbial protein production (Harvatine and Allen, 2005), ultimately altering AA supply to the mammary gland. Although FA supplementation can impact the yield of milk components, it most directly influences milk fat.

A recent meta-analysis reported increases in milk yield and a tendency to increase milk fat yield with FA supplementation, with different responses to different types of FA supplements (Ca-salts of palm or prilled FA supplements; Rabiee et al., 2012). However, results were not separated out by FA profile. Production responses to individual FA and different FA profiles has been a subject of recent interest. As degree of unsaturation of FA supplements increase, decreases in milk yield and milk fat concentration and yield have been observed (Harvatine and Allen, 2006; Relling and Reynolds, 2007). Reduction in milk fat synthesis in the mammary gland with UFA is due to changes in biohydrogenation pathways increasing specific CLA that can have direct effects in the mammary gland and decrease de novo FA synthesis (Bauman et al., 2011).

Amino Acid Digestion and Metabolism

Amino Acid Digestion and Metabolism in Ruminants

An overview of ruminant digestion and metabolism of dietary protein is shown in Figure 2.4. Dietary proteins fed to dairy cattle are typically categorized as either rumen degradable protein (RDP) or rumen undegradable protein (RUP), which together make up the postruminal supply of AA absorbed in the small intestine. Postruminally digested protein and AA absorbed by the intestine make up metabolizable protein (MP) supplied to the cow. AA, and not protein, are the required nutrients for dairy cattle (NRC, 2001). RDP provides a mixture of free AA, peptides, and ammonia for microbial growth and synthesis of microbial protein (NRC, 2001). RDP is made up of fermentable feed proteins plus endogenous proteins from saliva, lysed

ruminal microorganisms, and sloughed epithelial cells. Rumen microorganisms break down peptides to AA, then incorporate the AA into microbial protein (Wallace, 1996). Peptides and free AA released from degradation of true proteins in the rumen stimulate microbial protein synthesis (Russell et al., 1992). Rumen bacteria form a complex with feeds and perform extracellular proteolysis which produces oligopeptides which are degraded further into free AA and small peptides. The rumen bacteria cleave the small peptides to produce free AA, which can then be utilized for protein synthesis or deaminated into ammonia and carbon skeletons, and the ammonia can then be utilized for resynthesis of AA or diffused out of the bacterial cell (Broderick, 1998). Protozoa are less abundant in the rumen than bacteria, but because of their size, they make up a large portion of total microbial biomass in the rumen (Jouany, 1996; Jouany and Ushida, 1999). Protozoa ingest particulate matter (bacteria, fungi, and small feed particles) instead of forming a complex with it. They have higher specific proteolytic activity than that of bacteria (Nolan, 1993), and are therefore more active in degrading insoluble feed proteins such as soybean meal than soluble feed proteins from protein supplements (Hino and Russell, 1987; Jouany, 1996; Jouany and Ushida, 1999). Although protozoa can deaminate AA, they are not able to synthesize AA from ammonia, making them net exporters of ammonia (Jouany and Ushida, 1999). Protozoa also release large amount of AA, peptides, and peptidases into ruminal fluid (NRC, 2001). Ruminally synthesized microbial protein typically supplies most of the AA passing to the small intestine (Clark et al., 1992). In a literature review by Sok et al. (2017), total AA accounts for >80% of the protein in bacteria, and composition of half of the EAA differed between protozoa and bacteria, with bacteria having 42% lower lysine concentration than protozoa. Overall, microbial protein has an average EAA composition similar to that of milk and lean body tissue (Schwab and Broderick, 2017). Some of the peptides and AA not incorporated

into microbial protein may escape ruminal degradation to ammonia and become sources of absorbed AA (NRC, 2001). Protein that does not undergo rumen digestion makes up the RUP fraction of protein absorbed in the small intestine (NRC, 2001).

Rumen protected proteins and protein supplements are important in dairy cow rations because of the low content of digestible RUP in most feedstuffs. In diets containing high amounts of forages, the basal diet often contains adequate RDP but is deficient in RUP. Most protein supplements in North American are protected from rumen degradation mainly through heat treatment (NRC, 2001). Heat processing denatures proteins and forms Maillard reaction and protein-protein cross-links, decreasing rumen protein degradation (NRC, 2001). However, careful heating conditions are required to optimize the content of digestible RUP (Schwab, 1995). Damaging protein by overheating creates indigestible Maillard products and protein complexes, reducing the intestinal digestibility of RUP (Van Soest, 1994), and causes significant losses of arginine, cystine, and lysine (Parsons et al., 1992).

Absorbed AA from synthesized microbial protein, RUP, and endogenous protein are essential for the synthesis of milk and tissue proteins. Absorbed AA are also required as precursors for the synthesis of other body metabolites, and serve as precursors for gluconeogenesis, sources of metabolic energy when oxidized, and can be converted to FA (Lobley, 1992). There are ten AA that are considered essential in nutrition because they cannot be synthesized by animal tissues or if they can (His and Arg), they cannot be synthesized at a sufficient rate to meet requirements, especially for high levels of milk production (NRC, 2001). When essential AA are absorbed in the profile required by the animal, the requirement for total AA is reduced and the efficiency of use for protein synthesis is improved (Heger and Frydrych, 1989). AA, specifically methionine and lysine, are commonly fed in ruminally protected form to

supply a balanced profile of essential AA to dairy cows (NRC, 2001). Methionine and Lysine have been identified as being the most limiting AA in dairy cattle due to the concentration of these AA provided by modern TMR diets and the relatively high requirements of cows (NRC, 2001). Methionine was identified as the first-limiting AA for lactating cows when high forage diets were fed, smaller amounts of corn were fed, or when most of the supplemental RUP was provided by soybean products or animal derived proteins (Armentano et al., 1997; Rulquin and Delaby, 1997; Schingoethe et al., 1988). Recent research has brought to attention the role of other EAA, such as histidine, in AA supplementation and their impact on milk protein synthesis and gene expression (Lee et al., 2012; Vyas and Erdman, 2009; Arriola Apelo et al., 2014).

However, if protein supplementation exceeds the requirement of the dairy cow, efficiency of incorporation into milk protein decreases. Excess AA are catabolized and excreted as urea via ureagenesis, an energetically demanding process (Lapierre et al., 2002; Reed et al., 2017). Diets formulated with an increased EAA supply without oversupplying MP can increase postabsorptive nitrogen efficiency and support mammary gland extraction of AA (Haque et al., 2012; Arriola Apelo et al., 2014). Therefore, dietary strategies are often designed to supply a more "ideal" AA profile without oversupplying protein which may increase the efficiency of protein production in the mammary gland and decrease nitrogen excretion.

Nitrogen recycling is a unique metabolic process that occurs in ruminants. Many reviews have been published detailing specifics of nitrogen recycling mechanisms and implications (Lapierre and Lobley, 2001; Lapierre et al., 2005; Reynolds and Kristensen, 2008). In short, nitrogen in the form of urea, mainly from hepatic oxidation of AA, recycles back into the rumen via saliva and absorption across the rumen wall from the blood and is used for microbial protein synthesis (Houpt, 1959; Cocimano and Leng, 1967). The amount of recycled N used for

microbial protein synthesis is primarily determined by factors affecting the efficiency of microbial protein synthesis and microbial requirements (Reynolds and Kristensen, 2008).

Effects on Nutrient Digestibility

Since adequate protein levels are required to maintain proper rumen function, deficiencies of RDP in dairy cows can decrease total-tract digestibility of DM and NDF (Lee et al., 2012; Giallongo et al., 2016). When protein is supplied at or above requirement, there is little impact on the digestibility of other nutrients (Allen, 2000).

Digestion and Absorption of Dietary Amino Acids

Mixed microbial protein has a high apparent digestibility in dairy cattle (NRC, 2001). There is limited research on the intestinal digestibility of ruminally synthesized microbial protein in dairy cows. Two different studies used infusions of freeze-dried preparations of ruminal bacteria in the abomasums of sheep and determined an average digestibility of microbial AA to be 85% (Storm and Ørskov, 1983) and 87% (Tas et al., 1981). The NRC (2001) estimates an intestinal digestibility of 80% for RUP and rumen-protected AA supplements, with variability in digestibility due to RUP source and supplement. Generally, forages have lower RUP digestibility (65-75%), while byproducts and soybean meal have higher digestibility (80-90%). Bone, fish, and feather meal are considered low-quality protein sources due to their relatively low RUP digestibility (~65%) compared with other byproducts (NRC, 2001). Blood meal's protein quality is variable and can also have lower intestinal digestibility (Erasmus et al., 1994).

Effects of Dietary Amino Acid Supplementation

Effects on DMI

DMI was not influenced by duodenal infusions of AA or casein (Schwab et al., 1992) or by ruminally protected methionine and lysine (Donkin et al., 1989; Rogers et al., 1987). MP deficient diets have been observed to decrease DMI which was hypothesized to be a result of impaired rumen function and a decrease in fiber digestibility due to ammonia and RDP deficiency (Lee et al., 2011; 2012). Allen (2000) concluded that improved AA supply does not affect DMI in dairy cows if rumen function is maintained across treatments.

Effects on Mammary Synthesis of Milk Components

Ruminally protected methionine (Wang et al., 2010; Yang et al., 1986; Zanton et al., 2014) and methionine and lysine (Socha et al., 2005) have been observed to increase milk fat percentage and yield. In contrast, other studies did not observe an effect of supplemented methionine and lysine on milk fat concentration (Lee et al., 2012; Patton et al., 2015). The effect of methionine on milk fat may be influenced by basal protein level of the diet. Socha et al. (2005) observed that methionine supplementation increased milk fat content in a basal diet with 18.5% CP, but not in a basal diet with 16% CP.

Methionine may increase de novo synthesis of FA in the mammary gland (Pisulewski et al., 1996; Christensen et al., 1994). In contrast, other studies did not observe an effect of supplemented methionine and lysine on FA composition of milk (Casper et al., 1987; Chow et al., 1990; Rulquin and Delaby, 1997). In recent research, the increase in milk fat in response to AA supplementation has been mainly attributed to the effect of methionine on methyl group donors and choline synthesis (Pinotti et al., 2002). Apolipoproteins and phospholipids, along

with the presence of LCFA, are required for the synthesis of chylomicrons and very low density lipoproteins (Bauchart et al., 1996). The synthesis of phosphatidylcholine (lecithin) requires choline, and the synthesis of apolipoproteins requires AA. A portion of dairy cow requirements for methionine are due to its role as a methyl donor for choline synthesis (Sharma and Erdman, 1988), and choline has been suggested as a limiting nutrient for milk fat synthesis (Erdman, 1994).

Protein supplementation can increase milk lactose yield when AA increase hepatic gluconeogenesis, increasing whole-body appearance of glucose (Galindo et al., 2011). Previous studies report that increasing postruminal AA supply increased total milk yield, whole-body glucose appearance, and lactose yield (Clark et al., 1977; Lemosquet et al., 2009b; Galindo et al., 2011).

Methionine and lysine supplementation have variable results on milk protein content, but generally increase milk protein yield (Schwab and Broderick, 2017; Vyas and Erdman, 2009). These effects are due to increasing arterial supply of AA available to the mammary gland for milk protein synthesis. Mammary removal of individual EAA is impacted by physiological condition (Schwab et al., 1992), hormonal status (Mackle et al., 1999) and arterial concentration of EAA (Doepel et al., 2004), with an average fractional removal of 43% for EAA (Hanigan et al., 1992). There is slight variation in mammary affinity for individual EAA, with methionine and lysine having higher affinities than some other EAA (Arriola Apelo et al., 2014). Arterial supply of EAA can affect the fractional removal of individual EAA by the mammary gland (Bequette et al., 2000; Hanigan et al., 2002).

Regarding the efficiency of milk protein production, there are numerous metabolic interactions between energy and AA supply (Lobley, 2007). Previous studies observed that

increasing the supply of glucogenic nutrients improves post-absorptive transfer efficiency of AA from the gastrointestinal tract to the mammary gland, resulting in greater yields of milk and milk protein (Lemosquet et al., 2009a; Cantalapiedra-Hijar et al., 2015). Although AA supplementation typically does not impact plasma insulin concentrations (Doepel and Lapierre, 2010; Griinari et al., 1997), insulin influences the yield and concentration of milk protein across many studies (Winkelman and Overton, 2013; Mackle et al., 1999; Griinari et al., 1997). Mackle et al. (1999) utilized a hyperinsulinemic-euglycemic clamp with or without infusions of casein plus branched-chain AA, and observed that insulin by itself increased milk protein yields by 15%, and when combined with abomasal infusion of casein plus branched-chain AA, milk protein yield increased by 25%. However, infusion of casein plus branched-chain AA without the insulin clamp did not affect the concentration or yield of milk protein (Mackle et al., 1999). Griinari et al. (1997) also utilized abomasal infusions of casein and hyperinsulinemic-euglycemic clamps, and observed an increase in milk protein yield with insulin alone, and a greater milk protein response with casein infusions paired with the insulin clamp. Both studies saw the impact of insulin increasing milk protein yield did not alter the relative proportions of milk whey and casein, and insulin decreased concentrations of plasma urea nitrogen (Mackle et al., 1999; Griinari et al., 1997). Milk protein yield responses have not been observed when the insulin response is acute (Hove, 1978; Laarveld et al., 1985; Tesseraud et al., 1992), while a response is seen when the insulin response is chronic and euglycemia is maintained (Griinari et al., 1997; Mackle et al., 1999). This highlights the dependency of milk protein responses on both AA supply and physiological signals, with insulin strongly influencing mammary synthesis of milk protein. The mechanisms by which insulin impact milk protein synthesis are not fully understood, but it is hypothesized that it could be a consequence of changes in the IGF system

(McGuire et al., 1995), the effects of insulin on the activation cascade for milk protein synthesis (Winkelman and Overton, 2013; Arriola Apelo et al., 2014), or insulin increasing the number of ribosomes in mammary tissue for milk protein synthesis (Bolster et al., 2004; Proud, 2006; Mahoney et al., 2009).

Fatty Acid and Amino Acid Interactions

FA supplements increase the yields of milk and milk fat, but typically have a neutral or negative impact on milk protein yields (Rabiee et al., 2012). Rumen-protected protein sources are fed to help increase AA availability for absorption and mammary gland utilization, but if there is not enough energy for milk synthesis supplied by other nutrients, the AA may be oxidized as a source of energy (Bequette et al., 2002; Lapierre et al., 2006). Therefore, the supply of non-AA energy precursors may optimize AA for milk protein synthesis (Raggio et al., 2006; Rius et al., 2010a). In fact, energy intake is directly linked to milk protein production (Doepel et al., 2004). Previous studies have examined infusions of propionate, starch, or glucose with or without AA or casein infusions (Lemosquet et al., 2009a; Rius et al., 2010b; Nichols et al., 2016), but there is a paucity of research on the interaction of specific FA and AA on milk component production.

Recent research (Nichols et al., 2018a,b; 2019) investigated the effects of fat and protein supplementation with different sources of energy (aminogenic vs lipogenic or glucogenic vs lipogenic). Neither study observed any interactions between fat and protein on milk production or composition. Nichols et al. (2018) observed a tendency for an interaction between protein and fat supplementation on milk nitrogen efficiency, where fat increased milk nitrogen efficiency

more at a low protein level than at a high protein level. However, the diets in these studies were designed to be isoenergetic with restricted DMI, which may influence production results differently than if these treatments were fed ad libitum. Also, the fat supplements used in these trials contained high levels of C18:0 and C18:1, which at similar inclusion rates can have negative effects on rumen fermentation, DMI, and milk production (Allen, 2000; Coppock et al., 1991; de Souza et al., 2018a).

Inclusion of different ratios of FA, especially C16:0, C18:0, and *cis*-9 C18:1 have variable impacts on nutrient digestibility, energy partitioning, and milk production due to inclusion rate, production level of the cow, stage of lactation, or other dietary nutrients. Supplementing C16:0 and *cis*-9 C18:1 increases the yield of milk and ECM (Rico et al., 2014; Western et al., 2020b; de Souza et al., 2018a). Rico et al. (2014) supplemented highly enriched C18:0 and C16:0 and found that C16:0 supplementation increased the yields of ECM and milk fat compared with C18:0. Similarly, Western et al. (2020a) found that a FA supplement containing 80% C16:0 tended to increase yields of ECM compared with a FA treatment of 30% C16:0 and 50% C18:0. Additionally, de Souza et al. (2019) altered the ratio of C16:0 and *cis*-9 C18:1 in FA blends and observed that increasing the amount of *cis*-9 C18:1 increased ECM and milk yield in high producing cows, while C16:0 increased these variable in low producing cows. While FA supplementation often increases yields of milk and milk fat, typically FA supplementation does not increase milk protein yield (Rabiee et al., 2012). Interestingly, increases in milk protein yield were observed with C16:0 supplementation compared with a nonfat control and other FA supplements in studies where the basal diet contained high quality blood meal (de Souza et al., 2019; Western et al., 2020a). Importantly, C16:0 and *cis*-9 C18:1 supplementation has been observed to impact plasma insulin concentrations in various studies

(de Souza et al., 2018a; Piantoni et al., 2013) which influences milk protein synthesis (Arriola Apelo et al., 2014).

Conclusions

The metabolic flexibility of the mammary gland allows for variable responses to dietary supplementation and nutritional strategies, which support milk production under periods of nutrient deficiency, but also creates complexity in manipulating the production of milk fat and milk protein. Although considerable research has examined production responses to protein and fat, responses may be impacted by individual AA and FA supply. Recent research has highlighted the importance of examining the supply of different protein and fat supplements, but a paucity of studies have investigated the interactions between the two.

Therefore, our objective was to investigate the interaction between AA (methionine and lysine) and FA (C16:0 and *cis*-9 C18:1), and their effects on the yields of milk fat and protein. Exploring the relationships between specific ratios of FA and AA will advance our understanding of milk component production responses and allow for more informed decision making for dairy farmers and nutritionists.

APPENDIX

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

Feedstuff	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
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

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

Table 2.2. AA profile and FA % DM of common feed ingredients.

Feedstuff	Arg, % AA	His, % AA	Ile, % AA	Leu, % AA	Lys, % AA	Met, % AA	Phe, % AA	Thr, % AA	Val, % AA	AA, % DM
Alfalfa Hay	0.72	0.31	0.80	1.27	0.90	0.27	0.86	0.73	0.97	15.4
Alfalfa Silage	0.34	0.21	0.89	1.37	0.53	0.28	0.75	0.53	1.06	15.0
Beet Pulp	0.25	0.21	0.31	0.47	0.38	0.12	0.28	0.33	0.45	6.47
Corn Gluten Feed	0.93	0.59	0.70	1.64	0.52	0.32	0.73	0.73	1.06	18.0
Corn Silage	0.14	0.11	0.27	0.59	0.22	0.10	0.26	0.24	0.35	5.69
Cottonseed	2.72	0.70	0.85	1.43	1.12	0.38	1.35	0.77	1.14	23.0
Ground Corn	0.34	0.23	0.30	0.95	0.27	0.16	0.39	0.29	0.39	8.02
High Moisture Corn	0.10	0.12	0.28	0.91	0.19	0.15	0.34	0.23	0.39	7.18
Soybean Meal	3.83	1.39	2.59	4.07	3.39	0.72	2.74	1.99	2.70	52.3
Soyhulls	0.57	0.31	0.48	0.80	0.81	0.14	0.46	0.42	0.56	11.2

[1]Compilation of data from Lock Laboratory and Agricultural Experiment Station Chemical Laboratory.

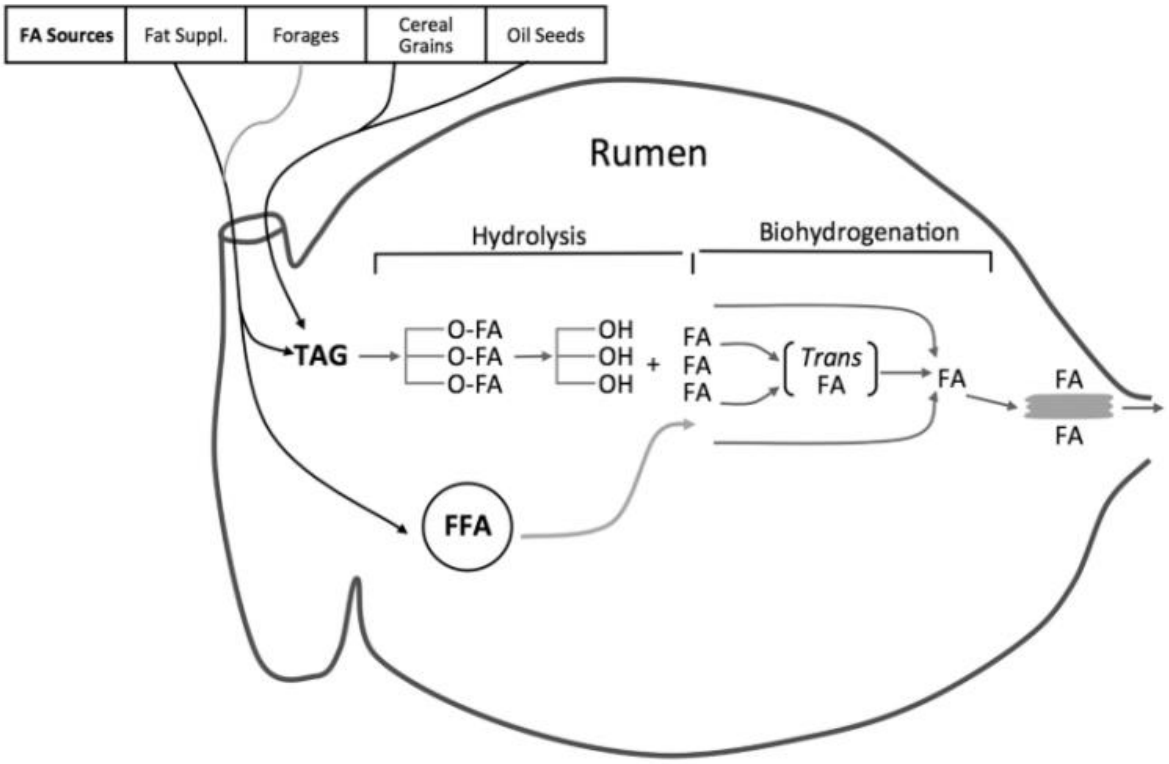

Figure 2.1. Metabolism of dietary lipids in the rumen. Triglycerides (TG), glycolipids (GL), phospholipids (PL), trans fatty acids (trans FA), mixture of fatty acids (FA), and volatile fatty acids (VFA). Adapted from Lock et al., 2006.

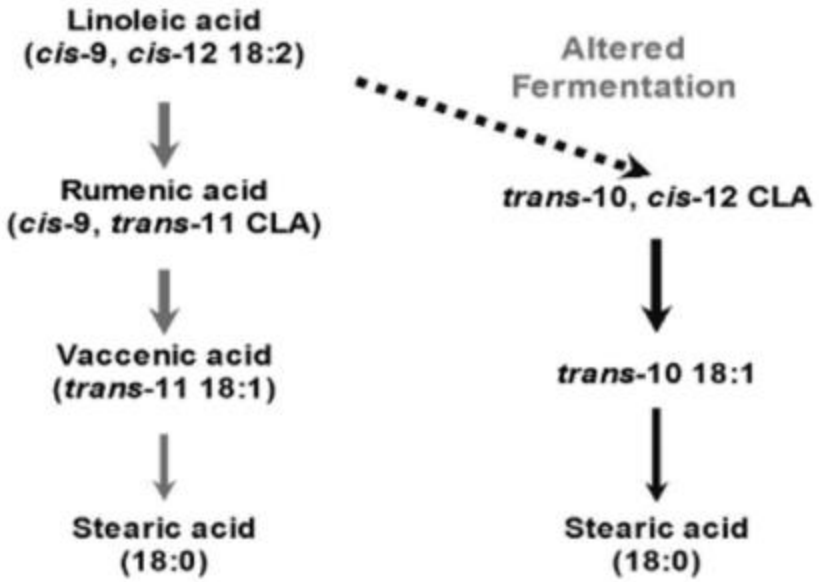

Figure 2.2. Biohydrogenation pathways of dietary lipids in the rumen. Adapted from Bauman et al., 2003.

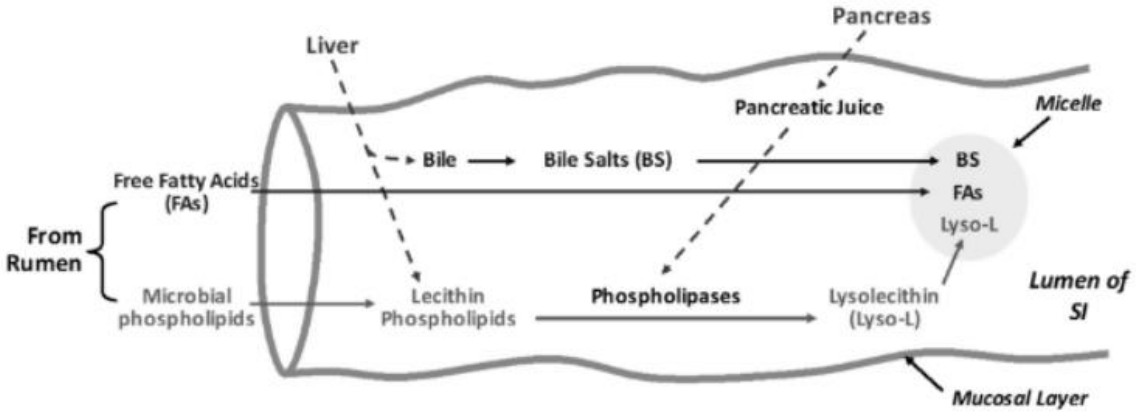

Figure 2.3. Fat digestion in the small intestine of ruminants. Adapted from Lock et al., 2006.

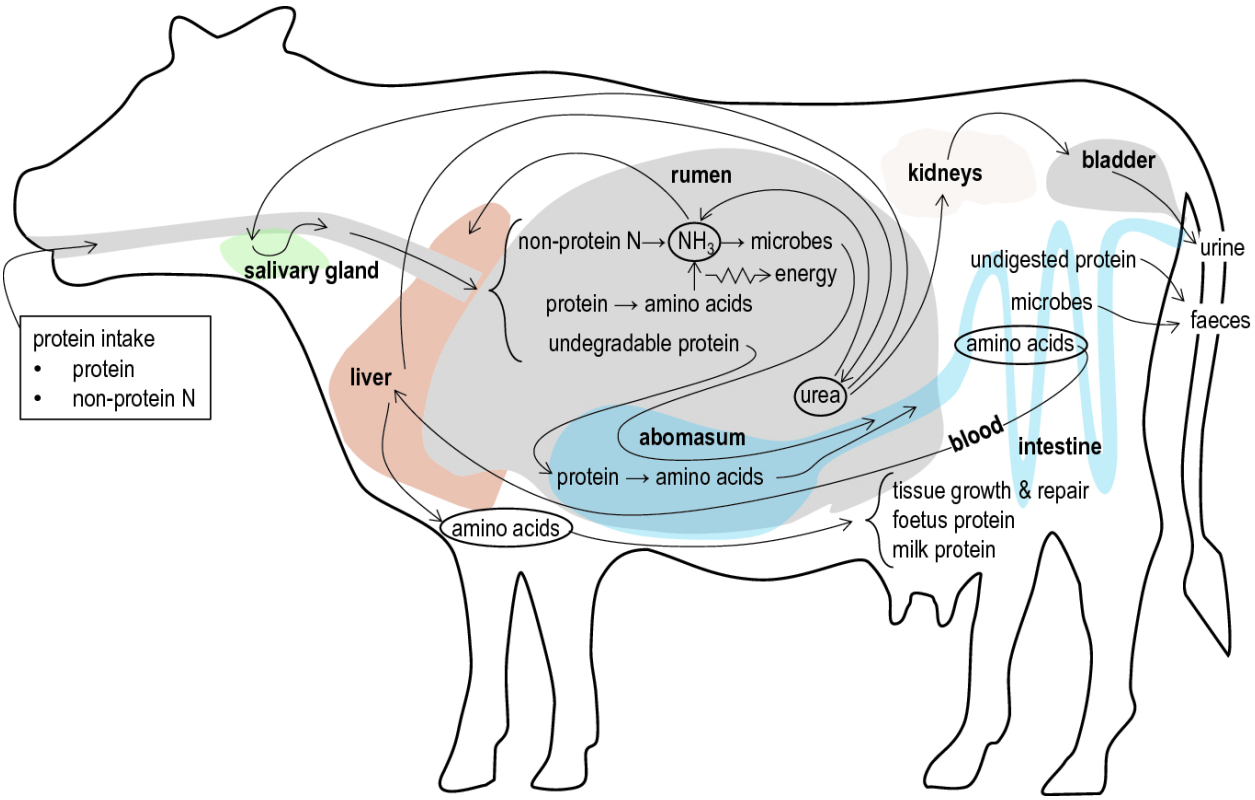

Figure 2.4. Metabolism of dietary protein in dairy cattle. Adapted from Pérez-Barbería F. (2020) The Ruminant: Life History and Digestive Physiology of a Symbiotic Animal.

CHAPTER 3

A low forage diets increased the yields of milk fat and protein of mid lactation dairy cows compared with typical midwestern forage inclusion diet

Abstract

Examining the effect of lower forage diets and alternative fiber sources in lactating dairy cow diets is important since forage quality and inventory can vary greatly depending on growing conditions, years, and locations. Therefore, we determined the effect of feeding diets similar in neutral detergent fiber (NDF), starch, and crude protein (CP) with differing amounts of forage on the yields of milk and milk components of mid-lactation dairy cows. Thirty-two Holstein cows (132 ± 68 DIM) were used in a crossover design with two consecutive 28 d periods, with sample and data collection during the final 5 d of each period. Treatment diets were: 1) control diet (CON) containing high forage (55.5% diet DM; forage NDF 19.2% diet DM) and no supplemental fatty acids or supplemental amino acids; and 2) low forage diet (LF) containing low forage (36.6% diet DM; forage NDF 12.7% diet DM), including supplemental fat (1.5% diet DM; 82% C16:0-enriched supplement) and rumen-protected methionine and lysine. Diets were balanced for similar NDF (30.2% diet DM), starch (26.7% diet DM), and CP (16.2% diet DM). The statistical model included the random effect of cow and fixed effects of diet, period, and their interaction. Results are presented in the sequence CON vs LF. There was no effect of treatment on milk yield, milk fat content, or body weight (BW). Compared with CON, LF increased dry matter intake (DMI; 30.8 vs 31.8 kg/d), milk fat yield (1.78 vs 1.84 kg/d), milk protein yield (1.47 vs 1.56 kg/d), milk protein content (3.24% vs 3.41%), energy-corrected milk (ECM; 48.3 vs 50.2 kg/d), and body condition score (BCS; 3.2 vs 3.3). Our results demonstrate that feeding a low forage diet supplemented with

amino acids and a C16:0-enriched FA supplement increased DMI and the yields of milk fat and protein, without changes in body weight.

Introduction

With changes in availability of high-quality forages due to unusual cropping seasons, geographic location, or increasing herd size with fixed forage inventories, it is important to explore the effects of low forage diets and alternative fiber sources to forage in dairy nutrition. Previous studies have taken many different approaches to formulating low forage diets, such as increasing the proportion of byproducts in low forage diets with low starch content (Hall and Chase, 2014), comparing low starch diets with different amounts of forage (Farmer et al., 2014), altering the starch:NDF ratio in low and high forage diets (Pereira and Armentano, 2000), or comparing the effects of nonforage fiber sources on nutrient digestibility (Clark and Armentano, 1997; Mooney and Allen, 1997; Boguhn et al., 2010). However, the majority of studies investigated the effects of low forage diets in lower producing cows averaging 33 kg/d milk yield (Clark and Armentano, 1997; Hall and Chase, 2014), and it is important to examine the effects of low forage diets in high producing cows with greater nutrient requirements and DMI.

Many studies have observed an increase in DMI with low forage (Kalscheur et al., 1997; Weiss and Pinos-Rodríguez, 2009; Farmer et al., 2014) associated with a higher rumen turnover rate caused by the higher NDF digestibility of non-forage fiber sources (Allen, 2000), which can lead to a decrease in rumen digestion of nutrients (Kendall et al., 2009). To mitigate the negative effects of excessive starch fermentation under these conditions, many studies decreased starch content (Pereira and Armentano, 2000). However, particularly in high producing cows, sufficient starch content is required to support ruminal starch fermentation and provide energy for microbial

growth, which when suppressed could reduce microbial protein yield and total tract starch digestibility, negatively impacting the yield of milk and milk protein (Allen 2000). Milk protein yield is directly linked to energy intake (Doepel et al., 2004) and increasing the supply of amino acids (AA), specifically methionine and lysine, to the mammary gland can increase milk protein production (Rius et al., 2010a; Schwab and Broderick, 2017). To our knowledge, there is no research looking at the potential for AA to maximize milk protein yields under low forage situations.

In contrast, various studies have investigated the effects of FA supplementation in low forage diets. Weiss and Pinos-Rodriguez (2009) fed high-producing cows (average 46 kg of milk per day) low- and high-forage diets with similar total NDF (~32% of diet DM) and starch (~29% of diet DM) content with or without supplemental FA. In the low forage diet, supplementation of a C18:0-enriched FA supplement (2.3% of DM) increased milk yield versus the low-forage diet without supplemented FA (Weiss and Pinos-Rodriguez, 2009). Similarly, Ylioja et al. (2018) observed that DMI and milk yield tended to increase with added fat in low forage diets. Recent research suggests that dairy cows have different metabolic and production responses when fed different combinations of C16:0, C18:0, and cis-9 C18:1. Under typical forage conditions C16:0 supplementation consistently increases milk production and NDF digestibility compared with non-FA supplemented control diets and diets supplemented with other supplements with different blends of FA (de Souza and Lock, 2018b; Western et al., 2020a).

Therefore, the objective of our present study was to evaluate the effects of a low forage diet balanced for total NDF, starch, and CP on nutrient digestibility and production of high producing, mid-lactation dairy cows. Our hypothesis was that a low forage diet containing a C16:0-

enriched FA supplement and rumen-protected methionine and lysine would maintain or surpass the yields of milk and milk components compared with a typical midwestern diet.

Materials and Methods

Design and Treatments

Experimental procedures were approved by the Michigan State University Institutional Animal Care and Use Committee. Thirty-two multiparous, mid-lactation Holstein cows (mean ± SD: 132 ± 39 DIM, 50.8 ± 4.4 kg/d milk, 702 ± 54 kg of BW), at the Michigan State University Dairy Cattle Teaching and Research Center were used in a crossover design. The study was completed from October to December 2019. All animals received a common diet during a 7 d preliminary period. Cows were randomly assigned to treatment sequences in a crossover design experiment with two consecutive 28 d periods.

Treatments were 1) control (CON) diet containing 19.2% forage NDF and no supplemental fat or supplemental AA and 2) low forage diet (LF) containing 12.7% forage NDF, including supplemental FA (1.5% diet DM; 82% C16:0-enriched supplement) and rumen-protected methionine and lysine (0.1% diet DM and 0.2% diet DM, respectively). Although the diets differed in fiber and starch sources, they were formulated to contain similar total NDF, starch, and CP. The diets differed in RUP and RDP content as a result of keeping CP constant with the addition of supplemental AA to the LF diet. The ingredient and nutrient composition of the diets fed as TMR are presented in Table 3.1. Cows (n=16) in treatment sequence A received CON in period 1 and LF in period 2 and averaged 50.8 ± 4.71 kg with a range in milk yield between 40.7 and 59.7 kg/d.

Cows (n=16) in treatment sequence B received LF in period 1 and CON in period 2 and averaged 50.8 ± 4.16 with a range in milk yield between 42.9 and 58.8 kg/d.

Dry matter concentrations were determined twice weekly for forages and diets were adjusted accordingly. Diets were mixed separately daily in a mixer wagon. Cows were milked twice daily (0400 and 1500 h) and housed in tiestalls throughout the experiment. Stalls were bedded with sawdust and cleaned twice daily. Access to feed was restricted from 0800 to 1000 h for collection of orts and administration of new feed. Cows were fed at 1000 h daily at 115% expected intake, with water available ad libitum in each stall.

Data and Sample Collection

Samples and data for production results were collected during the last 5 d of each treatment period (d 24 to 28). During this time, samples of all diet ingredients (0.5 kg) and orts from each cow (1.0 kg) were collected daily and composited by period for analysis. Milk yield was recorded and samples were collected at each milking. One aliquot was collected in a sealed tube without preservative at -20°C until analyzed for FA composition. The second aliquot was stored with preservative (Bronolab W-II liquid, Advanced Instruments, Norwood, MA) and stored at 4°C for milk component analysis. Blood (~15 mL) and fecal (~400 g) samples were collected every 15 h resulting in 8 samples/cow/period representing every 3 h over a 24-h period to account for diurnal variation. Blood samples were 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. Feces were stored at -20°C until dried and composited on an equal DM basis for each cow/period. Body weight was measured for each cow 3 times a week for the duration of the trial. On the last day of each period 3 trained investigators determined BCS on a 5-point scale in 0.25 increments (Wildman et al., 1982).

Sample Analysis

Diet ingredients, orts, and fecal samples were dried at 55°C in a forced-air oven for 72 h for DM determination. Dried samples were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Samples of feed ingredients and orts were analyzed for ash, indigestible NDF, NDF, CP, starch, and FA concentration as described by Boerman et al. (2017). Samples of feed ingredients were analyzed for AA concentrations at the University of Missouri, Agricultural Experiment Station Chemical Laboratory according to the AOAC (2006; Official Method 982.30 E(a,b,c)). Indigestible NDF was determined after 240 h of in vitro fermentation (Goering and Van Soest, 1970). Indigestible NDF was used as an internal marker to predict fecal output to determine apparent total-tract digestibility (Cochran et al., 1986).

Plasma insulin concentrations were determined by ELISA (Bovine Insulin ELISA; Mercodia AB, Uppsala, Sweden) at the Michigan State University Veterinary Diagnostic Laboratory (East Lansing). Individual milk samples were analyzed for fat, true protein, lactose, and MUN concentrations by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan Dairy Herd Improvement Association (North Star DHI, Grand Ledge, MI). Yields of ECM, 3.5% FCM, and milk components were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each collection period. Energy-corrected milk was calculated as: ECM = [(0.324 x kg milk) + (12.95 x kg milk fat) + (7.20 x kg milk protein)]. Fat-corrected milk was calculated as: 3.5% FCM = [(0.4324 x kg milk) + (16.216 x kg milk fat)]. Milk samples used for analysis of FA composition were composited based on milk fat yield (d 24-28 of each period). Milk lipids were extracted, and FA-methyl esters prepared and quantified using GLC described by Lock et al. (2013). Yield of individual FA (g/d) in milk fat was calculated by using milk fat yield and FA concentration to determine yield on a

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

Statistical Analysis

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

$$Y_{ijk} = \mu + C_i + P_j + T_k + P_jT_k + e_{ijk}$$

Where Y_{ijk} = dependent variable, μ = overall mean, C_i = random effect of cow (i = 1 to 32), P_j = fixed effect of period (j = 1 to 2), T_k = fixed effect of treatment (k = 1 to 2), P_jT_k = interaction between period and treatment, and e_{ijk} = residual error. The interaction between period and treatment was removed for all variables when it was not significant ($P > 0.15$). Normality of the results were tested using box plots, normal probability, and homogeneity of variances. Main effects were declared significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. All data was expressed as least square means and standard error of means, unless otherwise specified.

Results

Diets and Nutrient Composition

Treatment diets were similar in NDF, starch, and CP content, and differed in the content of DM, forage NDF, and total FA (Table 3.1). The CON diet contained 51.3% DM, 19.2% forage NDF, and 2.43% FA, whereas the LF diet contained 61.9% DM, 12.7% forage NDF, and 4.21% FA (Table 3.1). Compared with CON, LF increased C16:0, C18:0, and *cis*-9 C18:1 by an additional

1.29% DM, 0.09% DM, and 0.17% DM, respectively, and increased methionine and lysine by an additional 0.60% MP and 0.60% MP, respectively.

Nutrient Intake and Total-tract Digestibility

Compared with CON, LF increased DMI (1.0 kg/d; P < 0.01) and tended to increase NDF digestibility (2.3; P = 0.09; Table 3.2). No treatment differences were observed for DM digestibility (P > 0.15).

Production Responses

Compared with CON, LF increased the yields of milk fat (0.06 kg/d; P = 0.02), milk protein (0.09 kg/d; *P* < 0.01), 3.5% FCM (1.4 kg/d; *P* = 0.01), and ECM (1.9 kg/d; *P* < 0.01; Table 3.3). LF also increased BCS (0.06; *P* = 0.02), and tended to increase BW change (*P* = 0.09) and BCS change (*P* = 0.08). LF decreased the content of milk lactose by 0.09% units (*P* < 0.01) and increased milk protein by 0.17% units (*P* < 0.01) compared with CON. No treatment differences were observed for milk yield, lactose yield, fat content, or BW (*P* > 0.30).

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 FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis in the mammary gland and extraction from plasma. The yields and concentrations of milk fat according to source are shown in Table 3.4. Yields and concentrations of selected individual FA are shown in Table 3.5 and Table 3.6, respectively. Compared with CON, LF decreased the yield of de novo FA (15 g/d; P = 0.03) and increased the yields of mixed FA (44 g/d; P < 0.01) and preformed FA (25 g/d; P < 0.01). LF increased mixed milk FA predominately due to an increase in the yield of C16:0 in milk

fat ($P < 0.01$). LF increased preformed milk FA predominately due to an increase in the yield of unsaturated 18 carbon FA ($P < 0.01$). Concentrations of milk FA followed the same pattern as yields.

Plasma Insulin

Compared with CON, LF increased plasma insulin concentration by 12% ($P < 0.01$; Table 3.7).

Discussion

Due to increasing consumer demand for milk fat and protein, milk prices in most markets are driven by fat and protein yields. Therefore, we need to focus on increasing production of milk components. With changes in availability of high-quality forages due to unusual cropping seasons, geographic location, or increasing herd size with fixed forage inventories, it is important to explore the effects of lower forage and alternative fiber sources on milk production. However, challenges can arise in maintaining yields of milk fat and protein in situations when forage inventories are limited. Variable responses to low forage diets have been observed, depending on source of nonforage fiber and other dietary factors. Many studies have altered the supply of critical nutrients between low- and high-forage diets, such as total NDF and starch content (Hall and Chase, 2014; Farmer et al., 2014). These studies observed increases in DMI without changes in milk production, resulting in a decrease in feed efficiency in the low forage treatments. Other studies have tried to increase milk production through the addition of supplemental fat in low forage diets (Ylioja et al., 2018; Piantoni et al., 2015; Weiss and Pinos-Rodriguez, 2009). While Ylioja et al. (2018) and Piantoni et al. (2015) did not observe increases in milk fat yield or ECM, Weiss and Pinos-Rodriguez (2009) reported that cows fed low-forage diets increased milk

protein yield and fat supplementation increased milk yield in low-forage diets. The increase in milk protein yield was attributed to a greater DMI providing more glucose precursors and AA to support milk lactose and protein yields. To our knowledge, no studies have examined AA supplementation in low forage diets. Typically, studies investigating the effects of low forage diets alter the proportion of byproducts, starch, or NDF, and some have supplemented fat to increase the energy density of the ration and support milk fat production.

Our aim was to evaluate if milk component yields could be maintained or increased in low forage diets with additional AA and FA supplementation compared with a traditional midwestern diet. In order to support milk component production, we recognized the importance of maintaining rumen health by providing enough starch, NDF, and protein in the diet. In our study, dietary forage content was reduced from 55.5% diet DM to 36.6% diet DM. We achieved this by replacing forage (alfalfa silage and corn silage) with nonforage fiber sources (beet pulp, soyhulls, and cottonseed). We replaced high moisture corn with ground corn to decrease the supply of rapidly fermentable starch in the LF treatment. Additionally, we altered the amount of ground corn and soybean meal between treatments to balance the supply of NDF, starch, and CP, and supplemented AA and FA to support the production of milk and milk components in the LF treatment. We utilized a C16:0-enriched FA supplement due to recent research supporting that C16:0 supplementation increases milk yield, milk fat yield, and NDF digestibility compared with other FA supplements and non-FA supplemented controls fed to mid-lactation cows (de Souza and Lock, 2018b; Western et al., 2020a). We increased RUP by increasing methionine and lysine available for absorption in the LF treatment, and decreased RDP to keep CP values similar to the CON treatment. This allowed for the LF treatment to have an increased supply of methionine and

lysine to support milk protein synthesis without oversupplying protein and potentially decreasing nitrogen efficiency.

LF increased DMI, which is similar to previous results with low forage diets (Kalscheur et al., 1997; Mooney and Allen, 1997; Clark and Armentano, 1997). In low starch diets (21% of DM), replacing dietary forage with byproducts increased DMI (Farmer et al., 2014). Similarly, Weiss and Pinos-Rodrigues (2009) observed that cows fed wheat middlings and soybean hulls in partial replacement of corn silage and alfalfa increased DMI compared with a high-forage diet. Increased DMI in low forage diets can be attributed to a decreased supply of forage NDF and smaller particle length, leading to a decrease in physical fill and rumen retention rate (Allen, 2000). In addition, nonforage fiber sources increase DMI by increasing NDF digestibility (Mooney and Allen, 1997) and accelerating passage rate of nutrients from the rumen (Bhatti and Firkins, 1995). While we did not measure rumination or average particle length, the effects of low forage diets on these variables have been well studied in previous research (Cotanch et al., 2014; Allen, 2000). Although fat supplementation has variable impacts on DMI depending on FA profile (Allen, 2000; Rabiee et al., 2012), C16:0 supplementation in LF in the current study likely did not influence or contribute to the increase in DMI observed. When C16:0 is supplied at 1.5% of diet DM, previous studies have observed either no response or an increase in DMI compared with a non-FA supplemented control diet (de Souza et al., 2018a; de Souza and Lock, 2018; Western et al., 2020a).

Although we observed no difference in milk yield between treatments, LF increased ECM yield because it increased the yields of milk fat and protein compared with CON. Previous studies investigating the effects of low forage diets with varying nutrient compositions did not see a positive effect on the yields of milk fat or protein (Ylioja et al., 2018; Hall and Chase,

2014). In contrast, Weiss and Pinos-Rodriguez (2009) observed an increase in milk protein yield in low forage diets compared with high forage diets due to increased DMI providing more nutrients to the mammary gland and supporting an increased production of microbial protein. Under typical forage conditions, C16:0 supplementation increases milk fat yield compared with a non-FA supplemented control (Piantoni et al., 2013; Lock et al., 2013; de Souza et al., 2018a). C16:0-enriched FA supplementation in LF supported the increase in milk fat yield by providing additional FA for milk fat synthesis. Although most of our studies involving C16:0-enriched supplements (fed at <2.0% diet DM) have observed increases in ECM yield (Lock et al., 2013; de Souza et al., 2018a), this increase was driven by milk fat responses while milk protein yield was unaffected. However, increases in milk protein yield were observed with C16:0 supplementation compared with a non-FA supplemented control diet and other FA supplements in studies where the basal diet contained high quality blood meal (de Souza et al., 2019; Western et al., 2020a). LF increased milk protein yield, likely partially caused by increases in DMI providing enough energy from starch for microbial protein production, combined with AA supplementation supplying enough nutrients for milk protein synthesis in the mammary gland. The increase in insulin concentration observed with LF could also support the synthesis of protein through its action on milk protein synthesis in the mammary gland (Winkelman and Overton, 2013).

Weiss and Pinos-Rodriguez (2009) observed no effects of forage inclusion and fat supplementation on the concentrations of de novo or preformed milk FA. However, at 125 DIM both fat supplementation and low forage diets decreased de novo and increased preformed milk FA. Similarly, we observed an increase in the yields of mixed and preformed milk FA and a decrease in de novo milk FA yield in response to LF. FA supplementation alters milk FA content

and yield and is impacted by the FA being supplemented. In a recent review, Dorea and Armentano (2017) observed that supplementation of C16:0 increased total milk FA, primarily due to an increase in mixed source FA. The reduction of de novo milk FA in our trial agrees with responses to a C16:0-enriched FA supplement observed in some trials (Rico et al., 2014; Western et al., 2020a), but not others (de Souza and Lock, 2018; Lock et al., 2013). The reduction in de novo synthesis in LF was probably due to a substitution effect, which often occurs when there is an increase in preformed milk FA from FA supplementation (Glasser et al., 2008; He et al., 2012). The increase in DMI with LF, coupled with the higher dietary FA content in the diet provided more long-chain FA for incorporation into milk FA. While methionine supplementation increases de novo synthesis of milk FA in some studies (Pisulewski et al., 1996; Christensen et al., 1994), other studies report no impact on milk FA (Rulquin and Delaby, 1997; Casper et al., 1987; Chow et al., 1990). In contrast, rumen-protected methionine and lysine supplementation to Comisana ewes increased 16-carbon FA and reduced C4:0 FA concentrations in milk fat (Sevi et al., 1998). Although we did not observe a treatment effect on the concentration of C4:0 milk FA, LF did decrease C4:0 yield compared with CON. Overall, the milk FA responses in LF is expected when increasing C16:0 and total dietary FA content.

We observed that LF increased plasma insulin concentration compared with CON. Insulin responses to low forage diets in previous studies have typically been attributed to altered starch content between the low- and high-forage treatments (Pereira and Armentano, 2000), while in the current trial starch was kept constant between treatments. Supplementation of C16:0 has been observed to increase plasma insulin compared with a non-FA supplemented control diet in some trials (Piantoni et al., 2013; Harvatine and Allen, 2006), while in others it had no effect on plasma insulin (Western et al., 2020b; de Souza et al., 2018a). de Souza et al. (2016) observed

a tendency for an increase in insulin response when palmitic acid when supplemented as a replacement for soyhulls but not when supplemented as a replacement for dry ground corn, suggesting an interaction between basal diet composition and C16:0 supplementation. Additionally, increases in DMI are associated with elevated plasma insulin concentrations (Choi and Palmquist, 1996).

Replacing forage with nonforage fiber sources decreases total-tract NDF digestibility in some studies (Farmer et al., 2014; Kalscheur et al., 1997), but not others (Cunningham et al., 1993). Many byproducts and nonforage fiber sources are more digestible than forage fiber, but higher passage rates with smaller particle sizes may limit their digestibility (Bhatti and Firkins, 1995; Dann et al., 2007). Although we did not measure the effects of LF on rumen fermentation, we did observe an increase in DMI, suggesting a higher rate of passage of nutrients through the rumen. However, we observed an increase in NDF digestibility, likely due to the effects of C16:0 supplementation. Previous studies have observed that C16:0 supplementation increased NDF digestibility (Piantoni et al., 2013; de Souza et al., 2018b; Rico et al., 2017). Dietary C16:0 incorporates into rumen bacteria membranes, reducing bacterial synthesis of C16:0 and sparing ATP for bacterial growth, which in turn increases NDF digestion (Vlaeminck et al., 2006; Hackmann and Firkins, 2015). Further research is required to determine the relationship between nonforage fiber sources and C16:0 supplementation on NDF digestibility.

Although the LF treatment increased DMI, ECM also increased. In contrast to previous studies, we observed an increase in the yields of milk fat and milk protein, which could be attributed to supplying starch and NDF in adequate amounts in the low forage diets, and the addition of AA and C16:0-enriched FA supplements. While a factorial design would be needed to test the specific effects of AA and FA supplementation, this was not the focus of our study.

Rather, our focus was to determine if we can formulate low forage diets to increase the yields of milk fat and protein. We demonstrated that low forage diets can be formulated to increase ECM compared with traditional midwestern diets. Accounting for the change in DMI between the two treatments, cows on LF consumed 5.5 kg/d less forage DM than cows on CON. Over 28 d, this equated to ~150 kg less forage DM fed per cow. However, long term studies are required to determine if feeding low forage diets can maintain rumen health and productivity for long-term implementation in the industry.

Conclusion

In high producing dairy cows, a diet containing only 12% forage NDP plus a C16:0-enriched FA supplement and bypass methionine and lysine increased DMI and the yields of milk fat and protein compared with a control diet containing 19% forage NDF. Cows on the LF treatment consumed 5.5 kg/d less forage DM compared with CON, yet maintained milk yield and increased ECM yield. Under certain circumstances where forage inventories are limited due to increasing cow numbers or unusual cropping seasons, low forage diets can be formulated to sustain, or even increase, milk component production in high producing cows.

APPENDIX

Table 3.1. Ingredient and nutrient composition of treatment diets[1]

	Treatments[1]	
	CON	LF
Ingredient, % DM		
Corn Silage	38.8	28.0
Alfalfa Silage	14.6	6.16
Alfalfa Hay	2.17	2.41
Beet Pulp	1.45	9.20
Ground Corn	3.74	17.24
High Moisture Corn	8.82	-
Corn Gluten	1.30	4.86
Soybean Meal	7.98	6.57
Soy Hulls	5.79	6.93
Cottonseed	5.49	8.13
Vitamin Mineral Mix[2]	1.74	1.78
DCAD[3]	0.48	0.47
CON Mix[4]	7.72	-
LF Mix[5]	-	6.79
C16:0-enriched FA Supplement[6]	-	1.43
Nutrient Composition, % DM		
DM[7]	51.3	61.9
NDF	29.9	30.5
Forage NDF	19.2	12.7
CP	16.3	16.0
RUP	6.2	5.9
RDP	11.0	10.4
MP[8]	11.1	11.0
Lys % MP	6.45	7.05
Met % MP	1.78	2.38
Starch	26.6	26.8
FA	2.43	4.21
16:0	0.48	1.76
18:0	0.05	0.15
cis-9 18:1	0.42	0.60
cis-9, *cis*-12 18:2	1.27	1.54
cis-9, *cis*-12, *cis*-15 18:3	0.15	0.09

[1]Treatments were 1) control (CON) diet containing high forage (forage NDF 19.2% diet DM) and no supplemental fat or supplemental amino acids (RUP 6.67% diet DM; RDP 10.4% diet DM); 2) low forage diet (LF) containing low forage (forage NDF 12.7% diet DM), including supplemental

Table 3.1 (cont'd)

fat (1.5% diet DM; 82% C16:0-enriched supplement) and supplemental amino acids (RUP 7.01% diet DM; RDP 9.69% diet DM)

[2]Vitamin and mineral mix contained 27.1% calcium carbonate, 22.2% calcium phosphate di, 16.3% ground corn, 15.4% magnesium oxide, 9.6% salt, 4.8% sodium carbonate, 1.7% selenium, and <1% of each of the following: soybean oil, Availa-4 (Zinpro, Eden Prairie, MN), manganese sulfate, zinc sulfate, selenium yeast, copper sulfate, cobalt carbonate, 9.2% EDDI (Vedco Inc., Saint Joseph, MO), vitamin E, vitamin A, and vit D3 500 (Baltivet, Dubingai, Lithuania).

[3]DCAD Plus (Dietary Cation-Anion Difference; Arm & Hammer, Swedesboro, NJ) containing 88.0% DM, 56.0% Potassium, and <0.01% of the following: calcium, phosphorus, magnesium, chlorine, sodium, sulfur, cobalt, copper, iodine, iron, manganese, selenium, zinc.

[4]Control mix contained 42.3% Amino Plus (Ag Processing Inc, Omaha, NE), 33.1% corn grain, 11.2% sodium sesquinate refined, 6.7% calcium carbonate, 3.9% DCAD Plus (Arm & Hammer, Swedesboro, NJ) 1.5% urea, 1.2% QLF 68 5 Custom (Quality Liquid Feeds, Dodgeville, WI).

[5]Test mix contained 44.0% corn grain, 13.2% sodium sequinate refined, 10.6% bypass protein (Caledonia Farmers Elevator), 8.7% calcium carbonate, 6.6% DCAD Plus (Arm & Hammer, Swedesboro, NJ), 5.5% Amino Plus (Ag Processing Inc, Omaha, NE), 4.9% urea, 3.6% AjiPro L (Ajinomoto Health & Nutrition North America, Inc., Chicago, IL), 1.6% QLF 68 5 Custom (Quality Liquid Feeds, Dodgeville, WI), 1.2% Smartamine M (Adisseo, Alpharetta, GA)

[6]Spectrum Fusion (Perdue Agribusiness, Salisbury, MD). This supplement contained (g/100 g of fatty acid) 0.58 of C14:0, 90.2 of C16:0, 0.60 of C18:0, 6.77 of *cis*-9 C18:1, and 93.0% total fatty acids.

[7]Expressed as percent of as fed.

[8]Metabolizable protein; Calculated using DMI of 30.8 kg/d (CON) and 31.8 kg/d (LF; NRC, 2001).

Table 3.2. Nutrient intake and nutrient digestibility for cows fed treatment diets (n=32).

	Treatment[1]		SEM	P-value[2]
Variable	CON	LF		Trt
DMI, kg/d	30.8	31.8	0.40	<0.01
Digestibility, %				
DM	62.8	60.7	0.65	0.02
NDF	39.2	41.5	1.11	0.16

[1]Treatments were 1) control (CON) diet containing high forage (forage NDF 19.2% diet DM) and no supplemental fat or supplemental amino acids (RUP 6.67% diet DM; RDP 10.4% diet DM); 2) low forage diet (LF) containing low forage (forage NDF 12.7% diet DM), including supplemental fat (1.5% diet DM; 82% C16:0-enriched supplement) and supplemental amino acids (RUP 7.01% diet DM; RDP 9.69% diet DM).

[2] P values associated with treatment.

Table 3.3. Milk yield, milk composition, BW, and BCS for cows fed treatment diets (n=32).

	Treatment		SEM	P-value
Variable	CON	LF		Trt
Milk yield, kg/d				
Milk yield	45.4	46.1	0.92	0.34
3.5% FCM	48.5	49.9	0.89	0.01
ECM	48.3	50.2	0.87	<0.01
Milk composition				
Fat, %	3.95	3.99	0.09	0.37
Fat, kg/d	1.78	1.84	0.04	0.01
Protein, %	3.24	3.41	0.04	<0.01
Protein, kg/d	1.47	1.56	0.03	<0.01
Lactose, %	4.93	4.84	0.02	<0.01
Lactose, kg/d	2.23	2.22	0.05	0.81
ECM/DMI	1.58	1.55	0.02	0.25
BW, kg	704	703	9.41	0.83
BWC*, kg	0.20	0.40	0.09	0.09
BCS	3.24	3.30	0.06	0.02
BCS Change*	0.02	0.08	0.02	0.08

[1]Treatments were 1) control (CON) diet containing high forage (forage NDF 19.2% diet DM) and no supplemental fat or supplemental amino acids (RUP 6.67% diet DM; RDP 10.4% diet DM); 2) low forage diet (LF) containing low forage (forage NDF 12.7% diet DM), including supplemental fat (1.5% diet DM; 82% C16:0-enriched supplement) and supplemental amino acids (RUP 7.01% diet DM; RDP 9.69% diet DM).

[2] P values associated with treatment.

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

[4] Energy-corrected milk; ECM = [(0.324 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)]. This equation corrects milk to a 0.68 Mcal/kg energy basis.

*Significant period*trt interaction.

Table 3.4. FA concentrations and yields by source of milk FA for cows fed treatment diets (n=32).

	Treatments[1]		SEM	P value[2]
Variable	CON	LF		Trt
Summation by Source[3], g/100 g FA				
De Novo	27.1	25.4	0.27	<0.01
Mixed	39.2	40.6	0.39	<0.01
Preformed	33.6	34.1	0.42	0.03
Summation by Source[3], g/d				
De Novo	454	439	11.8	0.03
Mixed	657	701	19.5	<0.01
Preformed	558	583	9.47	<0.01

[1]Treatments were 1) control (CON) diet containing high forage (forage NDF 19.2% diet DM) and no supplemental fat or supplemental amino acids (RUP 6.67% diet DM; RDP 10.4% diet DM); 2) low forage diet (LF) containing low forage (forage NDF 12.7% diet DM), including supplemental fat (1.5% diet DM; 82% C16:0-enriched supplement) and supplemental amino acids (RUP 7.01% diet DM; RDP 9.69% diet DM).

[2] P values associated with treatment.

Table 3.5. Milk fatty acid yield for cows fed treatment diets (n=32).

	Treatments[1]		SEM	P value2
Variable	CON	LF		Trt
Selected Individual FA[3], g/d FA				
C4:0	41.4	40.9	1.10	0.51
C6:0	32.4	31.2	0.96	0.04
C8:0	20.6	19.8	0.62	0.03
C10:0	58.1	56.3	1.95	0.13
C12:0	68.6	67.2	2.30	0.26
C14:0	219	210	5.35	<0.01
C16:0	633	676	18.7	<0.01
cis-9 C16:1	24.3	25.2	1.02	0.06
C18:0	144	142	3.73	0.55
trans-6 to 8 C18:1	3.50	4.11	0.10	<0.01
trans-9 C18:1	2.74	3.41	0.08	<0.01
trans-10 C18:1	5.70	7.53	0.35	<0.01
trans-11 C18:1	10.8	13.7	0.67	<0.01
cis-9 C18:1	269	278	4.95	0.06
cis-11 C18:1	9.04	9.71	0.35	<0.01
cis-9, *cis*-12 C18:2	34.8	43.6	0.75	<0.01
cis-9, *trans*-11 C18:2	4.77	6.02	0.31	<0.01
cis-9, *cis*-12, *cis*-15 C18:3	5.34	3.99	0.10	<0.01

[1]Treatments were 1) control (CON) diet containing high forage (forage NDF 19.2% diet DM) and no supplemental fat or supplemental amino acids (RUP 6.67% diet DM; RDP 10.4% diet DM); 2) low forage diet (LF) containing low forage (forage NDF 12.7% diet DM), including supplemental fat (1.5% diet DM; 82% C16:0-enriched supplement) and supplemental amino acids (RUP 7.01% diet DM; RDP 9.69% diet DM).

[2] P values associated with treatment.

Table 3.6. Milk fatty acid concentration for cows fed treatment diets (n=32).

Variable	Treatments[1]		SEM	P value[2]
	CON	LF		Trt
Selected Individual FA[3] , g/100 g FA				
C4:0	2.48	2.39	0.03	<0.01
C6:0	1.94	1.82	0.03	<0.01
C8:0	1.23	1.14	0.02	<0.01
C10:0	3.47	3.28	0.06	<0.01
C12:0	4.10	3.87	0.08	<0.01
C14:0	13.1	12.2	0.12	<0.01
C16:0	37.8	39.1	0.37	<0.01
cis-9 C16:1	1.46	1.47	0.04	0.84
C18:0	8.65	8.31	0.18	0.01
trans-6 to 8 C18:1	0.21	0.24	0.01	<0.01
trans-9 C18:1	0.17	0.20	0.01	<0.01
trans-10 C18:1	0.34	0.44	0.02	<0.01
trans-11 C18:1	0.66	0.80	0.04	<0.01
cis-9 C18:1	16.2	16.2	0.25	0.96
cis-11 C18:1	0.54	0.57	0.02	<0.01
cis-9, *cis*-12 C18:2	2.10	2.53	0.05	<0.01
cis-9, *trans*-11 C18:2	0.29	0.36	0.02	<0.01
cis-9, *cis*-12, *cis*-15 C18:3	0.32	0.24	0.01	<0.01

[1]Treatments were 1) control (CON) diet containing high forage (forage NDF 19.2% diet DM) and no supplemental fat or supplemental amino acids (RUP 6.67% diet DM; RDP 10.4% diet DM); 2) low forage diet (LF) containing low forage (forage NDF 12.7% diet DM), including supplemental fat (1.5% diet DM; 82% C16:0-enriched supplement) and supplemental amino acids (RUP 7.01% diet DM; RDP 9.69% diet DM).

[2] P values associated with treatment.

Table 3.7. Blood metabolites for cows fed treatment diets (n=32).

	Treatments[1]		SEM	P value[2]
Variable	CON	LF		Trt
Insulin, ug/L	0.64	0.72	0.03	<0.01

[1]Treatments were 1) control (CON) diet containing high forage (forage NDF 19.2% diet DM) and no supplemental fat or supplemental amino acids (RUP 6.67% diet DM; RDP 10.4% diet DM); 2) low forage diet (LF) containing low forage (forage NDF 12.7% diet DM), including supplemental fat (1.5% diet DM; 82% C16:0-enriched supplement) and supplemental amino acids (RUP 7.01% diet DM; RDP 9.69% diet DM).

[2] P values associated with treatment.

CHAPTER 4

Milk production responses to altering the ratio of palmitic and oleic acids in basal diets with low or high metabolizable protein content of methionine and lysine on the yield of milk and milk components

Abstract

We evaluated the effects of fatty acid (FA) supplements with different ratios of palmitic (C16:0) and oleic (*cis*-9 C18:1) acids in basal diets formulated for low or high metabolizable protein (MP) content of methionine (Met) and lysine (Lys) on the yields of milk and milk components of mid-lactation dairy cows. Thirty-six Holstein cows (53±14kg milk/d; 107±49 DIM) were equally allocated to a split plot receiving either a basal diet containing 18.1% CP (HP) (MP 11.6% diet DM, Lys 6.23% MP, Met 1.73% MP, RUP 6.98% diet DM) or containing 16.6% CP (LP) (MP 10.4% diet DM, Lys 6.68% MP, Met 2.19% MP, RUP 5.59% diet DM). Diets were balanced for similar starch (28.0% diet DM), RDP (10.2% diet DM), and NDF (28.5% diet DM). Within each plot a 3×3 Latin square arrangement of treatments was used with three 21 d periods, with sample and data collection during the final 5 d of each period. Treatments were 1) control diet (CON) containing no supplemental fat; 2) FA supplement containing 80% C16:0 + 10% C18:1 (PA); and 3) FA supplemented diet containing 60% C16:0 + 30% C18:1 (OA). FA supplements were fed at 1.5% DM and replaced soyhulls in CON. Compared with HP, LP decreased blood urea nitrogen (BUN) and milk urea nitrogen (MUN) concentrations and had no effect on DMI, milk yield, ECM, protein yield, body weight, body condition score (BCS), or plasma insulin. Compared with CON, FA treatments increased milk fat yield, ECM, and feed efficiency (ECM/DMI), and decreased DMI. Compared with PA, OA decreased DMI and plasma insulin. We observed a treatment by basal diet interaction for milk protein content where FA treatments decreased protein content more

in HP than LP. In conclusion, reducing MP content while supplementing Met and Lys reduced BUN and MUN, and maintained the production of milk and milk components. Addition of FA supplements increased fat yield, FCM, and ECM regardless of basal amino acid (AA) supplementation.

Introduction

Efficiency of milk fat and protein production is an area of increasing importance to the dairy industry due to milk income being driven by milk fat and protein yields. As a result, research has focused on feeding strategies to increase milk component yields and improve nutrient efficiency. FA supplements increase the yields of milk and milk fat, but typically do not increase milk protein yield (Rabiee et al., 2012). Strategies for improving milk protein yield include varying the supply of essential amino acids (EAA), metabolizable protein (MP), crude protein (CP), rumen-degradable protein (RDP), and starch (NRC, 2001). Balancing ratios of AA and MP to supply EAA without oversupplying protein improves nitrogen efficiency and increase milk protein yield (Haque et al., 2012; Lee et al., 2012; Arriola Apelo et al., 2014). Rumen-protected AA, specifically Met and Lys, are often fed to help increase AA availability for absorption and mammary gland utilization, but if there is insufficient energy supplied by other nutrients for milk synthesis, AA may be oxidized as a source of energy (Bequette et al., 2002; Lapierre et al., 2005). Previous studies have demonstrated that energy supply from glucose or glucose precursors can increase the use of available AA for milk protein synthesis (Lemosquet et al., 2009a; Rius et al., 2010b; Nichols et al., 2016). Glucogenic diets may improve milk protein yield by reducing AA catabolism in the mammary gland (Raggio et al., 2006; Rius et al., 2010a) and increasing plasma insulin concentrations (Nichols et al., 2016; Rius et al., 2010b). Increases

in plasma insulin concentrations are associated with increased milk protein yields (McGuire et al., 1995; Griinari et al., 1997; Arriola Apelo et al., 2014). Therefore, milk protein production is directly linked to energy intake (Doepel et al., 2004), and the supply of non-AA energy precursors may influence AA use for milk protein synthesis (Raggio et al., 2006; Rius et al., 2010a).

Glucogenic and lipogenic diets have been compared in multiple studies (Boerman et al., 2015c; Grum et al., 1996; Lapierre, 2020) with lipogenic diets typically improving milk fat output and feed efficiency, while glucogenic diets partition more energy towards adipose tissue accretion. Nichols et al. (2019) compared glucogenic and lipogenic substrates at low and high MP levels on energy and nitrogen partitioning. In this isoenergetic study, no interactions were observed between glucogenic or lipogenic infusions and AA supply on DMI or production responses. Similarly, Nichols et al. (2018) isoenergetically supplemented protein and fat to mid-lactation cows and found no interactions on milk production or composition. However, both of these studies supplemented protein and fat as sources of energy in isoenergetic diets fed at restricted intake instead of additional nutrients in diets formulated to meet energy requirements fed ad libitum. Other studies investigating fat and protein supplementation have also reported a lack of interaction between fat and protein on milk component yields (Chan et al., 1997; Hoffman et al., 1991). However, these studies supplied very high levels of CP, fat, or levels of C18:0 or *cis*-9 C18:1.

Recently, studies have highlighted the importance of FA profile of fat supplements in determining effects on the yields of milk and milk components. Inclusion of different ratios of FA, especially C16:0, C18:0, and *cis*-9 C18:1 have variable impacts on nutrient digestibility, energy partitioning, and milk production due to inclusion rate, production level of the cow, stage of lactation, or other dietary nutrients. Supplementing blends of C16:0 and *cis*-9 C18:1 increases the

yield of milk and ECM (Rico et al., 2014; Western et al., 2020b; de Souza et al., 2018a). Western et al. (2020a) found that a FA supplement containing 80% C16:0 increased yields of ECM compared with a FA treatment of 30% C16:0 and 50% C18:0. Additionally, de Souza et al. (2019) altered the ratio of C16:0 and *cis*-9 C18:1 in FA blends and observed that increasing the amount of *cis*-9 C18:1 increased ECM and milk yield in high producing cows, while C16:0 increased these variable in low producing cows. While FA supplementation often increases yields of milk and milk fat, typically FA supplementation does not increase milk protein yield (Rabiee et al., 2012). Interestingly, increases in milk protein yield were observed with C16:0 supplementation compared with a non-FA supplemented control and other FA supplements in studies where the basal diets contained high quality blood meal (de Souza et al., 2019; Western et al., 2020a). Importantly, supplementation with C16:0 and *cis*-9 C18:1 has been observed to impact plasma insulin concentrations in various studies (de Souza et al., 2018a; Piantoni et al., 2013), and insulin increases milk protein synthesis (Arriola Apelo et al., 2014).

Despite some authors examining effects of lipogenic and glucogenic diets with protein supplementation on milk production, there is a paucity of research on the interaction between specific FA and AA on milk production. The objective of our present study was to determine if supplementing different ratios of C16:0 + *cis*-9 C18:1 have differing effects on milk production and efficiency in lactating cows when they are fed basal diets formulated for low or high Met plus Lys content.

Materials and Methods

Design and Treatments

Experimental procedures were approved by the Michigan State University Institutional Animal Care and Use Committee. Thirty-six multiparous, mid-lactation Holstein cows (mean ± SD: 107 ± 26 DIM, 55.1 ± 6.9 kg/d milk, 742 ± 66 kg of BW), at the Michigan State University Dairy Cattle Teaching and Research Center were randomly assigned to a treatment sequence in a replicated split-plot 3 × 3 Latin square design balanced for carryover effects in three consecutive 21 d periods. The study was completed from January to April 2020. All animals received a common diet during a 14 d preliminary period.

This trial was designed to test the interaction between increasing Met and Lys content in lower MP basal diet and FA supplements with different ratios of C16:0 and *cis*-9 C18:1. Cows were assigned to a main plot, with 18 cows receiving a basal diet containing 18.1% CP (HP; MP 11.6% diet DM, Lys 6.23% MP, Met 1.73% MP, RUP 6.98% diet DM) and 18 cows receiving a basal diet containing 16.6% CP (LP; MP 10.4% diet DM, Lys 6.68% MP, Met 2.19% MP, RUP 5.59% diet DM). The AA supply of the treatment diets are presented in Table 4.1. The LP basal diet contained rumen-protected Met (Smartamine M; Adisseo, Alpharetta, GA) and Lys (Smartamine ML; Adisseo, Alpharetta, GA), along with an increased supply of soybean meal and decreased blood meal content. Within each basal diet split-plot, FA treatments were assigned within replicated 3 × 3 Latin squares so that each cow received each of the FA treatments but only one basal diet. The design of the experiment lessens the statistical power of the main plot factor (HP vs. LP basal diets) 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 were 1) control diet (CON) containing no supplemental fat; 2) FA supplement

containing 80% C16:0 + 10% *cis*-9 C18:1 (PA); and 3) FA supplemented diet containing 60% C16:0 + 30% C18:1 (OA). Both FA supplements were fed at 1.5% DM 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 4.2. The ingredient and nutrient composition of the diets fed as a TMR are provided in Table 4.3. Dry matter concentration was determined twice weekly for forages and diets were adjusted accordingly. Base diets were mixed in a wagon daily, with forages (corn silage and alfalfa silage) mixed in one base mix that was then split. Soybean meal, ground corn, cottonseed, high moisture corn, and vitamin-mineral mixes were added to the separate forage bases to produce the final bases for the HP and LP basal diets. The HP and LP base mixes had their own respective vitamin-mineral mixes that were formulated to supply different amounts and ratios of MP and AA in the LP basal diet. Then soyhulls, FA supplements, and basal diet were mixed in a tumble-mixer for each treatment diet. Cows were milked twice daily (0400 and 1500 h) and housed in tiestalls throughout the experiment. Stalls were bedded with sawdust and cleaned twice daily. Access to feed was restricted from 0800 to 1000 h for collection of orts and administration of new feed. Cows were fed at 1000 h daily at 115% expected intake, with water available ad libitum in each stall.

Data and Sample Collection

Samples and data for production results were collected during the last 5 d of each treatment period (d 17 to 21). During this time, samples of all diet ingredients (0.5 kg) and orts from each cow (1.0 kg) were collected daily and composited by period for analysis. Milk yield was recorded and samples were collected at each milking. One aliquot was collected in a sealed tube without preservative at -20°C until analyzed for FA composition. The second aliquot was stored with preservative (Bronolab W-II liquid, Advanced Instruments, Norwood, MA) and stored at 4°C for

milk component analysis. 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 store at -20°C until composited by cow/period. Body weight was measured for each cow 3 times a week (1600 h) for the duration of the trial. On the last day of each period 3 trained investigators determined BCS on a 5-point scale in 0.25 increments (Wildman et al., 1982).

Sample Analysis

Diet ingredients and orts samples were dried at 55°C in a forced-air oven for 72 h for DM determination. Dried samples were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Samples of feed ingredients and orts were analyzed for ash, NDF, CP, starch, and FA concentration as described by Boerman et al. (2017). Samples of feed ingredients were analyzed for AA concentrations at the University of Missouri, Agricultural Experiment Station Chemical Laboratory according to the AOAC (2006; Official Method 982.30 E(a,b,c)).

Plasma insulin concentrations were determined by ELISA (Bovine Insulin ELISA; Mercodia AB, Uppsala, Sweden), and plasma BUN concentrations were determined by mass spectroscopy at the Michigan State University Veterinary Diagnostic Laboratory (East Lansing). Individual milk samples were analyzed for fat, true protein, lactose, and MUN concentrations by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan Dairy Herd Improvement Association (North Star DHI, Grand Ledge, MI). Yields of ECM, 3.5% FCM, and milk components were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each collection period. Energy-corrected milk was calculated as: ECM = [(0.324 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)]. Fat-corrected milk was calculated as: 3.5% FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)]. Milk

samples used for analysis of FA composition were composited based on milk fat yield (d 17-21 of each period). Milk lipids were extracted, and FA-methyl esters prepared and quantified using GLC described by Lock et al. (2013). Yield 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) according to the following model:

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

Where Y_{ijk} = dependent variable, μ = overall mean, + $C(B)_{i(j)}$ = random effect of cow nested within 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), $P_k T_l$ = the interaction of period and treatment, $B_j T_l$ = the interaction of basal diet and treatment, $B_j P_k$ = the interaction of period and basal diet, and e_{ijk} = residual error. The interaction between period and treatment and between period and basal diet were removed for values where it was not significant ($P > 0.20$). $P_k B_j T_l$ was not significant for all variables and was removed from the model. Normality of the results were tested using box plots, normal probability, and homogeneity of variances. Main effects were declared significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. Interactions were declared significant at $P \leq 0.10$ and tendencies at $0.10 < P \leq 0.15$. All data were expressed as least square means and standard error of means, unless otherwise specified. Two orthogonal contrasts were evaluated: (1) the overall effect of FA supplements [CON vs. FAT (½ PA + ½ OA)]; and 2) the effect of the PA versus OA treatments (PA vs. OA). These contrasts were used to test the main effect of FA

treatments and interactions between FA treatments and basal diet. All data were expressed as least square means and standard error of the means, unless otherwise specified.

Results

Diets and Nutrient Composition

Treatment diets contained similar contents of DM, NDF, and starch. The HP basal diet contained 18.1% CP, 6.51% EAA, 11.6% MP (Lys 6.23% MP, Met 1.73% MP), whereas the LP basal diet contained 16.6% CP, 5.91% EAA, 10.4% MP (Lys 6.68% MP, Met 2.19% MP). The FA treatments increased FA content by 1.34% diet DM compared with CON, and contained similar amounts of total FA compared with each other; however, PA contained an additional 0.3% DM C16:0 while OA contained an additional 0.2% DM *cis*-9 C18:1.

Production Responses

There was no effect of basal diet on DMI or the yield of milk and milk components (all $P > 0.15$). Compared with HP, LP decreased MUN ($P < 0.01$). Compared with CON, FAT increased milk yield, 3.5% FCM, ECM, milk fat content, milk fat yield, BCS change, and feed efficiency (ECM/DMI) (all $P < 0.05$; Table 4.4), tended to increase milk lactose yield ($P = 0.07$), and decreased DMI, milk lactose content, BW, and BW change (all $P < 0.05$). There was no effect of FA treatment on milk lactose content, MUN, or BCS (all $P > 0.15$).

Compared with PA, OA decreased DMI and milk fat content (both $P = 0.01$) and tended to increase feed efficiency (ECM/DMI; $P = 0.06$). There was no difference between PA and OA for the yield of milk or milk components, MUN, BW, BCS, or BCS change (all $P > 0.10$; Table 4.4).

We did not observe any basal diet by FA treatment interactions for the yields of milk and milk components, but we did observe an interaction for milk protein content ($P = 0.10$; Table 4.4). Compared with CON, FAT decreased milk protein content in both HP and LP basal diets ($P < 0.01$), with a greater magnitude of difference in HP than LP. Compared with PA, OA decreased milk protein content in both HP and LP basal diets ($P < 0.01$; Figure 4.1), with a smaller magnitude of difference in HP than LP.

Milk FA Yield and Concentration

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 FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis in the mammary gland and extraction from plasma. Compared with HP, LP did not affect the yields of de novo or mixed milk FA ($P > 0.10$) and decreased preformed milk FA yield ($P = 0.05$; Table 4.5). FAT decreased the yield of de novo milk FA ($P < 0.01$) and increased the yields of mixed ($P < 0.01$) and preformed milk FA ($P < 0.01$) compared with CON. Compared with PA, OA decreased the yield of de novo ($P < 0.01$) and mixed milk FA ($P < 0.01$), and increased preformed milk FA yield ($P < 0.01$). There was no effect of basal diet on the concentration of de novo, mixed, or preformed FA ($P > 0.20$). Compared with CON, FAT decreased de novo milk FA concentration ($P < 0.01$), and increased mixed ($P < 0.01$) and preformed milk FA concentration ($P = 0.03$). OA decreased de novo ($P < 0.01$) and mixed milk FA concentration ($P < 0.01$), and increased preformed milk FA concentration ($P < 0.01$) compared with PA.

We observed tendencies for basal diet by FA treatment interactions for the yields of mixed ($P = 0.11$) and preformed milk FA ($P = 0.13$). Compared with CON, FAT increased mixed milk FA yield in HP and LP, but the magnitude of change was greater in HP ($P < 0.05$; Figure 4.2).

Compared with PA, OA decreased mixed milk FA yield more in HP ($P < 0.01$) than in LP ($P = 0.03$). FAT increased preformed milk FA yield in both HP and LP compared with CON, but the magnitude of change was greater in HP ($P < 0.05$; Figure 4.2). Compared with PA, OA increased preformed milk FA yield more in LP ($P < 0.01$) than in HP ($P = 0.02$). We observed an interaction between basal diet and FA treatments for de novo milk FA concentration ($P = 0.07$; Figure 4.3). FAT decreased de novo milk FA concentration in HP and LP compared with CON, but the magnitude of change was greater in HP ($P < 0.05$). Compared with PA, OA decreased de novo milk FA concentration more in LP ($P < 0.01$) than in HP ($P = 0.04$). Selected individual milk FA concentrations and yields are shown in Table 4.6 and Table 4.7, respectively.

Blood Metabolites

Compared with HP, LP decreased BUN ($P < 0.01$), and did not impact plasma insulin ($P > 0.20$; Table 4.8). FAT tended to decrease BUN ($P = 0.09$) but did not affect plasma insulin ($P = 0.60$) compared with CON. Compared with PA, OA decreased plasma insulin ($P < 0.01$) and tended to decrease BUN ($P = 0.10$). We did not observe any basal diet by FA treatment interactions for BUN or plasma insulin ($P > 0.20$).

Discussion

FA supplements increase the yields of milk and milk fat, but typically have no effect on milk protein yield (Rabiee et al., 2012). However, we have observed increases in milk protein yield with C16:0 supplementation compared with nonfat supplemented control diets and other FA supplements in studies where the basal diets contained blood meal (de Souza et al., 2019; Western et al., 2020a). Therefore, our study was designed to test the interaction between FA

supplements containing different blends of C16:0 + *cis*-9 C18:1 FA and basal diets formulated for low or high MP content of Met and Lys. Although many studies have observed an interaction between dietary energy and AA supplementation on milk protein yield (Lobley, 2007; Rius et al., 2010b; Hanigan et al., 1998), these studies typically used glucose or glucose precursors as an energy source and milk protein responses to energy supplementation from FA supplementation are limited and variable. Previous research on the interactions between fat and protein included high inclusion rates of CP (~18% diet DM; Hoffman et al., 1991; Chan et al., 1997) or UFA from tallow, oil, or grease (Canale et al., 1990; Hoffman et al., 1991; Cant et al., 1993). Recent discoveries regarding AA requirements of lactating dairy cows and the effects of different FA and blends of FA on milk production and energy partitioning have highlighted that oversupplying AA or FA may reduce nutrient efficiency, DMI, and negatively impact milk production (Rico et al., 2017; Allen, 2000; Reed et al., 2017).

Recent research has investigated the relationship between lipogenic and glucogenic precursors with protein in isoenergetic diets (Nichols et al., 2019) and the impact of energy from starch and from fat on AA requirements (Lapierre, 2020). Nichols et al. (2019) utilized isoenergetic abomasal infusions of glucose and a FA supplement containing ~43% C16:0 + 43% *cis*-9 C18:1 with or without EAA, and concluded that FA supplementation can support milk production and metabolism largely independent of protein level. However, Nichols et al. (2019) experimental design and approach to FA and AA supplementation differed greatly from our study. While Nichols et al. (2019) was designed to provide isoenergetic infusions of lipogenic substrates with a high content of UFA, our study increased dietary FA from supplements with different FA profiles in cows fed ad libitum. We have observed that different FA profiles can affect energy partitioning and nutrient utilization in multiparous dairy cows (de Souza et al.,

2018a; 2019; Western et al., 2020b). Specifically, FA supplements enriched in C16:0 and *cis*-9 C18:1 interact with production level (de Souza et al., 2019; Western et al., 2020b) and basal FA content (Burch, 2020). Additionally, we increased Lys and Met by 0.45% MP and 0.46% MP, respectively, while decreasing total MP in our LP basal diet, while Nichols et al. (2019) increased MP supply by infusing 844 g/d of EAA. Providing a more complete supply of EAA for absorption improves nitrogen efficiency and increases milk protein yield as long as protein is not oversupplied (Haque et al., 2012; Lee et al., 2012; Arriola Apelo et al., 2014). Higgs (2014) observed that cows maintained high levels of performance at lower levels of CP (~14 % diet DM) when balanced for EAA and provided an adequate supply of rumen nitrogen. However, oversupplying protein decreases nitrogen efficiency. Excess AA are catabolized and excreted as urea via ureagenesis, an energetically demanding process (Lapierre et al., 2002; Reed et al., 2017). Due to the flexibility of the mammary gland to different precursors, it is important to understand the relationship between FA profiles and AA utilization in the mammary gland on production responses and nitrogen efficiency in cattle fed ad libitum for application in feeding practices on farm. Our results show that increasing methionine and lysine supply as % MP can sustain milk protein yield with a lower MP and both FA treatments increased yields of milk and milk fat. However, altering the ratio of FA did not interact with AA supplementation to alter yield of milk or milk components.

We did not observe an effect of basal diet on DMI. Interestingly, Nichols et al. (2019) observed when diets were fed at 90% of estimated requirements, high MP increased DMI, while lipogenic infusions decreased DMI. We did not observe interactions between FA treatments and basal diet for DMI. Our results are similar to Hoffman et al. (1991), who did not observe an interaction between supplemental FA and AA on DMI. In contrast, Chan et al. (1997) observed

an increase in DMI with supplemental FA containing approximately 53% C18:0 + 33% C16:0 for cows fed a low quality protein but not for cows fed a high quality protein. Potential differences in results could be attributed to the FA profile supplemented in different studies. DMI is variable for cows supplemented with dietary fat, due to the type of supplement being fed (Rabiee et al., 2012), and the degree of saturation (Harvatine and Allen, 2006). In general diets higher in UFA decrease DMI compared with diets supplemented with SFA and diets without FA supplementation (Christensen et al., 1994; Harvatine and Allen, 2005; de Souza et al., 2018a). Supply of UFA past the rumen can increase secretion of gut peptides, e.g. CCK, that signal for satiety and decrease DMI (Relling and Reynolds, 2007; Bradford et al., 2008). In agreement with these finding, we observed that OA decreased DMI compared with PA, driving the overall effect of FAT compared with CON. Previous studies have observed variable responses in DMI to differing ratios of C16:0 and *cis*-9 C18:1 interacting with production level (Western et al., 2020b; de Souza et al., 2019) and FA content of the basal diet (Burch, 2020; de Souza, 2018a). Further research needs to be conducted to determine the interactions between FA profile and basal AA supplementation on DMI.

Contrary to our hypothesis, we did not observe main effects or interactions between basal diet and FA treatment on milk protein yield. Similarly, previous studies did not observe interactions between fat and protein supplementation on milk protein yields (Nichols et al., 2018a; Nichols et al., 2019). Our LP basal diet may have limited responses to FA supplementation by supplying MP at 10.4% of diet DM, limiting the AA availability for the synthesis of milk and milk components with higher energy intakes. It is possible that the cows could be mobilizing muscle protein to sustain milk yield; however, a longer-term study is needed to measure the effects of treatment on tissue protein degradation. Higgs (2014) suggests that

supplying EAA as a percentage of metabolizable energy may be a more accurate predictor of milk protein yield than on a MP basis. Also, the supply of Lys and other EAA may have restricted milk protein synthesis in the LP basal diet. On the other hand, we decreased MP supply in our LP basal diet, and still maintained milk protein yield across basal diets. However, we observed a basal diet by FA treatment interaction for milk protein content, with FA treatments decreasing protein content in both HP and LP. Compared with OA, PA increased protein content more in LP than HP, indicating that in contrast to HP, PA maintained milk protein content at a level comparable to CON in LP while OA decreased milk protein content compared with CON in both basal diets. Compared with PA, OA decreased DMI, which could have reduced milk protein synthesis by providing less AA for absorption.

Supplying a more optimum concentration of EAA in MP to lactating dairy cows has been observed to increase nitrogen efficiency and decrease concentrations of blood urea nitrogen (BUN) and milk urea nitrogen (MUN; Haque et al., 2012; Lapierre et al., 2002; Lapierre, 2019). Compared with HP, LP decreased BUN and MUN, indicating that the LP basal diet increased nitrogen efficiency compared with HP. Previously, tendencies for interactions between fat and protein supplementation on milk nitrogen efficiency have been observed (Nichols et al., 2018a; Nichols et al., 2019). Our results differ from Nichols et al. (2018b; 2019), who observed a tendency for fat to increase milk nitrogen efficiency more at a low protein level than at a high protein level. However, they supplied isoenergetic diets and supplemented AA at the higher MP level, while we only supplemented AA at a lower MP level in diets fed ad libitum. Also, Nichols et al. (2018b, 2019) supplied different FA profiles than in the current study. We observed no effect of FA supplements on MUN; however, FAT tended to decrease BUN. Interestingly, OA tended to decrease BUN compared with PA, suggesting the tendency for the overall effect of

FAT was driven by OA. The response of BUN to OA could be attributed to the decrease in DMI observed with OA, causing a decrease in intake of RUP and reduced absorption. The relationship between profile of FA supplements and nitrogen efficiency is poorly characterized and deserve further research.

We observed no interaction between basal diet and FA treatments on milk yield or ECM. Similarly, Nichols et al. (2019) reported no interaction between MP level and lipogenic infusions on milk production or milk composition under isoenergetic conditions. However, we did observe main effects of FAT on ECM. Compared with CON, FAT increased milk yield and ECM, with no difference between OA and PA. Previous studies in our lab have observed different ECM responses to altering the ratio of C16:0 and *cis*-9 C18:1 due to production level (de Souza et al., 2019; Western et al., 2020b). Higher producing cows responded more positively to blends with a higher content of *cis*-9 C18:1, while lower producing cows responded more positively to blends higher in C16:0 (de Souza et al., 2019; Western et al., 2020b). The cows at the start of our current study averaged 53 kg/d milk yield and we observed no difference between PA and OA treatments for the yields of milk or ECM. Similarly, de Souza et al. (2019) observed no production differences between differing ratios of C16:0 and *cis*-9 C18:1 in cows averaging 53 kg/d of milk yield. Importantly, we hypothesized that cows would have responded differently to PA and OA, which may be a reason why we did not observe an interactions between FA treatment and basal AA on the yields of milk and milk components in this current study.

Although we did not observe interactions between basal diet and FA on milk fat yield, there were multiple interactions on the yields of milk FA. Yields of mixed and preformed milk FA increased with FAT supplementation, with PA and OA responding differently in HP and LP basal diets. Compared with OA, PA tended to increase mixed milk FA yield more in HP than LP.

In contrast, PA increased preformed milk FA yield with HP but not LP, while OA increased preformed milk FA yield compared with PA in both basal diets. In contrast, Nichols et al. (2018) did not observe protein by fat interactions on the yield of milk FA. Previous studies reported that FA blends with higher levels of *cis*-9 C18:1 linearly increased preformed milk FA yield while a blend with a higher level of C16:0 increased mixed milk FA (de Souza et al., 2019; Burch, 2020). Typically, protein supplementation impacts milk FA by providing energy for de novo FA synthesis or spares FA from catabolism, allowing for more preformed FA to be incorporated into milk fat (Christensen et al., 1994; Lapierre et al., 2012). However, the interactions between FA profile and AA supplementation remain poorly characterized.

Energy metabolism and production responses to both protein and fat supplementation are complex and can impact each other through various mechanisms. Although insulin impacts energy metabolism and milk component production, many previous studies did not measure insulin responses to fat and protein supplementation (Hoffman et al., 1991; Chan et al., 1997; Cant et al., 1993). Recently, Nichols et al (2018) reported a decrease in plasma insulin concentrations in response to fat supplementation at a high protein level but not at a low protein level, whereas in a subsequent study (Nichols et al., 2019) no response to lipogenic infusion and MP level was observed. In agreement with Nichols et al. (2019), we did not observe an interaction between FA and AA supplementation on plasma insulin. However, OA decreased plasma insulin compared with PA. Interestingly, there was no overall effect of FAT compared with CON, indicating that PA increased insulin and OA decreased insulin compared with CON. Generally, increasing *cis*-9 C18:1 increases plasma insulin concentrations in high producing cows (de Souza et al., 2018a; de Souza et al., 2019), but interactions between insulin responses and production level have been observed (de Souza et al., 2019). Similar to the current study,

Burch (2020) observed *cis*-9 C18:1 decreased plasma insulin compared with C16:0 and a non-fat supplemented control in cows averaging 50 kg/d milk yield. The OA treatment probably decreased insulin concentrations due to decreased DMI (Choi and Palmquist, 1996). FAT decreased BW and BW change, and increased BCS change compared with control, with no differences between FA supplements even though we observed different insulin responses between FA supplements. This could be due to C16:0 inducing insulin resistance in muscle cells and *cis*-9 C18:1 improving insulin sensitivity (Yuzefobych et al., 2010). Recently, C16:0 FA supplementation has been linked to elevated circulating ceramide levels in mid-lactation dairy cows, which acts as an insulin antagonist in bovine adipocytes, inhibiting lipogenesis and promoting milk production (Rico et al., 2016; 2018).

Possible limitations of our study may explain why we did not observe FA by AA interactions predicted by our hypothesis. To avoid oversupplying protein and reducing nitrogen efficiency with Met and Lys supplementation, we decreased overall MP content of the LP basal diet. Although we observed increases in milk fat yield with FA supplementation, we may have limited milk protein yields by not supplying enough MP, Lys, and other EAA for milk protein production. Additionally, we did not conduct digestibility analysis. However, we would not expect large effects of the FA supplements on nutrient digestibility based on previous work (Western et al., 2020b; Burch, 2020). Further research is needed to examine milk production responses to EAA supplementation at different levels of MP and interactions with different FA profiles.

Conclusions

A basal diet containing AA supplementation decreased MUN, BUN, and did not impact DMI or the yields of milk and milk components compared with a basal diet without AA supplementation. Both FA treatments decreased DMI (average of 0.6 kg/d) and increased ECM (average of 1.1 kg/d). Compared with PA, OA decreased DMI (0.6 kg/d) and plasma insulin (0.07 ug/L). We did not observe interactions between basal AA supplementation and supplementation of FA supplements with different ratios of C16:0 + *cis*-9 C18:1 on the yield of milk or milk components. Overall, FA supplementation increased milk production regardless of basal diet, and AA supplementation maintained protein production with a reduced MP supply. Production responses in our trial may have been limited by average production level, stage of lactation, or restricted MP supply in the AA supplemented basal diet.

APPENDIX

Table 4.1. AA supply of treatment diets[1] using actual feed chemistry and dry matter intakes.

	Basal Diet[2]					
	HP			LP		
Item	CON	PA	OA	CON	PA	OA
AA supply, g/d						
Arg	280	275	269	257	252	247
His	136	133	130	116	114	112
Ile	225	220	216	209	205	201
Leu	468	460	451	405	398	390
Lys	261	255	250	244	238	233
Met	80.1	78.8	77.2	93.4	92.0	90.2
Phe	262	257	252	232	228	223
Thr	187	183	179	172	169	165
Val	290	284	279	258	253	248
AA supply, g/100g						
Arg	5.54	5.55	5.55	5.58	5.59	5.59
His	2.69	2.69	2.69	2.53	2.52	2.52
Ile	4.45	4.45	4.45	4.55	4.55	4.55
Leu	9.27	9.29	9.30	8.79	8.81	8.82
Lys	5.17	5.15	5.15	5.30	5.28	5.27
Met	1.59	1.59	1.59	2.03	2.04	2.04
Phe	5.18	5.20	5.20	5.03	5.05	5.05
Thr	3.70	3.70	3.70	3.74	3.74	3.74
Val	5.74	5.74	5.75	5.60	5.61	5.61

[1]CON = no FA supplementation, PA = 1.5% of DM to provide approximately 80% C16:0 + 10% *cis*-9 C18:1, and OA = 1.5% of DM to provide approximately 60% C16:0 + 30% *cis*-9 C18:1.
[2]Basal diets containing either high CP without supplemental Met and Lys (HP) or low CP with supplemental Met and Lys (LP).

Table 4.2. FA profile of FA supplements[1]

Item	Fat Supplement[2]	
	C16:0-enriched FA Supplement	Ca-salt of palm FA supplement
Total FA content, % DM	94.8	80.5
FA profile of each FA supplement, g/100 g of FA		
C14:0	0.58	0.81
C16:0	88.9	64.5
C18:0	0.71	4.56
cis-9 C18:1	7.79	24.7
cis-9, *cis*-12 C18:2	1.50	4.39
cis-9, *cis*-12, *cis*-15 C18:3	0.05	0.04

[1]Average (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 4.3. Ingredient and nutrient composition of treatment diets[1]

	Basal Diet					
	HP			LP		
Item	CON	PA	OA	CON	PA	OA
Ingredient, % DM						
Corn Silage	37.3	37.3	37.3	37.4	37.4	37.4
Haylage	11.0	11.0	11.0	11.0	11.0	11.0
Soybean Meal	6.84	6.84	6.84	4.09	4.09	4.09
Ground Corn	16.7	16.7	16.7	17.1	17.1	17.1
Cottonseed	5.42	5.42	5.41	5.09	5.09	5.09
High Moisture Corn	7.41	7.41	7.41	7.33	7.33	7.33
Soybean Hulls	5.29	3.88	3.62	7.98	6.58	6.31
HP Mix	10.0	10.0	10.0	-	-	-
LP Mix	-	-	-	10.0	10.0	10.0
C16:0-enriched FA	-	1.42	-	-	1.42	-
C18:1-enriched FA	-	-	1.70	-	-	1.70
Nutrient Composition, % DM[5]						
Forage NDF	22.1	22.1	22.1	22.1	22.1	22.1
NDF	31.9	31.0	30.8	33.4	32.5	32.3
Starch	27.0	27.0	27.0	27.2	27.2	27.2
CP	18.1	17.9	17.9	16.6	16.4	16.4
RUP	6.98	6.88	6.88	5.59	5.49	5.49
RDP	10.3	10.2	10.2	10.2	10.1	10.1
MP	11.6	11.5	11.5	10.4	10.2	10.2
Lys, %MP	6.23	6.16	6.16	6.68	6.60	6.60
Met, %MP	1.73	1.71	1.71	2.19	2.16	2.16
AA	15.0	14.9	14.8	13.7	13.5	13.5
EAA	6.51	6.45	6.43	5.91	5.85	5.84
FA	2.33	3.65	3.68	2.31	3.64	3.66
16:0	0.41	1.60	1.29	0.40	1.59	1.28
18:0	0.06	0.06	0.12	0.08	0.08	0.14
cis-9 18:1	0.43	0.53	0.77	0.42	0.52	0.76
cis-9, *cis*-12 18:2	1.23	1.24	1.28	1.20	1.21	1.25
cis-9, *cis*-12, *cis*-15 18:3	0.15	0.15	0.15	0.15	0.15	0.15

[1]CON = no FA supplementation, PA = 1.5% of DM to provide approximately 80% C16:0 + 10% *cis*-9 C18:1, and OA = 1.5% of DM to provide approximately 60% C16:0 + 30% *cis*-9 C18:1.
[2]Basal diets containing either high CP without supplemental Met and Lys (HP) or low CP with supplemental Met and Lys (LP).
[3]HP Mix contained 46% Amino Plus (Ag Processing Inc, Omaha, NE), 9.8% corn gluten meal, 9.0% blood meal, 3.2% urea. Nutrient composition: 43.5 % CP, 4.6 % crude fiber, 5.8 % ADF, 5.0

Table 4.3. (cont'd)

% Ca, 4.3 % HP, 1.2 % Mg, 1.9 % K, 2.3 ppm Se, 23,200 IU/lb Vitamin A, 5,670 IU/lb Vitamin D3, 132 IU/lb Vitamin E, and <1% of each of the following: crude fat, P, and S.

[4]LP Mix contained 3.8% blood meal, 60% soybean meal, 3.2% urea, 0.6% Smartamine M (Adisseo, Alpharetta, GA), 0.6% Smartamine ML (Adisseo, Alpharetta, GA). Nutrient composition: 41.1 % CP, 4.2

% crude fiber, 4.8 % ADF, 4.5 % Ca, 4.3 % HP, 1.2 % Mg, 2.2 % K, 2.3 ppm Se, 23,200 IU/lb Vitamin A, 5,670 IU/lb Vitamin D3, 132 IU/lb Vitamin E, and <1% of each of the following: crude fat, P, and S.

[5]Palmitic acid supplement (Spectrum Fusion, Purdue Agribusiness, Salisbury, MD). Supplement is a blend including most of the saturated FA as a prill and most of the unsaturated FA as a Ca-salt. The supplement contained (g/100 g of fatty acid) 0.58 of C14:0,88.9 of C16:0, 0.71 of C18:0, 7.79 of *cis*-9 C18:1, and 94.8% total fatty acids.

[6]Oleic acid Ca-salt supplement (Mega-max; Volac Wilmar Feed Ingredients Limited, Hertfordshire, UK) The supplement contained (g/100 g of fatty acid) 0.81 of C14:0, 64.5 of C16:0, 4.56 of C18:0, 24.7 of *cis*-9 C18:1, and 80.5% total fatty acids.

[7]Expressed as a percent of as fed.

Table 4.4. Dry matter intake, milk production, milk composition, BW, and BCS for cows fed treatment diets (n=36)[1]

	FA Treatment[2]				Basal Diet[4]			P-value[5]			Contrasts[6]	
Variable	CON	PA	OA	SEM[3]	HP	LP	SEM[3]	Basal diet	FA	Basal diet × FA	CON vs FAT	PA vs OA
DMI, kg/d	33.6	33.3	32.7	0.41	33.2	33.2	0.55	0.97	<0.01	0.95	<0.01	0.01
Milk yield, kg/d												
Milk yield	52.6	53.3	53.6	1.00	54.2	52.2	1.40	0.32	0.03	0.37	0.01	0.46
3.5% FCM[7]	54.2	55.7	55.7	0.84	56.5	54.0	1.17	0.13	<0.01	0.48	<0.01	0.89
ECM[8]	54.1	55.2	55.1	0.83	55.8	53.8	1.16	0.23	<0.01	0.66	<0.01	0.77
Fat, kg/d	1.94	2.01	2.00	0.03	2.04	1.94	0.04	0.12	<0.01	0.31	<0.01	0.55
Protein, kg/d	1.65	1.65	1.63	0.03	1.65	1.64	0.04	0.96	0.17	0.78	0.25	0.13
Lactose, kg/d	2.56	2.59	2.60	0.05	2.64	2.52	0.07	0.20	0.19	0.58	0.07	0.79
Milk composition, %												
Fat, %	3.71	3.80	3.74	0.06	3.76	3.74	0.09	0.83	<0.01	0.46	0.02	0.02
Protein, %	3.15	3.11	3.05	0.03	3.04	3.17	0.05	0.05	<0.01	0.09	<0.01	<0.01
Lactose, %	4.87	4.86	4.86	0.02	4.88	4.84	0.02	0.28	0.05	0.58	0.02	0.64
MUN*, mg/dL	15.8	15.9	15.7	0.27	17.1	14.5	0.31	<0.01	0.71	0.66	0.84	0.42
NUE[9]	0.278	0.285	0.283	0.00	0.27	0.29	0.00	<0.01	0.01	0.32	<0.01	0.50
ECM/DMI	1.61	1.66	1.68	0.02	1.68	1.63	0.02	0.10	<0.01	0.38	<0.01	0.06
BW, kg	752	750	750	11.2	760	741	16.0	0.41	0.07	0.28	0.02	0.80
BWC*, kg	0.62	0.43	0.10	0.13	0.28	0.49	0.11	0.17	0.02	0.92	0.03	0.08
BCS	3.25	3.27	3.24	0.05	3.32	3.19	0.06	0.14	0.25	0.84	0.45	0.13
BCS change	-0.02	0.02	0.03	0.02	0.00	0.01	0.01	0.62	0.12	0.71	0.04	0.91

[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).

Table 4.4. (cont'd)

[2]CON = control; PA = 1.5% of FA supplement to provide approximately 80% of C16:0 + 10% of *cis*-9 C18:1; OA = 1.5% of FA supplement to provide approximately 60% of C16:0 + 30% of *cis*-9 C18:1.
[3]Greatest SEM
[4]Basal diets containing either high CP without supplemental Met and Lys (HP) or low CP with supplemental Met and Lys (LP).
[5]P-values refer to the ANOVA results for the fixed effects of treatment and period.
[6]Pre-planned contrasts included CON versus FAT: the comparison between the control treatment (CON) and the average [1/2 (PA + OA)] of the FA treatments (FAT); and OA versus PA: the comparison between the PA and OA treatments.
[7]Fat-corrected milk; 3.5% FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].
[8]Energy-corrected milk; ECM = [(0.324 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)].
[9]NUE = (N milk/N intake).
*Period by basal interaction observed ($P < 0.15$).

Table 4.5. FA concentrations and yields by source of milk FA for cows fed treatment diets (n=36)[1]

	FA Treatment[2]				Basal Diet[4]			P-value[5]			Contrasts[6]	
Variable	CON	PA	OA	SEM[3]	HP	LP	SEM[3]	Basal diet	FA	Basal diet × FA	CON vs FAT	PA vs OA
Summation by Source[7], g/100 g FA												
De Novo	28.3	25.6	24.8	0.31	26.1	26.4	0.42	0.53	<0.01	0.07	<0.01	<0.01
Mixed	37.7	41.0	39.9	0.34	39.7	39.5	0.47	0.80	<0.01	0.23	<0.01	<0.01
Preformed	33.9	33.2	35.2	0.32	34.1	34.1	0.44	0.95	<0.01	0.23	0.03	<0.01
Summation by Source[7], g/d												
De Novo	514	481	465	9.32	495	479	12.7	0.35	<0.01	0.76	<0.01	<0.01
Mixed	688	775	752	16.3	758	719	22.7	0.22	<0.01	0.11	<0.01	<0.01
Preformed	616	629	659	9.08	652	617	12.2	0.05	<0.01	0.13	<0.01	<0.01

[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 *cis*-9 C18:1; OA = 1.5% of FA supplement to provide approximately 60% of C16:0 + 30% of *cis*-9 C18:1.
[3]Greatest SEM
[4]Basal diets containing either high CP without supplemental Met and Lys (HP) or low CP with supplemental Met and Lys (LP).
[5]P-values refer to the ANOVA results for the fixed effects of treatment and period.
[6]Pre-planned contrasts included CON versus FAT: the comparison between the control treatment (CON) and the average [1/2 (PA + OA)] of the FA treatments (FAT); and OA versus PA: the comparison between the PA and OA treatments.
[7]De novo FA originated from mammary de novo synthesis (<16 carbons), preformed FA originated from extraction from plasma (>16 carbons), and mixed FA originated from both sources (C16:0 plus *cis*-9 C16:1

Table 4.6. Milk fatty acid composition (g/100 g FA) for cows fed treatment diets (n=36)[1]

	FA Treatment[2]				Basal Diet4			P-value[5]			Contrasts[6]	
Variable	CON	PA	OA	SEM[3]	HP	LP	SEM[3]	Basal diet	FA	Basal × FA	CON vs FAT	PA vs OA
Selected individual fatty acids, g/100g												
C4:0	2.87	2.92	2.99	0.04	2.98	2.87	0.06	0.18	<0.01	0.18	<0.01	<0.01
C6:0	2.10	2.00	1.99	0.03	2.05	2.00	0.04	0.38	<0.01	0.23	<0.01	0.24
C8:0	1.32	1.20	1.17	0.02	1.23	1.23	0.03	0.92	<0.01	0.13	<0.01	<0.01
C10:0	3.70	3.19	3.01	0.07	3.23	3.36	0.10	0.37	<0.01	0.19	<0.01	<0.01
C12:0	4.26	3.62	3.35	0.09	3.62	3.86	0.12	0.17	<0.01	0.27	<0.01	<0.01
C14:0	13.2	11.9	11.5	0.12	12.2	12.3	0.16	0.64	<0.01	0.12	<0.01	<0.01
C16:0	36.6	39.9	38.8	0.34	38.5	38.4	0.47	0.88	<0.01	0.23	<0.01	<0.01
cis-9 C16:1	1.11	1.21	1.14	0.03	1.20	1.11	0.04	0.12	<0.01	0.05	<0.01	<0.01
C18:0	8.70	8.31	8.54	0.17	8.67	8.36	0.23	0.33	<0.01	0.69	<0.01	0.01
cis-9 C18:1	16.4	16.5	18.1	0.18	17.0	17.1	0.24	0.92	<0.01	0.87	<0.01	<0.01
trans-6 to 8 C18:1	0.21	0.21	0.25	0.01	0.22	0.23	0.01	0.12	<0.01	0.03	<0.01	<0.01
trans-9 C18:1	0.14	0.14	0.17	0.00	0.14	0.15	0.00	0.27	<0.01	0.66	<0.01	<0.01
trans-10 C18:1	0.35	0.34	0.38	0.02	0.34	0.37	0.02	0.48	<0.01	0.98	0.42	<0.01
trans-11 C18:1	0.66	0.64	0.73	0.03	0.69	0.67	0.03	0.67	<0.01	0.71	0.09	<0.01
cis-9, *cis*-12 C18:2	2.10	2.00	2.10	0.04	2.09	2.05	0.05	0.58	<0.01	0.04	<0.01	<0.01
cis-9, *trans*-11 C18:2	0.31	0.32	0.36	0.01	0.33	0.34	0.02	0.73	<0.01	0.78	<0.01	<0.01
cis-9, *cis*-12, *cis*-15 C18:3	0.28	0.25	0.25	0.00	0.25	0.26	0.01	0.60	<0.01	0.33	<0.01	0.77

[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 *cis*-9 C18:1; OA = 1.5% of FA supplement to provide approximately 60% of C16:0 + 30% of *cis*-9 C18:1.
[3]Greatest SEM
[4]Basal diets containing either high CP without supplemental Met and Lys (HP) or low CP with supplemental Met and Lys (LP).
[5]P-values refer to the ANOVA results for the fixed effects of treatment and period.

Table 4.6. (cont'd)

[6]Pre-planned contrasts included CON versus FAT: the comparison between the control treatment (CON) and the average [1/2 (PA + OA)] of the FA treatments (FAT); and OA versus PA: the comparison between the PA and OA treatments.

Table 4.7. Milk fatty acid composition (grams per day FA) for cows fed treatment diets (n=36)[1]

	FA Treatment[2]				Basal Diet4			P-value[5]			Contrasts[6]	
Variable	CON	PA	OA	SEM[3]	HP	LP	SEM[3]	Basal diet	FA	Basal × FA	CON vs FAT	PA vs OA
Selected individual fatty acids, g/d												
C4:0	52.3	55.0	55.8	1.24	56.5	52.2	1.72	0.08	<0.01	0.07	<0.01	0.20
C6:0	38.3	37.7	37.1	0.89	39.0	36.5	1.22	0.14	0.05	0.91	0.03	0.22
C8:0	24.1	22.6	21.8	0.57	23.3	22.3	0.78	0.34	<0.01	0.99	<0.01	<0.01
C10:0	67.0	60.0	56.7	1.65	61.5	60.9	2.28	0.84	<0.01	0.70	<0.01	<0.01
C12:0	77.6	68.6	63.6	1.92	69.4	70.4	2.68	0.79	<0.01	0.96	<0.01	<0.01
C14:0	240	224	216	3.94	231	223	5.39	0.25	<0.01	0.88	<0.01	<0.01
C16:0	667	752	727	16.2	732	699	22.5	0.29	<0.01	0.25	<0.01	<0.01
cis-9 C16:1	20.3	22.8	21.3	0.69	22.8	20.1	0.95	0.05	<0.01	0.01	<0.01	<0.01
C18:0	158	158	159	3.75	165	152	5.01	0.07	0.77	0.30	0.79	0.51
cis-9 C18:1	298	313	340	4.93	326	308	6.73	0.05	<0.01	0.17	<0.01	<0.01
trans-6 to 8 C18:1	3.82	3.98	4.66	0.10	4.12	4.18	0.12	0.70	<0.01	0.21	<0.01	<0.01
trans-9 C18:1	2.50	2.60	3.09	0.08	2.74	2.72	0.08	0.88	<0.01	0.55	<0.01	<0.01
trans-10 C18:1												
trans-11 C18:1	11.9	11.9	13.3	0.43	13.0	11.8	0.56	0.12	<0.01	0.98	<0.01	<0.01
cis-9, *cis*-12 C18:2	38.1	37.6	39.3	0.77	39.6	37.1	1.06	0.11	<0.01	0.03	0.16	<0.01
cis-9, *trans*-11 C18:2	5.65	5.94	6.65	0.22	6.21	5.95	0.28	0.53	<0.01	0.94	<0.01	<0.01
cis-9, *cis*-12, *cis*-15 C18:3	5.04	4.66	4.65	0.10	4.85	4.72	0.14	0.51	<0.01	0.55	<0.01	0.76

[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 *cis*-9 C18:1; OA = 1.5% of FA supplement to provide approximately 60% of C16:0 + 30% of *cis*-9 C18:1.
[3]Greatest SEM
[4]Basal diets containing either high CP without supplemental Met and Lys (HP) or low CP with supplemental Met and Lys (LP).
[5]P-values refer to the ANOVA results for the fixed effects of treatment and period.

Table 4.7. (cont'd)

[6]Pre-planned contrasts included CON versus FAT: the comparison between the control treatment (CON) and the average [1/2 (PA + OA)] of the FA treatments (FAT); and OA versus PA: the comparison between the PA and OA treatments.

Table 4.8. Blood metabolites for cows fed treatment diets (n=36)[1].

	FA Treatment[2]				Basal Diet[4]			P-value[5]			Contrasts[6]	
Variable	CON	PA	OA	SEM[3]	HP	LP	SEM[3]	Basal diet	FA	Basal diet × FA	CON vs FAT	PA vs OA
Insulin, ug/L	0.67	0.70	0.63	0.03	0.67	0.66	0.03	0.86	0.01	0.58	0.60	<0.01
BUN, mg/dL	15.7	15.6	15.4	0.26	17.2	14.0	0.35	<0.01	0.07	0.43	0.09	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 *cis*-9 C18:1; OA = 1.5% of FA supplement to provide approximately 60% of C16:0 + 30% of *cis*-9 C18:1.
[3]Greatest SEM
[4]Basal diets containing either high CP without supplemental Met and Lys (HP) or low CP with supplemental Met and Lys (LP).
[5]P-values refer to the ANOVA results for the fixed effects of treatment and period.
[6]Pre-planned contrasts included CON versus FAT: the comparison between the control treatment (CON) and the average [1/2 (PA + OA)] of the FA treatments (FAT); and OA versus PA: the comparison between the PA and OA treatments

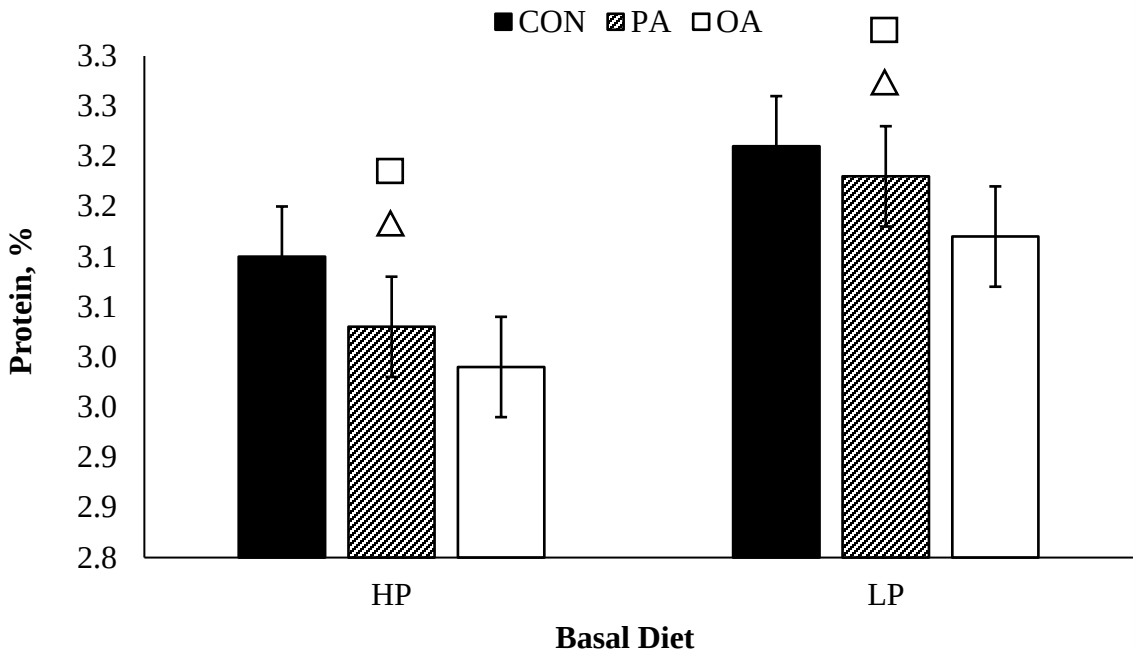

Figure 4.1. Interaction between basal diet and FA treatment for milk protein content for cows fed different FA treatments in both basal diets. An interaction between basal diet and FA treatment was detected for milk protein content (P = 0.09). Basal diets containing either high CP without supplemental Met and Lys (HP) or low CP with supplemental Met and Lys (LP).CON = non-FA supplemented control diet, PA = 1.5% of FA supplement to provide 80% C16:0 + 10% cis-9 C18:1, and OA = 1.5% of FA supplement to provide 60% C16:0 + 30% cis-9 C18:1. Error bars represent SEM. CON vs FAT (Δ) and PA vs OA (•) contrasts (P < 0.15) were to test the effects of FA supplementation and the difference between C16:0 and cis-9 C18:1, respectively.

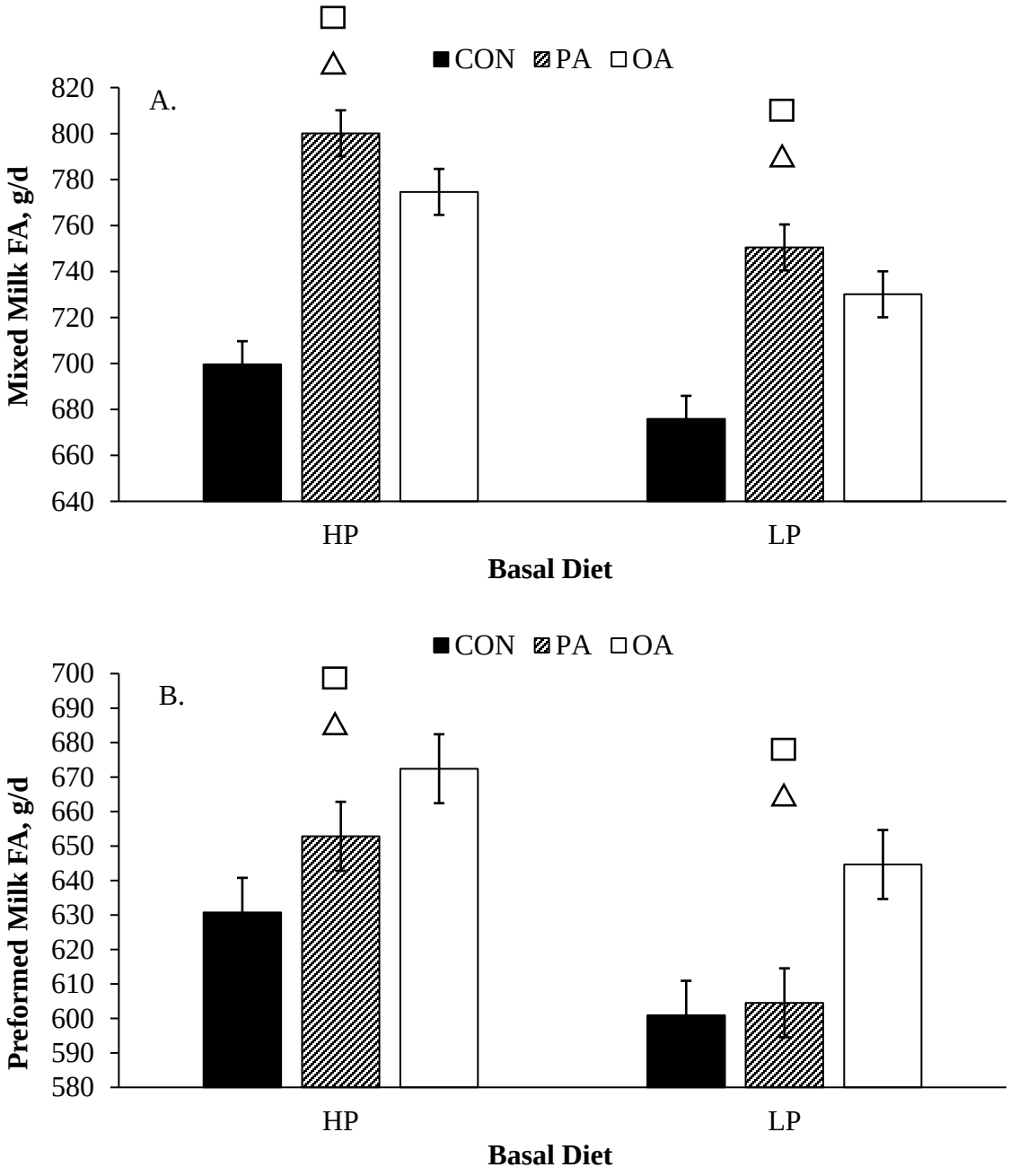

Figure 4.2. Interaction between basal diet and FA treatment for (A) mixed milk FA yield and (B) preformed milk FA yield for cows fed different FA treatments in both basal diets. A tendency for an interaction between basal diet and FA treatment was detected for mixed milk FA yield (P = 0.11) and preformed milk FA yield (P = 0.13). Basal diets containing either high CP without supplemental Met and Lys (HP) or low CP with supplemental Met and Lys (LP). CON = non-FA supplemented control diet, PA = 1.5% of FA supplement to provide 80% C16:0 + 10% cis-9 C18:1, and OA = 1.5% of FA supplement to provide 60% C16:0 + 30% cis-9 C18:1. Error bars represent SEM. CON vs FAT (Δ) and PA vs OA (•) contrasts (P < 0.15) were to test the effects of FA supplementation and the difference between C16:0 and cis-9 C18:1, respectively.

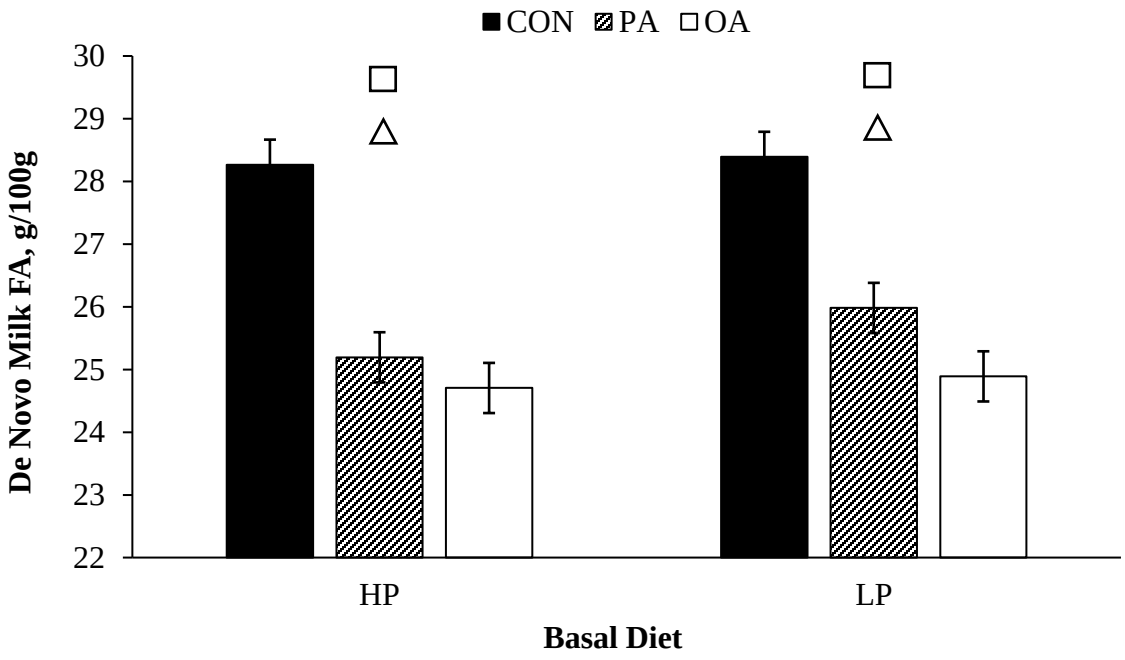

Figure 4.3. Interaction between basal diet and FA treatments on concentration of de novo milk FA for cows fed different FA treatments in both basal diets. An interaction between basal diet and FA treatments was detected for de novo milk FA concentration (P = 0.07). Basal diets containing either high CP without supplemental Met and Lys (HP) or low CP with supplemental Met and Lys (LP). CON = non-FA supplemented control diet, PA = 1.5% of FA supplement to provide 80% C16:0 + 10% cis-9 C18:1, and OA = 1.5% of FA supplement to provide 60% C16:0 + 30% cis-9 C18:1. Error bars represent SEM. CON vs FAT (Δ) and PA vs OA (•) contrasts (P < 0.15) were to test the effects of FA supplementation and the difference between C16:0 and cis-9 C18:1, respectively.

CHAPTER 5

Overall Conclusions

Supplementation of FA and AA can increase the yields of milk and milk components and thus can help achieve farm production goals. Limited studies have investigated possible interactions between dietary FA and AA, and have not observed any interactions for the yields of milk fat or milk protein. The metabolic flexibility of the mammary gland allows for variable responses to dietary supplementation and nutritional strategies, which support milk production under periods of nutrient deficiency, but also creates complexity in manipulating the yields of milk fat and milk protein. Although considerable research has examined production responses to protein and fat, responses may be impacted by individual AA and FA supply. The objective of this thesis was to determine milk production responses of high-producing dairy cows supplemented with C16:0 and rumen-protected methionine and lysine in low forage diets, as well as the effect of basal diets with or without AA supplementation and their interactions with different ratios of C16:0 + *cis*-9 C18:1. Together, these studies examined the interaction between the predominant FA included in commercially available FA supplements and dietary AA content on performance of lactating dairy cows.

In Chapter 3, a low forage diet containing a C16:0-enriched FA supplement and bypass methionine and lysine increased DMI and the yields of milk fat and protein compared with a control diet containing typical midwestern forage inclusion rates. Cows on the LF treatment consumed 5.5 kg/d less forage DM compared with CON, yet maintained milk yield and increased ECM. Thus, these results indicate that under certain circumstances where forage inventories are limited, low forage diets can be formulated to sustain, or even increase, milk fat and protein yields in high producing cows.

In Chapter 4, a basal diet containing low CP and supplemental methionine and lysine decreased MUN, BUN, and did not impact DMI or the yields of milk and milk components compared with a basal diet containing high CP without supplemental methionine and lysine. Both FA treatments decreased DMI and increased ECM. Compared with PA, OA decreased DMI and plasma insulin. We did not observe interactions between basal AA supplementation and supplementation of FA supplements with different ratios of C16:0 + *cis*-9 C18:1 on the yield of milk or milk components. These results indicate that FA supplementation increased milk production regardless of basal diet, and AA supplementation maintained protein production with a reduced MP supply.

Both chapters support recent research completed in our lab and other labs. Although we cannot determine if the production responses observed were specifically from FA and AA supplementation or other dietary components in Chapter 3, we observed that a low-forage diet containing supplemental C16:0, methionine, and lysine increased the yields of milk fat and milk protein compared to typical midwestern diet without FA and AA supplementation. Similarly, Schwab and Broderick (2017) observed that supplemental methionine and lysine can increase milk protein yield, and Western et al. (2020a) found that C16:0 supplementation increased milk fat yield compared to a non-FA supplemented control. Our study in Chapter 4 observed C16:0 and *cis*-9 C18:1 supplementation increased milk yield and milk fat yield, similar to Burch (2020). Although our method of supplementing FA and AA differed from Nichols et al. (2019), we also did not observe any interactions between FA and AA supplementation on the yields of milk fat and milk protein. We possibly did not observe an interaction in this trial due to the average production level or stage of lactation of the cows, or restricted MP supply in the LP basal diet. In conclusion, blends of C16:0 and *cis*-9 C18:1 can increase can increase the yields of

milk and milk fat regardless of basal AA content, and milk protein yield can be maintained with methionine and lysine supplementation in lower MP diets. This work allows for more precise feed management decision making to increase milk production yields with different basal dietary nutrient contents. Further research is needed to determine metabolic interactions between specific FA supplementation and supply of EAA.

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