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INVESTIGATING THE BEHAVIOR OF FATTY ACIDS FROM WHOLE COTTONSEED AND FAT SUPPLEMENTS IN THE RUMEN OF DAIRY CATTLE AND THE EFFECTS ON MILK FAT PRODUCTION

A Thesis in

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ABSTRACT

Dietary fat is an important component on the diets of lactating dairy cattle. Concentration of dietary fat can be increase by feeding oilseeds or enriched fat supplements which allows cattle to partition energy differently which can lead to increases in milk fat concentration, milk fat yield, or milk yield. One common oilseed fed to dairy cattle is cottonseed, as it is high in fiber but also relatively high in fat compared to other common feedstuffs. Fat supplements are very high in fat (>95%) but are more expensive so dairy producers often strike a balance and may feed both fat supplements and oilseeds to dairy cattle.

The first objective of this thesis was to investigate the effects of cottonseed on milk production in dairy cattle. Previous research feeding cottonseed fed inclusion rates of cottonseed in excess of 15% of dry matter intake (**DMI**), but cattle were producing less milk and consuming less so therefore, the actual mass of the cottonseed consumed may not be as high as contemporary dairy cattle. This thesis fed cottonseed at up to 9.9% of DMI and found that cottonseed inclusion into the diets of multiparous cattle did not affect milk yield or milk composition but led to a decrease in DMI indicating that it could be safely fed to mature cows. In primiparous cattle, cottonseed inclusion induced milk fat-depression, indicating that the level of unsaturated fatty acids in the diet was greater than the biohydrogenation potential of the ruminal microbes of these animals. The second part of this thesis was to examine the effects of increased concentrations of an unsaturated fatty acid (*cis*-9 C18:1; oleic acid) in a prilled fat supplement on the milk production and milk composition in dairy cows. Previous research suggests that oleic acid may increase digestibility of dietary fatty acids and consequently increase the amount of preformed fat for milk fat synthesis. This experiment indicated that fat supplementation in multiparous cows may decrease milk yield and DMI but was no effect of increased levels of oleic acid on other production

components in dairy cattle. Further investigation of the data collected for each half of the thesis is required to determine the effects on the respective methods of fat supplementation on fatty acid digestibility in lactating dairy cattle.

Keywords: cottonseed, milk fat, fatty acid, milk fat depression

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LIST OF ABBREVIATIONS

ALA - α-linolenic acid

BH - biohydrogenation

BHBA - beta-hydroxy butryric acid

CLA - conjugated linoleic acid

DGAT1 - diacylglycerol acyltransferase 1

DHIA - Dairy Herd Improvement Association

DMI - dry matter intake

dNDF - digestible neutral detergent fiber

EE - ether extract

FA - fatty acid

FASN - fatty acid synthetase

GHR - growth hormone receptor

HSL - hormone sensitive lipase

LA - linoleic acid

LPL - lipoprotein lipase

MFA - monounsaturated fatty acid

MFD - milk fat depression

MgO - magnesium oxide

NEB - negative energy balance

NEFA - non-esterified fatty acid

OA - oleic acid

PA - palmitic acid

PUFA - poly unsaturated fatty acids

QTL - quantitative trait locus

SA - stearic acid

SCD - Δ^9-desaturase

SCFA - short chain fatty acid

SFA - saturated fatty acid

SNP - single nucleotide polymorphism

SREBP-1 - sterol regulatory element binding protein-1

TAG - triacylglycerol

TMR - total mixed ration

TTD - total tract digestibility

UFA - unsaturated fatty acids

VLDL - very low-density lipoprotein

WCS - whole cottonseed

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Chapter 1

Introduction

Dairy cattle milk yield has steadily increased in recent decades. As production increases, the cow's energetic demand also increases so more energy-dense and more digestible feedstuffs are required for the diets of high producing dairy cattle to support lactation. The value of milk fat has increased in recent years leading to an increase in research surrounding the role of diertary lipids of dairy cows. Lipids fed to cattle generally originate from plant sources, palm oil-based dry fat supplements have gained popularity in recent years as cultivation of palm trees has risen steadily. However, some of these fat supplements are often criticized for having a low digestibility. High-fat forage sources are an alternative to saturated fat supplements but often contain high levels of polyunsaturated fatty acids (**PUFA**). These are altered through biohydrogenation in the rumen of dairy cattle and high levels of PUFAs can pose an increased risk for diet-induced milk fat depression (**MFD**).

Dry fat supplements were first developed in the 1970s as calcium salts and were a blend of saturated and unsaturated fatty acids. In recent years, it was discovered that palmitic acid (**PA**) seems to increase milk fat concentration more than other other fatty acids (**FA**) (Piantoni et al., 2013; Chamberlain and DePeters, 2017; de Souza and Lock, 2018). Consequently, the level of PA in dried fat supplements began to increase. However, recent work shows highly enriched PA fat supplements have a lower digestibility due to the crystalline FA structure, resulting in a higher melting point of the supplement. Blends of PA and stearic acid (**SA**) have been commonly used in dry fat prills for many decades, but the digestibility of SA may be lower in some of these supplements. Consequently, alternate methods for increasing the digestibility of fat supplements has been a focus in recent years. Research trials have examined including lysolecithin to increase

emulsification capacity of the small intestine or adding oleic acid (**OA**) to the fat supplement. Oleic acid is hypothesized to both decrease melting point of the fat supplement and potentially act as an emulsifier for other fatty acids in the duodenum. However, some OA will be biohydrogenated in the rumen and therefore its efficacy to increase the digestibility of dry fat supplements is currently unknown.

Whole linted cottonseed (**WCS**) is generally a cheaper source of dietary FA than commercial dry fat supplements. In addition, WCS contributes a large amount of NDF to a TMR and can help promote a healthier rumen by stimulating more rumination and thus better buffering in the rumen. The fat in cottonseed is contained within the fibrous seed coat and is potentially more slowly available in the rumen. This allows producers to feed greater quantities of unsaturated FA without greatly increasing the risk for MFD as the majority of FA in WCS are unsaturated FA. WCS does have certain drawbacks that limit its inclusion in dairy cattle diets. WCS and other cottonseed products contain a natural pigment called gossypol that is toxic to animals when ingested above certain levels. The levels are species-dependent, but ruminants appear to be more resistant to gossypol toxicity than non-ruminants due to the rumen microbes' ability to metabolize the compound.

However, little work has been completed to examine the effect of processing of feeds and source of lipids on rumen availability of lipids. In addition, the effects of FA profile on dry fat supplement formulation requires further investigation to properly examine how to maximize the efficacy of dry fat supplements. Therefore, the objective of this thesis was to characterize the availability of FA from cottonseed, examine production responses of feeding of cottonseed, and investigate methods for increasing the digestibility of a palm fatty acid supplement.

Chapter 2

Literature Review

Feed costs are generally the highest single production expense on a dairy farm, therefore feeding the most economical feeds is important for improving profitability. Maximizing the return on investment in feed requires understanding the behavior of feeds and nutrients in the feed after they are ingested and move through the digestive tract. Rumen behavior and total tract digestibility (**TTD**) are two items that are important to consider when balancing diets and calculating the value of feeds. Currently, this is an area that is receiving much more attention in regard to dietary fat in the rations of dairy cattle as all plant-based ingredients contain some level of fat, and certain ingredients, like oilseeds, can contain up to 20% of DM as fat. Therefore, dietary fat behavior in the rumen and digestive tract should be characterized for all feeds to examine the causes of differences in FA digestibility between various fat supplements and feeds that could contribute a high load of fat to a dairy cattle ration.

Milk Fat

It is necessary to discuss regulation of milk fat before further examining the effects of dietary fat in the rumen and post-absorptive mechanisms in the cow. Milk fat is predominantly triglycerides (>95%) coated with phospholipids and specific proteins that make up the milk fat globular membrane (Bauman and Griinari, 2003). Milk FA originate from two primary sources and are commonly categorized as preformed and de novo synthesized. Preformed FA are taken up as FA by the mammary gland from the blood. They either originate directly from absorption in the gut or come from export from other peripheral tissues such as the liver or adipose tissue. The FA released from other peripheral issues may have originated from absorbed FA and may differ from

their absorbed form because of desaturation and possible elongation. On the other hand, de novo FA are completely synthesized by the cow in the mammary gland from short chain fatty acids (**SCFA**) (Loften et al., 2014) by fatty acid synthase which can synthesize FA up to 16 carbons in length.

The FA in milk can be separated into three categories based on chain length. All of the even-carbon, straight chain FA less than 16 carbons in length are assumed to originate from de novo (C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, and C14:1) (Bauman and Griinari, 2003). The 16 carbon FA are considered a "mixed source" as they originate either from de novo synthesis or preformed uptake. Lastly, FA that are C18 or longer originate from preformed FA uptake because the mammary gland has little to no activity of elongase enzymes that synthesize 18 carbon FA from 16 carbon FA.

De novo synthesis of FA occurs primarily in the mammary gland (Dils, 1986) from acetate and butyrate. These originate from rumen fermentation and are absorbed across the rumen epithelium into the bloodstream. Very little acetate is metabolized within the epithelium although some is converted to butyrate. Butyrate is converted to beta-hydroxy butyric acid (**BHBA**) by the rumen epithelium and liver and extensively oxidized by the rumen epithelium resulting in little delivered to the mammary gland. BHBA can be used as a four-carbon molecule for the initiation of de novo FA synthesis and acetate is used for further elongation of the new FA and provides half of the reducing equivalents needed for de novo FA synthesis (Bauman and Griinari, 2003).

Preformed FAs follow a different path into the mammary gland (Mansbridge and Blake, 1997). They are absorbed in the small intestine and move into the bloodstream as chylomicrons. Within the blood, they may be taken up from chylomicrons by the mammary gland and other tissues that have lipoprotein lipase (**LPL)** expression. If they are taken up by adipose or liver tissue,

they may be recycled back to the bloodstream as either non-esterified FAs (**NEFAs**) or very low-density lipoproteins (**VLDLs**), respectively, when they would then be available for uptake by mammary epithelial cells and secreted into milk.

Regulation

Fat synthesis in the mammary gland is regulated primarily by sterol regulatory element binding protein-1 (**SREBP-1**) (Harvatine et al., 2009). SREBP-1 is a transcription factor that influences the expression of genes important for the metabolism of fats. One cell culture experiment showed that important enzymes related to de novo FA synthesis including acyl-coenzyme A (**CoA**) synthetase, acetyl-CoA carboxylase, FA synthetase, and isocitrate dehydrogenase are regulated by SREBP-1 expression (Ma and Corl, 2012). In addition, it was demonstrated that SREBP-1 expression is decreased during periods of MFD (Bauman et al., 2008) indicating the lower level of SREBP-1 was strongly associated with lower milk fat synthesis. This condition will be discussed more in depth later. The enzymes whose expression is regulated in part by SREBP-1 are primarily involved in de novo fat synthesis. Isocitrate dehydrogenase and acetyl-CoA carboxylase provide substrates for FA synthesis whereas acyl-CoA synthetase and FA synthetase are two of enzymes that contribute to FA elongation.

Milk fat production in the dairy cow is partially dependent on de novo FA synthesis but preformed FAs account for nearly half the FA in milk. Generally preformed FAs are fully saturated after leaving the rumen due to biohydrogenation (**BH**). However, up to 25% of the individual FA in milk fat are unsaturated (Chilliard et al., 2007). In the mammary gland, hepatic tissue, and adipose of dairy cattle, Δ^9-desaturase (**SCD**) reconverts SFA into monounsaturated FA (**MFA**) forming cis-9 isomers of FA. As MFA can originate from multiple sources, the pathway of FA

transfer to milk fat can occur in several ways. Monounsaturated FA synthesized in hepatic tissues would be packaged as VLDLs before export into the blood. Lipolysis of MFA from adipose tissue would leave as NEFAs in the blood. The NEFAs can then be taken up or repackaged by hepatic tissue. Triglycerides in LDL and VLDL can be metabolized by any cells expressing LPL.

Lipoprotein lipase expression is mediated by several factors including blood insulin level. Insulin increases LPL expression and concurrently limits lipolysis by suppressing hormone sensitive lipase (**HSL**). Hormone sensitive lipase hydrolyzes bonds between glycerol and FA in the adipose cells which allows for the export of NEFAs and glycerol (Rodwell et al., 2018). In a dairy cow, excessive insulin can suppress milk fat by limiting the availability of preformed FA for milk fat through both uptake of FA in triglycerides and decreased mobilization through lipolysis.

Factors Impacting Milk Fat Percentage

Milk fat production is impacted by many factors that are classified as either dietary factors or non-dietary factors. Dietary factors like digestible neutral detergent fiber (dNDF) level, dietary fat percent, and dietary FA profile can all change the quantity and distribution of FA in milk. Higher dNDF provides greater acetate and potentially more BHBA for de novo fatty acid synthesis. Lower dietary fat can alter the source of FA for milk fat by increasing the concentration of de novo fatty acids. In addition, higher levels of PA, SA, or polyunsaturated 18-carbon FA can increase the quantity of preformed FA in milk and, potentially, increase the total quantity of overall milk fat production.

Parity

Parity is a non-dietary factor which has an impact on total kilograms of milk fat produced. A past summary of a Dairy Herd Improvement Association (**DHIA**) database suggests milk fat percentages are the same between primiparous and multiparous dairy cattle, but multiparous cows

make a higher volume of milk (Schutz et al., 1990). Therefore, the total quantity of milk fat produced daily is increased in the multiparous cattle. This tendency was reinforced in recent research trials which studied primiparous and multiparous animals and reported no difference for milk fat concentration between parities (Drackley et al., 2003; de Souza and Lock, 2018).

Seasonality

Environment and season also play an important role in the secretion of milk fat. It has been established that there is annual rhythm of milk fat production (Salfer et al., 2019). Salfer et al. (2019) concluded that milk fat percentage generally peaks around January 1 of each year and reaches a minimum around 6 months later in dairy cattle managed in the Northern Hemisphere. It also suggests that average milk fat concentration fluctuates throughout the year and minimum and maximum milk fat concentrations can differ by as much as 0.25 percentage units depending on the geographical location within the United States. The annual rhythm of milk fat yield was also characterized and generally peaks in late February as milk yield peaks in April. Furthermore, these trends are consistent across all parities, reinforcing prior research that parity does not affect milk fat percentage. Exact causes of these patterns are unknown but environmental changes such as average daily temperature and day length may be factors.

Stage of Lactation

Milk fat percentage varies greatly over the course of a lactation. In fresh and early lactation animals, dry matter intake (**DMI**) cannot support all the necessary energy required for milk production leading to negative energy balance (**NEB**). NEB in animals suppresses insulin levels and therefore adipose tissue mobilization into the blood is increased due to an increase in hormone sensitive lipase (**HSL**) activity. Increased blood NEFA concentration allows for greater preformed FA concentrations early in lactation, particularly C18:0 (Stoop et al., 2009), and milk fat

percentage is also increased in the early post-partum period as milk production is not yet at its peak. The increase in milk yield to its peak contributes to the decrease in milk fat concentration due to dilution factors, and milk fat percentage reaches a minimum at the peak of milk yield. As milk production declines with persistency of lactation, the milk fat percentage rises, but total milk fat production decreases (Schutz et al., 1990).

Genetics

Genetics are another non-dietary factor that impact the secretion of milk fat. Heritability estimates for milk fat percent are around .45 (Welper and Freeman, 1992). The main contributor to the effect of genetics on milk fat is in the diacylglycerol acyltransferase 1 (**DGAT1**) gene at the K232A single nucleotide polymorphism (**SNP**) (Signorelli et al., 2009). Signorelli et al. (2009) also reported the F279Y SNP on the growth hormone receptor (**GHR**) gene also has a positive impact on milk fat production in dairy cattle but its effect is less than that of K232A. In a genome-wide association study, it was concluded that two other quantitative trait loci (**QTL**) in addition to the previously mentioned regions account for over 46% of the milk fat percentage in dairy cattle (Wang et al., 2012). Very little research has examined genetic effects on de novo FA synthesis. Some data suggests that over 9% of the genetic variation in caprylic acid is due to a QTL on BTA17 (Duchemin et al., 2014).

Circadian Pattern of Milk fat Production

Daily patterns exist in milk fat production throughout the day. Observational data shows that milk fat production is greater during the day than at night by an average of .04 kg per cow per 12 hr milking interval (Hargrove, 1994). Milk fat percentage is lesser at evening milkings

compared to morning milkings in cows milked 2x/day as milk yield was greater during the night and therefore dilution factors would further lower component percentages (Quist et al., 2008). Quist et al. (2008) also reported that milk fat concentration was highest in cows milked at night compared to morning or afternoon when the animals were milked 3x/day. These conclusions were reached after examining milk samples collected from commercial herds comparing evening (PM) milkings to morning (AM) milkings although the exact times of the milkings were not reported. The data was confirmed recently by Salfer and Harvatine (2020) where authors reported a tendency for milk fat concentration to peak shortly after midnight was found. This study did periodically restrict feed intake to examine the effects of timing of feed intake on the milk yield and milk components, but found that milk fat percentage did follow the same pattern as previous studies.

Dietary Interventions to Maximize Milk fat

Several ration formulation strategies and feeding strategies affect milk fat. Briefly, there is the production of acetate from dietary fiber, added dietary fat, FA profile of the dietary fat, and diet-induced MFD. The first three strategies are utilized to increase milk fat percentage while the latter is utilized to avoid a reduction in milk fat production.

Acetate

Acetate is produced in the rumen during the fermentation of dietary carbohydrates or can be ingested with silage as a byproduct of silage fermentation. Previously, it was thought that the acetate: propionate ratio was a contributing factor in diet-induced MFD. However, research surrounding high grain and high starch diets showed that propionate production was increased while acetate production was maintained, thus lowering the proportion (Bauman and Griinari,

2003). More recent work suggests that increasing acetate supply in the rumen increases de novo FA synthesis and therefore overall milk fat production. In a study ruminally infusing sodium acetate, milk fat production was increased with the largest increase seen in de novo and mixed FA production (Urrutia and Harvatine, 2017). In a separate feeding trial, similar results were obtained by feeding acetate in the TMR as sodium acetate to increase acetate supply in the rumen (Urrutia et al., 2019). This experiment also reported an increase in mixed and de novo fatty acids.

Supplemental Dietary Fat

Dietary fat has been added to dairy cattle total mixed rations (**TMR**) for many years with sources ranging from whole oilseeds like soybeans cottonseed, and canola, or animal and plant byproduct oils like tallow, calcium salts, or palm-based supplements. Added dietary fat sources are generally supplemented at 2 to 4% of DM in a ration but total ether extract (**EE**) concentration in a TMR rarely exceeds 7% of the ration. Early research examining additional dietary fat found that oils tended to increase dietary fat less than lipids fed as oilseeds or but decreased milk fat concentration when compared to unsupplemented diets.

Research on dietary fats has had different objectives over time. Some have focused on increasing energy intake, some on changing fatty acid profile of meat and milk, and more recently work has focused on increasing milk fat yield. The early research was conducted using fish oils rather than plant oils so the FA profile of the oils would be different and contain higher levels of PUFA providing greater potential to decrease milk fat production (Moore et al., 1945). Tallow was another common fat supplement but it also had a tendency to decrease milk fat production (Miller et al., 2009). Consequently, feeding oils and tallow was curtailed and palm fats, calcium salts of palm fats, and oilseeds gained popularity as they have fewer deleterious effects (Palmquist and

Jenkins, 1980) with greater nutritional value (Rabiee et al., 2012). In a separate meta-analysis of various oilseeds and processing methods, it was also suggested that soybean and sunflower oil decreased milk fat compared to a control (unsupplemented) diet, but, the same analysis reported no difference in milk fat when comparing added linseed and rapeseed oil to an unsupplemented diet (Glasser et al., 2008a). Glasser et al. (2008a) and Rabiee et al. (2012) did report that oilseeds may decrease milk fat percentage, but this includes any processing of oilseeds aside from solvent extraction of oils. Both papers did suggest that reacted palm fats and prilled fats increase milk fat compared to unsupplemented diets.

Fatty Acid Profile and Milk fat Production

The FA profile of a diet can have impacts on overall milk fat production of dairy cattle. PA has been shown to have a very positive effect on milk fat production whereas 18-carbon FA including SFA, MFA, and PUFA have less of an effect (Chamberlain and DePeters, 2017) and seem to be stored as adipose tissue more readily than 16-carbon fats as greater quantities of 18C FA are absorbed but are not secreted as milk fat. In addition, as dietary preformed FA concentrations increase, de novo FA synthesis tends to decrease and milk fat production can remain unchanged, decrease or increase. A reduction in milk fat may be the result of excessive dietary PUFA leading to altered BH and inhibition of milk fat synthesis.

Prevention of Biohydrogenation-Induced Milk fat Depression

Biohydrogenation of unsaturated FA (**UFA**) in the rumen of cows was first discussed in 1945 (Moore et al., 1945). This paper noted that iodine index of milk fat collected from cows given a large dose of cod liver oil was greater than that of cows given the same quantity of oil over 12

timepoints throughout the day. This indicates that cows can convert MFA and PUFA to SFA but there are limits to ruminal BH. However, an overload of unsaturated FA or other ruminal conditions like sub-acute ruminal acidosis can alter microbial populations and the predominant BH pathway. Intermediates of the alternate BH pathway may flow from the rumen and inhibit milk fat synthesis in the mammary gland. This process will be discussed more in depth later.

Fatty Acid Digestion in Dairy Cattle

Fatty acid digestion in dairy cattle is starkly different than FA digestion in non-ruminants due the microbial populations in the rumen. The differences in digestive tract anatomy also changes the locations of important processes related to FA digestion: namely lipolysis, hydrolysis, and emulsification.

Fatty Acid Behavior in the Rumen

The dairy cow's rumen becomes the primary spot for rapid and nearly complete hydrolysis of FA from the glycerol backbone in a triacylglycerol (**TAG**). The NEFA may then be incorporated into the membranes of microbes but are predominantly adsorbed onto feed particles in the digesta (Bauchart, 1993). In addition, the negatively charged carboxylic head of the NEFA allows for interaction with cations (generally calcium and magnesium) in the rumen digesta, forming inert soaps of fatty acids to a certain extent. This ionic interaction can provide some protection from ruminal BH of unsaturated FA but calcium will dissociate from FA and the dissociation may be higher in fat sources that contain higher levels of unsaturated FA (Sukhija and Palmquist, 1990). Therefore, a higher percentage of ingested unsaturated FA will undergo BH in the rumen prior to passage to the abomasum.

Fatty Acids in the Small Intestine

Dairy cattle have very little capacity for hydrolysis of FA in the small intestine so only a limited amount of TAG can be hydrolyzed in the small intestine due to limited pancreatic lipase (Lock et al., 2006). However, esterified lipids are extensively hydrolyzed by microbes in the rumen and remaining TG entering the small intestine are in a limited enough quantity that intestinal and pancreatic lipases are enough to hydrolyze them. In the duodenum, bile salts, lecithin, and bicarbonate secretions raise the pH of the digesta and begin the emulsification process (Doreau and Chilliard, 1997). The NEFA that were hydrolyzed in the rumen dissociate from fiber particles and form micelles in conjunction with lysolecithin. It has been suggested in work conducted in ovine that most FA in the micelles are absorbed in the middle and lower jejunum as micelles primarily form after the duodenum (Leat and Harrison, 1975). Absorptive capacity in the small intestine has certain limits for FA absorption (Van der Honing and Tamminga, 1986), but intake of FA does not appear to ever reach these levels (Doreau and Chilliard, 1997). Micelles are absorbed into the enterocytes and packaged as chylomicrons before entering lacteals. The chylomicrons are transported through the lymphatic system to arterial blood and transported throughout the body of an animal and digested in processes similar to cholesterols (Van Soest, 2018).

Fatty Acid Sources

Nearly every feed consumed by dairy cattle contains some level of FA, including oilseeds processed with solvent extraction to remove oils. There are certain feeds that naturally contain higher levels of certain FA and the FA profile of feeds often varies between sources. Dry fat supplements are another, more recent source of FA that allows producers to better control the FA

profile of feed, but this does not come without its challenges. Some of the most common sources and any challenges with feeding them will now be discussed.

Soybeans

There are many different seeds and byproducts that contain a relatively high level of fat. Generally, these feeds are oilseeds, but they are often processed with a solvent extraction or extrusion to remove the oil for other purposes. One primary example of such a feed is soybean meal. Raw soybeans contain about 19% EE. After processing, soybeans generally contain between 1 to 8% EE (NRC, 2001) depending on whether the beans were processed chemically or mechanically.

Recent work developing soybeans has led to the release of high-oleic soybeans as high-oleic oils provide a longer oil life in deep fryers used in human food production (Waltz, 2010). This new technology changes the FA profile of the soybeans by reducing the amount of linoleic acid (**LA**) and α-linolenic acid (**ALA**) and increasing the oleic acid levels in the beans. Recent animal feeding trials suggest that high oleic soybeans provide value in animal feed by increasing milk fat percentage and total milk fat production when the beans were either extruded or fed whole (Lopes et al., 2017). This suggests that inclusion of high oleic soybeans into the diets of dairy cattle provides more value than conventional soybeans.

Canola

Canola was developed as a variant of rapeseed with FA profile that contains increased levels of OA and lower levels of LA. It is rarely fed as a whole seed to dairy cattle in the USA as the oil is commonly used for human food production. Canola is processed into a meal and the oil

is extracted prior to inclusion in TMRs for dairy cattle so canola is considered a protein source for cattle, rather than as an energy source, and only contains about 5% EE (NRC, 2001). In addition, protein sources are not the primary component in dairy cattle rations and so the overall contribution from canola to dietary fat is limited, if it is fed. Nevertheless, there will be some level of FA intake if any canola products are included in the TMR.

Whole Cottonseed

The final oilseed that is fed to dairy cattle at high rates is WCS and it contains around 19% EE. Cottonseed can be processed to produce cottonseed oil for human use, cottonseed hulls, and cottonseed meal for animal consumption. The by-products from cottonseed oil production contain an EE level of 1 to 2.5% (NRC, 2001) but are more commonly used for other species as they can be expensive. WCS has been researched extensively, especially in the later part of the 20th century. Many trials report positive effects from feeding WCS when compared to unsupplemented diets or diets containing other oilseeds (Anderson et al., 1979, 1984; Coppock et al., 1987; Harrison et al., 1995). Often, the result of these experiments showed that WCS maintained or increased milk fat and a titration experiment examining diets containing WCS inclusion from 0% up to 20% of DMI concluded that more WCS in the TMR further increased milk fat production (DePeters et al., 1985).

However, the cotton plant has a natural pigment found in high quantities within the seed coat called gossypol that acts as a pest repellant. This pigment is toxic to animals at certain doses so high inclusion rates of cottonseed products, including WCS, are discouraged (Poore and Rogers, 1998; Myer et al., 2018). The maximal rate of inclusion of this by-product is variable as the gossypol availability and gossypol concentration changes between different cotton derivatives. Gossypol exists in two forms: free gossypol is the form primarily found in WCS and chemically

processed cottonseed and bound gossypol is gossypol bound to amino acids. Free gossypol is more toxic and more likely to cause gossypol toxicity, a condition that predominantly causes circulatory failure (Wang et al., 2009). Ruminants have lower susceptibility to gossypol toxicity than monogastric animals as the rumen microbes can detoxify and metabolize gossypol, but this is only seen in fully functioning ruminants. Therefore, calves are more susceptible to gossypol toxicity than lactating dairy cattle. Often, this is not a concern as WCS and cottonseed byproducts are primarily fed to adult cattle with developed rumens. Nevertheless, cottonseed byproducts have higher concentrations of free-gossypol and bound-gossypol so there are guidelines for any feeding cottonseed products based on gossypol concentration that suggest WCS should be no more than 15% of a diet (Arieli, 1998).

Dried Distillers Grains with Solubles

One high-fat, non-oilseed feed included in dairy cattle rations is distillers' grains with solubles (**DGS**). This byproduct of ethanol production often contains between 4 to 12% EE but unlike the previously mentioned feeds, the fat is not contained within the seed following the fermentation process. Consequently, the quantity of unsaturated FA entering the omasum is elevated by increased DGS inclusion in a TMR but does not increase as much as intake of unsaturated FA. Chibisa et al. (2013) fed wheat DGS (4.58% fat) at up to 20% of intake and elevated C18:2n6 intake by almost 200g/day but C18:2 in milk FA only increased by 18g/day. In addition, Leonardi et al. (2005) fed corn DGS at up to 15% of DMI and compared these treatments to a diet with corn oil which was iso-ether extract to the 15% DGS diet. There was a 3 percentage unit increase in C18:2 concentration in the diet but 15% DGS and corn oil diets only increased

C18:2 concentration in milk FA by 1 percentage unit and decreased overall milk fat concentration. Therefore, the protection of unsaturated FA from DGS in dairy cattle TMRs is relatively low.

DGS are primarily sourced from corn grain so the predominant fatty acid supplemented by DGS is linoleic acid followed by oleic acid (Leonardi et al., 2005). This can have important impacts on a cow's risk for diet-induced MFD. Nevertheless, DGS can be fed safely as one study showed no significant difference in pounds of milk fat production but did decrease milk fat percentage in milk at the highest inclusion rate of DGS (Leonardi et al., 2005). Multiple trials showed a similar increase in milk fat production but did not report a significantly lower milk fat percentage when feeding DGS compared to a control diet without DGS (Anderson et al., 2006; Kleinschmit et al., 2006). In both trials, the control diet contained a lower level of EE than diets containing DGS and Kleinschmit et al., (2006) did report that diets containing DGS had greater levels of FA than the control diet. This would increase the preformed FA level in the diet and potentially allow for the greater milk fat yield in cows fed diets containing DGS. Recent developments allow for the fractioning of corn prior to the fermentation process, yielding distillers grains with lower fat levels that can thus be fed to cattle at higher rates without increasing the risk for BH-induced MFD (Robinson et al., 2008; Christen et al., 2010).

Dry Fat Supplements

The main sources of dry fat supplements fed to dairy cattle are calcium-salts of palm fats or prilled saturated fats from byproducts of tallow or blended palm fat. Calcium salts were first developed in the 1980s to supplement energy in dairy cattle rations (Jenkins and Palmquist, 1982, 1984). These were demonstrated to provide some protection for UFA and reduce interactions between FA and rumen microbes. Prilled FA were developed later as alternate method for

increasing dietary energy but prilled fat supplements have a FA profile that is higher in total SFA (Grummer, 1988). Grummer (1988) and Schauff and Clark (1989) both reported no significant difference in milk fat percentage or milk fat production between the two products. However, Grummer (1988) reported that calcium salts of palm oils decreased milk protein when compared to a control diet whereas prilled fats did not yield the same milk protein decrease. Rabiee et al. (2012) noted a similar decrease in milk protein concentration when calcium salts were fed but only noted a 55 g decrease in milk protein yield. They found that prilled fats also decreased milk protein concentration but reported a 101 g increase in milk protein yield. Nevertheless, both types of fat supplements can be fed to dairy cattle with no adverse effects on cow health.

Fatty Acid Profile of Feed

It has been discovered that different FA in the diet have different effects in the cow. Most of the early research utilized abomasal and ruminal infusions to administer doses of enriched FA and then measure the biological outcomes. This slowly moved toward feeding trials and the research focused primarily on PA and SA, but more interest has recently been shifted toward OA. The focus moved towards SFA as they have relatively little effect on rumen fermentation when the dietary FA load is kept under 5%. However, issues with digestibilities of dry fat supplements caused focus to be turned to OA, because it can be a natural emulsifier and therefore increase overall FA digestibility.

Palmitic Acid

PA is one of the more popular SFA for supplementation in dairy cattle and some research suggests that PA increases milk fat concentration more than SA in dairy cattle (Rico et al., 2014b; Chamberlain and DePeters, 2017). However, Rico et al. (2014a) reported no change in milk fat

concentration in a diet supplemented with PA compared to a unsupplemented diet but the supplementation did increase FA digestibility and increase feed efficiency of the cattle. Nevertheless, these results compared the mean changes in FA production of both a prilled PA supplement and a calcium salt of PA to the control diet and reported no difference. This observation is confounded because high production dairy cows in Rico et al. (2014a) fed the diet containing the PA prill had a greater milk fat percentage than the cows fed the Ca-salt of PA. Further work by de Souza and Lock (2019) contrasts the results from Rico et al. (2014a) and showed that PA helps to increase milk fat percentage and milk fat production but this work was completed in fresh cows. de Souza and Lock (2019) also reported cows which consumed PA after their peak milk production retained the higher milk fat percentage and milk fat production which reinforces prior research by Piantoni et al. (2013) on the benefits of PA supplementation.

Stearic Acid

SA is another SFA that is commonly supplemented in TMR rations as many prilled fats are blends of PA and SA. Often, producers include SA in diets because it appears to be transferred to adipose tissue as OA rather than incorporated into milk fat thereby increasing the energy status of a cow. Increased energy status has been linked to better reproductive performance, so SA supplementation may have indirect positive effects on reproductive performance in dairy cattle. SA is the primary form of 18-carbon FA absorbed across the small intestine in the dairy cow as a result of BH of unsaturated FA in the rumen and is the FA absorbed in the greatest quantities in the dairy cow (Loften et al., 2014). However, some research reports the desaturation of SA to OA in the mammary gland to be 52% (Enjalbert et al., 1998), thus SA is not the predominant FA in milk fat. Enjalbert et al. (1998) also reported that SA infused into the duodenum had a numerically

increased milk fat percentage when compared to a control infusion containing 0g of FA. However, these infusion solutions were mixed with soy lecithin which may have increased micelle formation and increase absorption of the FA and its availability to the cow.

Oleic Acid

OA is a mono-unsaturated FA found in various concentrations among oilseeds and is recently been the focus of investigation for inclusion in dry fat supplements. The increased unsaturation index of OA compared to SA allows it to act as an emulsifying agent for aerosols (Choulis, 2011). It has not been fully proven that OA acts in the same manner in the small intestine but the potential for OA to act as an emulsifying agent in biological systems exists. Casper et al. (1988) compared different levels of OA ingested in the form of whole sunflower seeds and the results suggests that increased OA can increase milk fat percentage in dairy cattle. However, the trial compared high oleic sunflower seeds to regular sunflower seeds and the regular sunflower seeds contained a high level of LA which may have caused diet induced MFD. Other research suggests that large doses of OA entering the small intestine may inhibit DMI and milk production but smaller doses appear to increase milk fat percentage and milk fat production (Drackley et al., 2007). However, the dose levels in Drackley et al. (2007) which caused lower DMI were much higher than any biological dose of OA entering the small intestine.

Fatty Acid Digestibility

The FA traditionally fed to cattle are rarely in a highly enriched form as the common ingredients for fat supplements are oils and byproduct streams which do not have a pure FA profile. Therefore, the dry fat supplements fed to dairy cattle are often blends of FA. There are advantages

to feeding a blend of FA rather than an enriched FA as has been demonstrated recently with positive impacts on digestibility of certain individual FA in the supplement and this allows producers to supplement different blends of FA for the unique challenges of their operation (Cedeño et al., 2001; Shepardson, 2018; Western et al., 2020).

Fatty acid digestibility is a unique topic as one meta-analysis, Boerman et al. (2015), examined unsupplemented (control) diets and found no statistical differences between the digestibilities of any long-chain FA compared to SA. This begs the question as to what happens as specific FA are consumed in greater quantities, what happens as total FA intake increases, and whether the form in which these FA are consumed impacts the overall digestibility of FA in the dairy cow.

Palmitic Acid Digestibility

Several meta-analyses have been conducted examining the digestibility of the most common FA in dairy cattle rations. Although the estimates of FA digestibility vary, the consensus suggests that PA is more digestible than other FA commonly found in plants (Glasser et al., 2008b; Boerman et al., 2015). The difference in digestibility is partly explained by the higher rate of absorption of PA compared to other common FA in a diet (Harrison and Leat, 1972). However, this work was completed in sheep, limited research units were utilized, and FA were supplemented using concentrates rather than pure FA sources so more data is needed to reinforce the conclusions in the study. Estimates for PA digestibility in these meta-analysis range between 73 and 77% but experimental studies calculate the digestibility of PA to be as low as 73% (Schmidely et al., 2008; Boerman et al., 2015). Boerman et al. (2015) reported that the digestibility of PA was not greatly

affected by increased PA intake and that increased PA intake resulted in greater total FA absorption.

Stearic Acid Digestibility

Stearic acid digestibility is generally lower than that of PA and other 18-carbon FA and estimates put SA digestibility between 65% and 80% (Boerman et al., 2015). Although, Glasser et al. (2008b) examined total 18-carbon flow and estimated digestibility to be 85%, this included the unsaturated 18-carbon FA and cannot be used to describe SA digestibility. In addition, rumen BH of UFA increases the amount of SA entering the duodenum, therefore, it is difficult to accurately characterize the true digestibility of SA. Boerman et al. (2015) evaluated data reporting duodenal and ileal flow of FA, but very few studies report this data and therefore the digestibility of SA was numerically lower than other long-chain FA but was not statistically significant. However, some data suggests SA digestibility decreases considerably as the level of SA intake increases and one analysis suggests that increased SA intake decreases overall FA digestibility, but this study fed a highly pure supplement (98% SA) that contained trace levels of unsaturated FA (Boerman et al., 2015). Explanations for this trend have not yet been elucidated, but theories postulate that the cow lacks to the physiological capacity to adequately emulsify the total FA load entering the small intestine.

Oleic Acid Digestibility

UFA are generally more digestible than SFA as they appear to be absorbed more readily than SFA after forming a micelle (Ockner et al., 1972). Boerman et al. (2015) estimated OA digestibility to be more than 82% with the range of OA digestibilities in the 95th percentile

remaining within 2 percentage units across the studies included in the analysis. This includes both ileal and fecal collections and it is statistically higher than the digestibility for SA. For ileal collection studies, the estimate for OA digestibility is slightly lower and there is only a tendency for OA to be more digestible than SA. Glasser et al. (2008b) reported a slightly lower digestibility of 79% for all OA isomers but found a 2 percentage unit higher digestibility for trans-isomers of OA.

Digestibility of Fatty Acid Blends

Enriched dry fat supplements of SA (>85% pure) appear to have lower digestibilities than blends of FA (Shepardson, 2018). Other data suggests that blends of SA and PA reduce total FA and PA digestibility (Shepardson, 2018; Western et al., 2020). Nevertheless, the majority of research suggests a consensus that increased FA supplementation reduces FA digestibility. Piantoni et al. (2013) compared a 99% enriched PA supplement to a control diet containing soyhulls and found a 10% reduction in total FA digestibility and a nearly 20% decrease in PA digestibility when comparing the PA diet to the diet with soyhulls. Western et al. (2020) reported a 5% decrease in PA when comparing an 85% enriched PA source to a control diet but a large percentage of the other FA in the supplement were OA, so this may have positively impacted FA digestibility.

The reduced digestibility of highly enriched FA most likely results from increased melting points and the formation of crystalline matrix between SFA of the same chain length (Cedeño et al., 2001). Cedeño et al. (2001) also reported that blends of FA help reduce the matrix formation and that UFA reduce the matrix formation more than SFA. Lower levels of matrices in the FA prills causes a lower melting point and it is thought that lower melting points and lower

crystallization allows for greater absorption of FA in the small intestine. It was also reported that oleic acid was particularly effective at reducing the melting point of blends of FA (Figure 2-1) and, thus, the prills may be able to break apart more readily with a lower melting point. The mixture of OA and PA was lower than that of OA and SA but blends of all three FA were lower than any binary mixture of a SFA and OA.

Absorption efficiency may play a part in the digestibility of FA. A rat model suggested that a blend of UFA and SFA in the small intestine optimizes the absorptive efficiency of FA (McDonald and Weidman, 1987). As mentioned by Glasser et al. (2008b), this may explain why high levels of SA could cause lower digestibility as the ratio of SFA to UFA would be less than ideal for optimal absorptive efficiency. However, this would not explain why an increase is PA entering the small intestine would not yield the same decrease in digestibility as observed with SA.

Milk Fat Depression

MFD is a condition experienced by dairy cattle in which synthesis and secretion of FA in the mammary gland are downregulated. There have been several factors implicated in causing MFD that are often traced to rumen environment conditions and diet factors causing altered BH of PUFA. These factors range from a high quantity of UFA intake, sub-acute ruminal acidosis, to a small particle size in the diet of a dairy cow. Even though these conditions were known to decrease milk fat concentration, the mode of action causing BH-induced MFD was not elucidated until the early 2000s. Altered BH in the rumen causes the formation of conjugated linoleic acid (**CLA**) isomers that impact gene expression in the mammary gland (Bauman et al., 2008). The main causative agent is *trans*-10 *cis*-12 CLA. The presence of BH-induced MFD is often determined by

increased concentrations of *trans*-10 C18:1 in milk, as normal BH results in *trans*-11 CLA whereas altered-BH yields *trans*-10 isomers as the product (Figure 2-2).

Biohydrogenation-Induced Milk Fat Depression

Milk fat depression can be caused by changes in dietary forages. Grant et al. (1990) examined three different rations where particle size distribution of the diet was altered by feeding silages with different particle sizes. Cows fed the diets containing the finely chopped alfalfa silage resulted in a decrease in milk fat percent by 0.8 percentage units compared to cows fed the diet containing the coarse silage. Low forage inclusion in the diet is another causative agent for diet-induced MFD as shown by Kalscheur et al. (1997). Kalscheur et al. (1997) reported a 0.65 percentage unit decrease in milk fat percentage when comparing low- and high-forage diets but no decrease in daily milk fat production. The low forage diet resulted in a lower total tract NDF digestibility and lower FA digestibility which may have limited the quantity of de novo FA synthesis precursors and limited preformed FA for secretion, thus potentially limiting milk fat percentage. Grummer et al. (1987) also examined the effects of low forage, high-grain diets and found a 0.5% percentage unit decrease in milk fat production when comparing a low-forage diet to a control diet. In Grummer et al. (1987) and Kalscheur et al. (1997), the dietary NDF concentrations were 20 and 22%, respectively, much less than the 25% minimum recommendation set by the NRC (2001) so although the diets are not representative of those in the industry, the potential for low forage diets to create milk fat depression exists. For example, Rico and Harvatine (2013) examined the time-course for the induction of MFD and caused MFD feeding a 29.5% NDF diet. This study did couple a low forage diet with high levels of PUFA to achieve the reduction in milk fat production. Increased intake of PUFA has been shown to be a risk factor for diet-induced

MFD. Griinari et al. (1998) found that UFA addition to a high fiber diet (32.1% NDF) did yield a reduction in milk fat percentage. When the UFA were combined with a low fiber diet (14.8% NDF), the decrease in milk fat production was even greater.

Physiology of Diet Induced Milk Fat Depression

One of the primary methods causing MFD is trans-10, cis-12 CLA. Peterson et al. (2002) demonstrated this isomer to decrease milk fat secretion using a dose titration model and abomasally infusing the CLA into the cow and found a quadratic decrease in milk fat reduction as CLA infusion quantity increased. Baumgard et al. (2002) infused trans-10, cis-12 CLA and also reported a decrease in milk fat production. In addition, gene expression in the mammary gland was examined and found decreases in mRNA expression of fatty acid synthetase (FASN), acetyl CoA carboxylase, and SCD. Harvatine and Bauman (2006) examined the effect of trans-10, cis-12 CLA and found similar results to Baumgard et al. (2002) with decreases in FASN and SCD expression. They also reported LPL, SREBP-1, and spot 14 expression was decreased in the trans-10, cis-12 CLA treatment.

Economics of Milk fat

Milk pricing is based largely on the predominant use for milk in a geographical region, or federal order. Within these regions, there are separate classes of products that require different components from the milk (Nepveux, 2019). Domestic and global demand plays a large part in the overall prices of the classes of milk products and therefore, impact the price of milk components. Milk fat is one of the two most valuable fractions contributing to the mailbox price for milk. Historically, milk protein has been more valuable per head per day but in recent years, milk fat has

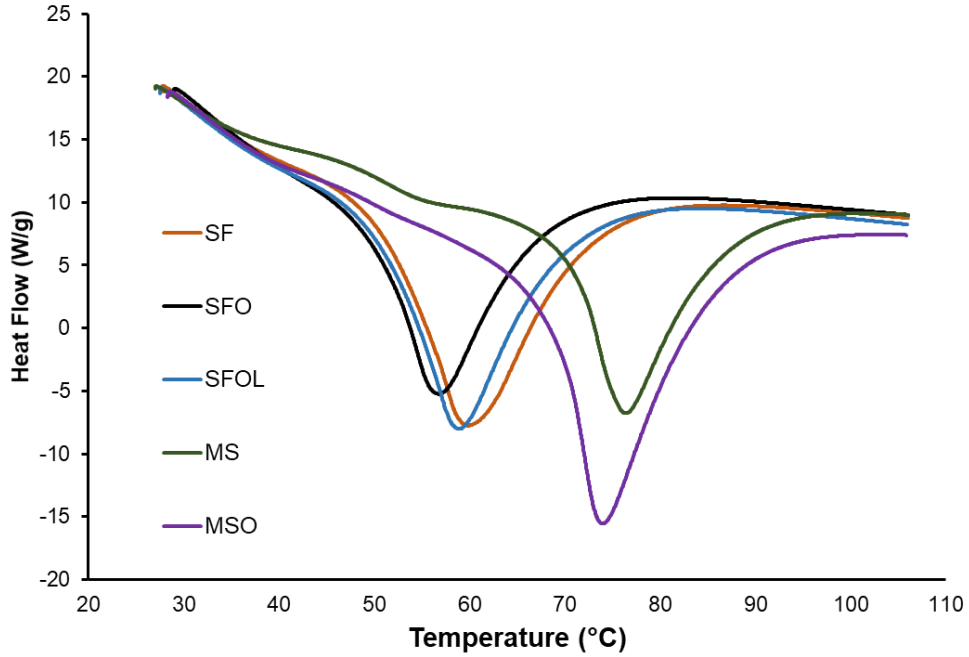
been the most valuable component averaging more than $6/head/day. This increased value drives producers to push for greater milk fat production whether that is through the means of increasing the milk fat concentration and maintaining milk yield or by maintaining milk fat concentration and increasing milk yield. Increasing milk fat concentration may be more efficient depending on market conditions as greater volumes of milk would yield increased hauling costs as milk is 87% water and holds no value in milk pricing.

Conclusions

Supplying energy demands of high producing lactating cows can be very difficult without compromising rumen health but additional dietary fat is a viable solution in the dairy industry. Additional fat can be added to a TMR via various oilseeds or through commercially-available dry fat supplements but there are drawbacks with each approach. Commercial fat supplements will be more expensive, but the FA profile can be adjusted to the needs of each individual producer. Oilseeds will be much cheaper and potentially add protein or NDF to the diet but regional availability may be limited and the FA profile may pose a greater risk for BH-induced MFD.

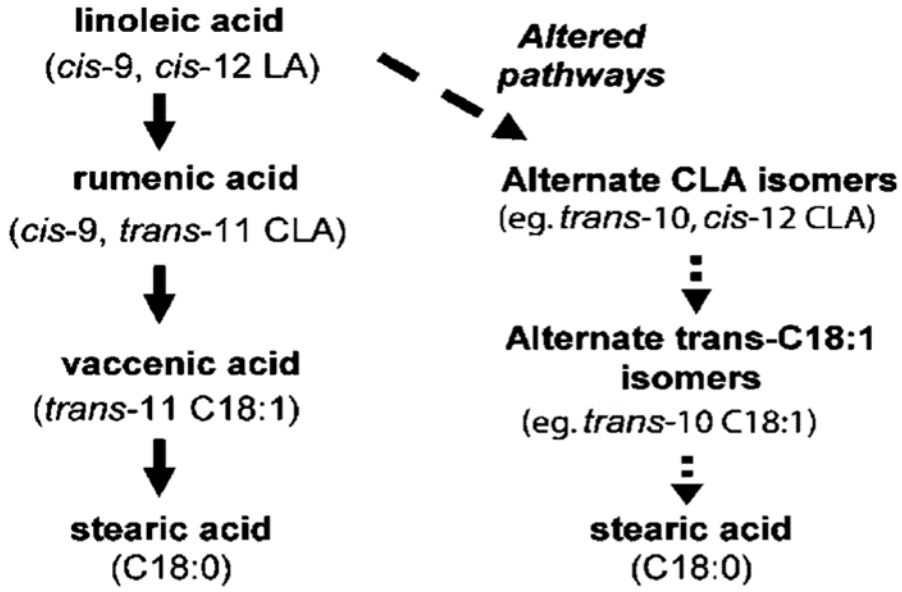
Research on oilseeds slowed for several decades but with recent developments in plant genetics and greater milk yields in cows, the applicability of the older research may be in question. Therefore, revisiting the data on feeding oilseeds like whole cottonseed to examine the recommendations is necessary to evaluate cow health and productivity, while advancing the knowledge surrounding the digestibility of these oilseeds. Very little research has examined the behavior of WCS in dairy cattle and even less work has been completed to examine the digestibility of dietary FA from WCS. In addition, diets without fat supplementation yield higher digestibilities of fats so it is necessary to examine the causes of this decrease and attempt to increase the digestibility of fats in supplemented diets. It is known that blending FA can increase the digestibility of specific FA, but many FA blends have not been evaluated in a research setting so there exists the potential for higher FA digestibilities.

Figure 2-1: Differential scanning calorimetry measuring the enthalpy and melting point of blends of FA.



The blends were primarily SA and PA but the SF contained 2.9% OA, the SFO contained 4.8% OA, the SFOL contained 6.6% OA, the MS contained 3.8% OA and the MSO contained 6.1% OA. Increased OA concentration decreased melting points of FA blends when comparing within unreacted products (SF, SFO, and SFOL) and within reacted products (MS and MSO) (Shepardson, 2018).

Figure 2-2: Diagram of normal and altered biohydrogenation in the rumen of dairy cattle.



Normal ruminal biohydrogenation of fatty acids follows the vertical pathway with solid arrows whereas altered biohydrogenation follows the pathway on the right of the image with dashed arrows (Harvatine et al., 2009).

Chapter 3

Evaluation of feeding increased levels of whole cottonseed on milk production, milk components, and milk fatty acid profile.

Abstract

Milk fat is a valuable milk component and around 60% of milk fatty acids (FA) originate from dietary fat. Oilseeds are an economical source of oil compared to dry fat supplements, but contain unsaturated FA that increase risk for diet induced milk fat depression. Cottonseed is expected to have a slower ruminal release rate of unsaturated FA and decrease diet-induced MFD. The hypothesis of the experiment was that increasing whole cottonseed in the diet would increase milk fat yield in high producing cows by providing additional dietary fat without induction of milk fat depression. Four primiparous and eight multiparous lactating Holsteins (136 ± 35 DIM and 127 ± 4 DIM, respectively) were arranged in a 4x4 Latin square design with 21 d periods. Treatments were 0, 3.4, 6.8, and 9.9% of DMI as whole cottonseed. Dietary NDF and protein were maintained by substitution for cottonseed hulls and soybean meal. Data was analyzed using the random effect of cow and period and fixed effect of treatment and preplanned contrasts tested the linear and quadratic effect of increasing cottonseed level. Milk yield was not modified by treatment. There was a treatment by parity interaction ($P < 0.05$) for milk fat percent and yield with no effect of cottonseed in multiparous cows, but milk fat percent and yield were quadratically decreased in primiparous cows. Importantly, multiparous cows were 4.05% and primiparous cows were 3.53% milk fat when fed the control diet. Milk FA profile indicated that cottonseed increased *trans*-10 C18:1 in primiparous cows ($P < 0.05$), but not multiparous cows. Milk fat increased at the highest level of cottonseed in primiparous cows ($P < 0.05$) due to an increase in yield of

preformed FA ($P < 0.05$). Apparent transfer rates of 18C FA decreased in all cows ($P < 0.05$), but this trend was more pronounced in primiparous cows. In conclusion, whole cottonseed maintained milk and milk component yield when fed at up to 9.9% of the diet to multiparous cows, but the primiparous cows in the current trial were more susceptible to diet-induced milk fat depression.

Introduction

Intake of an adequate supply of nutrients is paramount to supporting the lactation in a high producing dairy cow. There are many ways to increase the energy density in a ration and one of the most common methods is to increase the fat concentration in the diet. Various methods exist to accomplish this task and one cost-effective strategy is through the inclusion of cottonseed. Research about the inclusion of cottonseed in lactating diets conducted in the 1980s and into the early 1990s demonstrated that cottonseed can be safely fed at up to 15% of DMI in high producing dairy cattle (Anderson et al., 1979; Harrison et al., 1995; Arieli, 1998). However, very little work has been completed since that time and it is not clear if these recommendations are still realistic in the current high-producing cows. Previous work also focused on production responses and less on the digestibility of the fat in cottonseed. Thus, cottonseed was not analyzed in the meta-analysis by Glasser et al. (2008a) which examined the digestibility of fat from common oilseeds in various processed forms. Furthermore, less work has been done to examine the overall impact of dietary fat on milk production and milk component production. More recent research has examined specific sources of FA or specific FA and their impact on milk fat production and FA profile.

Cottonseed, like other oilseeds, contains a high level of unsaturated FA which is enriched in linoleic acid. Linoleic acid is over 50% of the total FA in whole cottonseed and cotton contains a relatively high level of fat with almost 17% of DM as fat (Bertrand et al., 2005; Dowd et al., 2010). Consequently, this poses an increased risk for dairy cattle to experience biohydrogenation induced milk fat depression as linoleic acid is one of the primary precursors for the trans-10, *cis*-12 conjugated linoleic acid (CLA) isomer which has been implicated in the onset of MFD (Bauman et al., 2008). However, Harrison et al. (1995) fed diets containing 12% of DM as whole cottonseed and observed an increase in milkfat concentration of over 0.25 percentage units in a comparison

of cottonseed inclusion to a control diet with basal levels of fat (2.5 and 3.5% on the two herds in the study, respectively). However, a substitution of cottonseed for raw soybeans showed that the neither oilseed increased milk yield or any milk component compared to the other (Abel-Caines et al., 1997). In addition, there are theories that the hull of the cottonseed may slow down the release of the fat within the rumen. Cows fed whole cottonseed yielded higher milk fat concentrations and higher milk fat production overall when compared to cows fed extruded soybeans (Anderson et al., 1984).

This experiment was conducted to re-evaluate the recommendations for whole cottonseed inclusion in dairy cattle and quantify the digestibility of the fat with increasing levels of cottonseed inclusion, and re-evaluate the effect of cottonseed on biohydrogenation-induced milk fat depression in contemporary dairy cattle. The hypothesis was that increased levels of cottonseed in the diet would supplement dietary fat intake and allow for greater milk fat concentration and milk fat yield without compromising milk yield.

Materials and Methods

Eight multiparous and four primiparous cows (136 ± 35 DIM and 127 ± 4 DIM, respectively) from The Pennsylvania State University Dairy Research and Teaching Center were housed in individual stalls and milked 2x/day. All treatments and procedures were pre-approved by the Penn State University Institutional Animal Care and Use Committee (PRAMS# 200946398). Cows were randomly assigned treatments in a 4x4 Latin square design. Treatment periods lasted 21 d and observations were collected in the last 4 d of each period. There was a 24 d covariate period for diet adaptation prior to the trial and the diet was reformulated after 10 d due to the occurrence of BH-induced MFD. Treatments were whole cottonseed (**WCS**) included in the diet at 0%, 3.3%, 6.6%, and 9.9% of DMI (Table 3-1). As WCS inclusion was increased, the

inclusion of a blend of soybean meal (**SBM**) and cottonseed hulls (**CSH**) was decreased so the treatment ingredients always provided 9.9% of DMI. SBM, CSH, and WCS were sampled prior to beginning the trial and the blend of SBM and CSH that was replaced by WCS was calculated based on ether extract-free, iso-NDF basis to the sample of WCS.

Feeding and Feed Sample Collection and Analysis

Feed was delivered to cows as a TMR and fed once per day while cows were milked in the morning. One base feed was mixed in a stationary mixer (Electra-Mix 1062; I.H. Rissler Manufacturing, Mohnton, PA), and treatment ingredients were then added to the base mix in a mobile apron mixer (Rissler 1050 Feedcart; I.H. Rissler Manufacturing, Mohnton, PA) and delivered immediately to the cows. Cows were fed at 110% of the intake of the previous day after intake was calculated and recorded. Dry matters for forages were calculated weekly (55ºC oven for 48 hrs) and diets were adjusted accordingly.

Samples of each ingredient were collected on d 18, 19, 20, and 21 and composited by period. Total mixed ration samples were collected on d 21 for Penn State Particle Separator (PSPS) analysis. Orts were collected during the last four days of each period using the quartering method and composited by period. Feed and orts samples were maintained in a -20ºC freezer after collection and thawed prior to being composited before returning to a -20ºC until freeze dried.

Orts and feed ingredients were freeze dried (Ultra 35-XL; Virtis CO. Inc., Gardiner, NY) and ground using a Wiley mill (A.H. Thomas CO., Philadelphia, PA) with a 4-mm screen. Subsamples were collected and analyzed for 105°C DM (overnight in a forced air oven) FA profile and total FA concentration of the samples were determined using direct methylation as described

by Sukhija and Palmquist (1988) including the modifications to the internal standard used by Rico and Harvatine (2013).

Milk Sampling and Analysis

Cows in the experiment were sample 2x per day at approximately 0700 and 1830 h in a parlor and milk yield was calculated using automated milk meters [Afimilk (SAE Afikim), Kibbutz Afikin, Israel]. Deviation in individual milk weights was adjusted based on a correction that accounts for the effect of milking (AM/PM), day, cow, and stall in the parlor for the last 7 d of each experimental period. Milk samples were collected at each milking for d 18 through d 21 of each experimental period, preserved at 4°C in a bronopol-based preservative, and submitted for analysis for fat and protein (DairyOne Lab, Ithaca, NY) by Fourier transform infrared spectroscopy. A subsample from each milking on d 20 and 21 was collected and composited based on milk weight within each period, then centrifuged at 1300 x g for 20 min at 4°C for collection of fat cake and then stored at -20°C. Fat cakes were analyzed using the procedure in Rico and Harvatine (2013) using a 3:2 hexane: isopropanol mixture. The FA are transmethylated using sodium methoxide and the resulting FAME are quantified by gas chromatography with a flame ionization detector and a capillary column [SP-2560; 100 m x 0.25 mm (i.d.) with a 0.2-μm film thickness; Supelco Inc., Bellefonte, PA].

Feed Sample Analysis

Feed ingredients were collected during the last four days of experimental period and then composited by period. Then, they were freeze dried (Ultra 35-XL; Virtis CO. Inc., Gardiner, NY) and ground using a Wiley mill (A.H. Thomas CO., Philadelphia, PA) with a 4-mm screen.

Subsamples were collected and the main sample was reground with a 1-mm screen for analysis. Individual samples of treatment ingredients, forages, and a representative composite of the dry concentrates in the ration [ground dry corn, vitamin-mineral mix, AminoPlus (Ag Processing Inc., Omaha, NE), sodium bicarbonate, and canola meal] were analyzed for CP, ADF, NDF, starch, 105°C DM, and FA concentration and profile. Book values for the urea and molasses were used (241% CP for urea (Optigen, Alltech Inc., Lexington, KY) and 6.3% CP, 0% NDF, and 11.0% ash for molasses). AOAC (2019) protocols 990.03 and 973.18 were used for CP and ADF, respectively. NDF was calculated according to Van Soest et al. (1991) using sodium sulfite, heat stable amylase, and an acetone rinse. Starch was determined enzymatically according to (Hall, 2009) and iNDF was determined by batch culture in vitro incubation. These were completed by Cumberland Valley Analytical Services (Waynesboro, PA). Samples were analyzed for 105°C DM (3 hr in a forced air oven) and FA profile and total FA concentration were determined using direct methylation as described by Sukhija and Palmquist (1988) including the modifications to the internal standard used by Rico and Harvatine (2013).

Statistical Analysis

Data was analyzed using the REML method of JMP Pro (version 14.0; SAS Institute Inc., Cary, NC) using the following model:

$$Y_{ijkl} = \mu + C_i + P_j + T_k + R_l + (T_k \times R_l) + e_{ijkl}$$

Where Y_{ijkl} represents the dependent variable of interest, μ is the overall mean, C_i is the random effect of cow (i = 1 to 16), P_j is the random effect of period (j = 1 to 4), T_k is the fixed effect of treatment (k = 1-4), R_l is the fixed effect of parity (j = primiparous or multiparous), T_k *x* R_l is the interaction of parity and treatment, and e_{ijkl} is the residual error. Studentized residuals outside of ±

3.0 were considered outliers and removed from analysis. Significance was claimed at $P \leq 0.05$ and trends at $P \leq 0.10$. A protected LSD was used for mean separation. Pre-planned contrasts comparing linear and quadratic effects of increased WCS inclusion were analyzed.

Results and Discussion

Production Response to WCS Inclusion

Responses to cottonseed inclusion are summarized in Table 3-2. Dry matter intake was linearly decreased ($P<0.05$) as WCS inclusion increased but this change was only observed in multiparous cows. Milk yield was unaffected by treatment or parity and no interaction for treatment by parity was detected. There was a trend for decreased milk fat concentration in primiparous cows compared to multiparous cows and an interaction of treatment by parity ($P < 0.05$) but no significant trend in milk fat concentration by cottonseed inclusion level was detected. There was a quadratic effect for milk fat concentration in primiparous cows ($P < 0.05$) with the lowest value observed at the 3.3% inclusion rate, but no linear or quadratic effect of cottonseed inclusion on milk fat concentration was observed in multiparous cattle. Milk fat production was greater in multiparous cows compared to primiparous cows ($P < 0.05$) and there was a trend for a quadratic effect for milk fat production in primiparous cattle. Milk protein concentration tended to be higher in multiparous cows even though there was a tendency for milk protein concentration to decrease in multiparous cows. Milk protein yield was greater in multiparous cattle ($P < 0.05$) but there was no treatment effect or interaction between treatment and parity. It was unaffected by levels of cottonseed inclusion. Milk urea nitrogen was unaffected by treatment, parity, and there was no interaction of cottonseed inclusion by parity.

Fatty Acid Profile in Milk Fat

Relative FA concentrations are summarized in Table 3-2 and the full FA profile can be found in Supplemental Table 3-1, Supplemental Table 3-2, Supplemental Table 3-3, and Supplemental Table 3-4. De novo FA concentration was decreased by diet and primiparous cows had lower de novo ($P < 0.05$) FA concentrations. There was a linear decrease in de novo FA concentration for multiparous cows but only a tendency for decreased de novo FA concentration in primiparous cows. Mixed FA concentration was affected by diet and tended to have a parity effect. There was a linear decrease in multiparous cows but primiparous cows tended to have quadratic response in mixed FA with increased WCS inclusion. Preformed FA concentration was significantly increased in multiparous cows fed increased levels of WCS but had lower concentrations of preformed FA ($P < 0.05$) when compared to primiparous animals. Primiparous animals tended to have greater concentrations of preformed FA as WCS intake increased. *trans*-10 C18:1 was significantly higher in primiparous cows and significantly increased as WCS intake increased. There was no effect of increased WCS intake on C18:1*c*10 in multiparous cows. *trans*-11 C18:1 was increased linearly in all cows ($P < 0.05$) and primiparous showed an increase in *cis*-9, *trans*-11 C18:2 but multiparous cows only tended to increase in *cis*-9, *trans*-11 C18:2. Overall, there was an increased concentration for most other *trans*-isomers of C18:1 in primiparous cattle with the exception of *trans*-15, C18:1. For all *trans*-isomers of C18:1 in the milk FA profile, there was a linear increase in primiparous cattle as cottonseed inclusion increased ($P < 0.05$). Apparent transfer rates of 18C FA are found in Table 3-3 and were decreased with higher WCS inclusion ($P < 0.05$) but were not different by parity. The decrease in 18C transfer rate only numerically decreased in multiparous cows but linearly and quadratically decreased with higher WCS inclusion in primiparous cows.

Integrative Discussion

The presence of BH-induced MFD in primiparous cows was unexpected and the causative agent is unclear as there was no visible sorting of feed. The results from the multiparous cows more closely resembled results predicted in our hypothesis but there was no increase in milk fat concentration or milk fat yield. However, milk protein concentration and yield were generally unchanged in multiparous and primiparous cattle.

The decrease in DMI is common in multiparous cattle when fat is increased in lactating dairy cattle diets. However, the lack of a change in DMI in primiparous cattle is a novel concept as primiparous cattle are rarely studied in research and only one other study reported intakes for primiparous cattle fed cottonseed (Harrison et al., 1995). They also reported no change in DMI in primiparous cows during the first period of their complete block trial but noted a decrease in DMI during the second period. Therefore, more data is required to determine the effects of cottonseed on DMI in primiparous cows. Nevertheless, the results indicate that fat supplementation, even when fat is added from oilseeds rather than from a dry fat supplement, still decrease DMI in multiparous cattle.

Milk yield was unaffected by treatment and parity, which does not match the notion that multiparous cows produce more milk per day than primiparous cattle. However, the cattle on the study had greater DIM and therefore the level of milk production in the multiparous cows would have dropped from their peak due to limited persistency in multiparous dairy cattle. Primiparous cows are more persistent than multiparous cows and therefore the cows maintained similar levels of production. In addition, one of the multiparous cows was a late-lactation animal and therefore would reduce the average production of the group of multiparous cows. The lack of a change in

milk fat yield in multiparous cattle is most likely due to the reduction in DMI, as higher levels of cottonseed would provide more energy for milk fat synthesis and allow for greater partitioning of energy toward overall milk yield. BCS of cows were not evaluated due to the short duration of each experimental period so it unclear whether adipose tissue deposition occurred in specific diets. However, the quadratic response of milk fat yield in primiparous cattle indicates the presence of BH-induced MFD due to the low milk fat concentration (2.8%) in the 3.4% cottonseed diet. The high concentration of *trans*-10 C18:1 in primiparous cattle further reinforces the induction of MFD in the primiparous cattle. In addition, *cis*-9, *trans*-11 C18:1 was increased in primiparous cows and this C18:1 isomer has been implicated in the altered BH pathway associated with MFD (Shingfield and Griinari, 2007). Therefore, we assume there was MFD in the primiparous cows. Generally, BH-induced MFD would decrease milk fat concentration as the flux of BH-intermediates increased. However, milk fat concentration initially decreased and then increased with increased levels of cottonseed. Therefore, it is possible that the increase in dietary FA had a greater effect than the negative effects of the BH intermediates as trans-10, cis-12 CLA only inhibits milk fat synthesis by up to 50% (de Veth et al., 2004), and an increase in plasma TAG from the cottonseed led to the increase in milk fat concentration between the 3.4% WCS diet and the 9.9% WCS diet but future analysis on blood samples from these animals is required to definitively tell if there was an increase in plasma TAG or NEFA concentrations. In addition, there may have been other dietary factors that caused MFD and further analysis of the TMR samples is required but the same diets were fed to the multiparous cows and the primiparous cattle so it is unclear why there was a difference in susceptibility to MFD between parities. One possible theory for the MFD in primiparous stems from the primary forage type in the TMR. Smith et al. (1993) examined diets fed to multiparous cows that contained a high corn silage percentage (50%) and two blends of corn

silage and alfalfa hay (37.5% and 12.5% or 25% and 25%, respectively) and fed cottonseed at 12% of DMI. Their results suggested that feeding cottonseed increased milkfat percentage but feeding cottonseed in conjunction with the high corn silage diet decreased milk fat concentration. However, we fed cottonseed at up to 9.9% of DMI in a diet with similar forage concentrations to the 37.5%:12.5% blend of corn silage:haylage in Smith et al. (1993) but did not see any changes in milk fat concentration similar to those reported in Smith et al. (1993). Thus, it is more perplexing that we observed BH-induced MFD as we were feeding a diet that Smith et al. (1993) suggested would pose less of a risk for reduced milk fat concentration.

When analyzing the overall FA profile, we noted that primiparous cattle had lower concentrations of de novo FA than multiparous cows. This is not a trend that has been widely researched and very few studies have reported FA profile for primiparous cows. However, there was a decrease in de novo FA concentration in all animals which matches studies where cottonseed oil was infused into the rumen of lactating cows and a decrease in de novo FA concentration was observed (Aprianita et al., 2014). Aprianita et al. (2014) also reported decrease in milk fat concentration and a numerical decrease in milk fat yield from infusion of cottonseed oil, but the quantity of infused oil was higher than the quantity of cottonseed oil consumed by the cows in our study so the results are not directly related. However, the trends for lower de novo FA concentration in milk fat are very similar across the two research trials.

Changes in mixed FA concentrations in milk fat in our trial are similar to those reported in other trials. Mohamed et al. (1988) fed cottonseed at 16.5% of DMI and observed a decrease in the concentrations of both C16:0 and C16:1 at 4.5 and 0.2 percentage units, respectively, when compared to a control diet. This is similar to the 3.2 percentage unit decrease we observed in multiparous cows when comparing our 0% cottonseed diet to the 9.9% cottonseed diet. Mohamed

et al. (1988) did not report parity for the cows in their study but the ruminally cannulated cows were used and therefore the animals may have started their second or subsequent lactation. Preformed FA concentration in milk fat increased in our study and that closely follows previous research. Arieli (1998) reviewed cottonseed inclusion in dairy cattle and reported that eight different trials noted an increase in C18 fatty acids concentrations when cottonseed was fed. However, these eight trials all fed cottonseed at higher rates than those in our research trial with the lowest rate of WCS inclusion at 12% (Smith et al., 1993). Nevertheless, the effect of WCS inclusion was the same general trend that resulted in a shift from de novo FA to preformed FA in milk fat.

The apparent transfer rates of 18C fatty acids to milk fat is quite interesting and plays into the differences between de novo, mixed, and preformed FA concentrations in primiparous compared to multiparous. Apparent transfer rates were not different between parities, but in an unsupplemented diet, primiparous cows achieved an apparent transfer rate of more than 100% in the 0% WCS diet compared to 78% in multiparous cows. This would suggest that primiparous cows partition dietary energy from fat for milk fat and use the fermentation products from anaerobic fiber and starch digestion as energy for growth, rather than de novo FA synthesis in the mammary gland. However, similar to what has been observed in Khiaosa-ard et al. (2015), transfer of all 18C FA from the diet to milk fat decreased as total intake of 18C FA increased. The calculated apparent transfer rate for 18C for all cows is much higher than the 49.7% reported in Enjalbert et al. (2000). However, they were calculating the apparent transfer of the infusion administered to cows only based on SA and OA concentrations, rather than overall dietary transfer rate for 18C FA. We calculated overall apparent transfer rate of all dietary 18C and that may contribute to the discrepancy for the calculated rates. However, the rates we calculated for all cows

were slightly lower than similar intakes of 18C FA in (Khiaosa-ard et al., 2015) but the apparent transfer rate of 18C in primiparous cattle fed 0% cottonseed was higher than that reported by Khiaosa-ard et al. (2015). It is unclear how MFD impacts the apparent transfer rate of rate of dietary FA to milk fat, but it is expected that the apparent transfer of 18C would be decreased if the enzymes responsible for fat synthesis in the mammary gland are downregulated and secrete less fat, even as dietary intake of 18C FA increases.

Milk protein yield and concentrations followed the results of previous research which examined feeding cottonseed (Harrison et al., 1995; Abel-Caines et al., 1997; Firkins et al., 2002). However, Harrison et al. (1995) fed cottonseed at 12% of DMI on two different herds and found a different response. They noted a decrease in milk protein concentration when feeding cottonseed during the second half of the trial on Herd 1 but an increase in milk protein yield. In Herd 2, milk protein concentration decreased in multiparous cows when fed cottonseed, but overall milk protein yield increased. Therefore, it is expected that milk yield per day increased when fed WCS, but daily milk yield was not reported in the paper. The cows on the current study were later in lactation upon its completion, and therefore the late lactation cows may be the cause for the tendency for milk protein concentration to decrease in multiparous cattle. However, the review of WCS feeding by Arieli (1998) reported that very few trials note a decrease in milk protein percentage or milk protein yield when fed to dairy cattle so it is unknown why we observed a tendency for a reduction in milk protein concentration in multiparous cows. The mean reduction we observed between the 0% WCS diet and the 9.9% WCS was lower than the mean reduction among the studies included in Arieli (1998) that reported a decrease in milk protein concentration (0.1 vs 0.3 percentage units, respectively).

Table 3-1: Diet summary and chemical composition of the total mixed rations containing increased inclusion of cottonseed.

	Whole Cottonseed			
Feed Ingredient, % of DM	0%	3.4%	6.8%	9.9%
Corn Silage[2]	38.6	39.1	39.1	38.9
Alfalfa Haylage[3]	16.4	16.6	16.6	16.1
Grass Hay/Straw[4]	1.7	1.7	1.7	1.7
Ground Corn	15.4	15.6	15.6	15.5
Canola Meal	9.3	9.4	9.5	9.4
Whole Cottonseed[5]	--	3.4	6.8	9.9
Cottonseed Hulls[6]	6.7	4.6	2.3	--
Soybean Meal[7]	3.5	2.4	1.2	--
AminoPlus	3.6	3.7	3.7	3.7
Molasses	2.5	2.5	2.5	2.5
Vit/Min Suppl.[8]	1.3	1.3	1.3	1.3
NPN (Optigen)	0.4	0.4	0.4	0.4
Sodium Bicarbonate	0.7	0.7	0.7	0.7
Nutrient, % of DM				
CP	17.2	17.2	17.1	17.0
NDF	32.7	32.5	32.3	32.0
ADF	22.8	22.6	22.5	22.1
Starch	24.2	24.2	24.2	24.3
Total FA	2.49	2.90	3.31	3.70

[1]Treatments were cottonseed included into the total mixed ration at 0% of DMI (0%), 3.4% of DMI (3.4%), 6.8% of DMI, (6.8%), and 9.9% of DMI (9.9%).
[2]Corn silage contained 8.9% CP, 39.4% NDF, 24.7% ADF, 33.5% starch, and 1.9% total FA.
[3]Haylage contained 19.7% CP, 39.3% NDF, 32.8% ADF, 1% starch, and 2% total FA.
[4]Dry Hay contained 8% CP, 67.7% NDF, 44.0% ADF, 1% starch, and 1.5% total FA.
[5]Whole cottonseed contained 21.8% CP, 46.9% NDF, 38.4% ADF, 0.0% starch, and 15.5% total FA.
[6]Cottonseed Hulls contained 7.9% CP, 75.9% NDF, 63.5% ADF, 0.4% starch, and 3% total FA.
[7]Soybean meal (solvent extracted) contained 52.5% CP, 9.1% NDF, 6.4% ADF, 0.4% starch, and 3.2% total FA.
[8]The vitamin and mineral premix contained (as-fed basis) 37.234% calcium carbonate, 29.0% dried distillers grains, 24.85% salt, 4.15% MgO, 2.4521% phosphorus, 0.5% mineral oil, 0.8% zinc sulfate, 0.3685% Vitamin E (227M U/lb), 0.3672% manganese sulfate, 0.2575% copper sulfate, 0.1575% ferrous sulfate, 0.12671% selenium, 0.03% Vitamin A (650M U/g), 0.0125% Vitamin D3 (500M IU/g), 0.0085% calcium iodate, and 0.005% cobalt carbonate. Composition on a DM basis: 7.5% CP, 7.1% NDF, 3.7% ADF, 15.1% Ca, 0.75% P, 2.48% Mg, 0.28% K, 0.42% S, 9.8% Na, 23 ppm Co, 651 ppm Cu, 796 ppm Fe, 54 ppm I, 1190 ppm Mn, 13 ppm Se, 1721 ppm Zn, 195,290 IU/kg Vit A, 62,500 IU/kg Vit D, and 1,864 IU/kg Vitamin E.

Table 3-2: Summary of daily milk production and milk composition of cows fed increasing level of cottonseed.

	Cottonseed Inclusion					P-values[2]				
	0%	3.4%	6.8%	9.9%	SE	Trt	Par	TxP	Linear	Quad
DMI (kg)										
Overall	31.4	30.2	29.9	29.6	1.7	0.12	<0.01	0.6	0.03	0.44
Prim	26.8	26.1	26.2	25.9	2.1				0.51	0.84
Mult	36.0	34.3	33.5	33.2	1.7				<0.01	0.28
Milk (kg)										
Overall	41.1	39.9	41.5	40.6	1.6	0.52	0.34	0.51	0.95	0.88
Prim	40.2	38.7	40.6	38.2	2.6				0.47	0.75
Mult	42.0	41.1	42.5	43.0	1.8				0.28	0.47
Fat (kg)										
Total	1.60	1.41	1.45	1.46	0.09	0.13	<0.01	0.08	0.21	0.11
Prim	1.42	1.10	1.22	1.21	0.14				0.12	0.06
Mult	1.69	1.72	1.68	1.71	0.10				>0.99	>0.99
Fat (%)										
Overall	3.78	3.51	3.51	3.56	0.2	0.17	<0.01	0.03	0.14	0.12
Prim	3.54	2.83	3.03	3.14	0.3				0.17	0.02
Mult	4.03	4.18	3.99	3.97	0.2				0.51	0.44
Prot (kg)										
Overall	1.27	1.21	1.27	1.24	0.05	0.43	0.05	0.78	0.76	0.57
Prim	1.18	1.11	1.20	1.12	0.09				0.66	>0.99
Mult	1.36	1.31	1.33	1.35	0.06				0.93	0.32
Prot (%)										
Overall	3.10	3.04	3.07	3.04	0.1	0.67	0.11	0.38	0.43	0.7
Prim	2.95	2.88	2.99	2.95	0.1				0.70	0.76
Mult	3.24	3.20	3.15	3.14	0.1				0.07	0.81
MUN										
Overall	15.3	14.2	13.6	14.3	1.3	0.17	0.21	0.57	0.11	0.12
Prim	15.0	13.0	13.2	13.7	1.6				0.25	0.12
Mult	15.6	15.4	14.6	14.9	1.3				0.21	0.61

[1]Treatments were cottonseed included into the total mixed ration at 0% of DMI (0%), 3.4% of DMI (3.4%), 6.8% of DMI, (6.8%), and 9.9% of DMI (9.9%).
[2]Trt - main effect of treatment; Par - main effect of parity; TxP - interaction of treatment and parity; Linear - preplanned contrast of the linear effect of increased cottonseed inclusion; Quad. - preplanned contrast for the quadratic effect of increased cottonseed inclusion.
[3]Prim - mean of primiparous cows; Mult - mean of multiparous cows.

Table 3-3: Apparent transfer of 18C fatty acids and milk fatty acid profile by source of fatty acids in milkfat from cows fed increasing levels of cottonseed.

	Whole Cottonseed					P-Values[2]				
18C Apparent Transfer (%)[3]	0%	3.4%	6.8%	9.9%	SEM	Trt	Par	TxP	Lin	Quad
Overall	98.1	74.3	72.8	65.6	0.07	<0.01	0.27	0.03	<0.01	0.13
Prim	118.0	74.5	71.6	68.1	0.11				<0.01	0.03
Mult	78.1	74.2	73.9	63.1	0.08				0.11	0.57
FA, % FA[4]										
∑<16 C	28.1	27.1	26.2	25.1	1.07	<0.01	<0.01	0.73	<0.01	0.97
Prim	25.7	24.9	24.5	23.6	1.49				0.09	0.94
Mult	30.5	29.2	28	26.7	1.14				<0.01	0.97
∑ 16 C	30.3	30.8	29.3	28.3	0.83	<0.01	0.06	0.07	<0.01	0.09
Prim	28.2	30.2	28.1	27.5	1.23				0.15	0.07
Mult	32.4	31.4	30.4	29.1	0.91				<0.01	0.79
∑>16 C	37.2	38	40.2	42.4	1.64	<0.01	<0.01	0.11	<0.01	0.33
Prim	41.7	40.6	42.8	44.5	2.25				0.06	0.27
Mult	32.7	35.4	37.5	40.4	1.74				<0.01	0.89
∑OBCFA[5]	3.54	3.38	3.51	3.42	0.18	0.51	0.1	0.09	0.58	0.7
Prim	3.55	3.52	3.66	3.75	0.24				0.22	0.68
Mult	3.53	3.24	3.36	3.1	0.19				0.01	0.92

[1]Treatments were cottonseed included into the total mixed ration at 0% of DMI (0%), 3.4% of DMI (3.4%), 6.8% of DMI, (6.8%), and 9.9% of DMI (9.9%).

[2]Trt - main effect of treatment; Par - main effect of parity; TxP - interaction of treatment and parity; Linear - preplanned contrast of the linear effect of increased cottonseed inclusion; Quad. - preplanned contrast for the quadratic effect of increased cottonseed inclusion.

[3]Apparent transfer rate - calculated percentage of all dietary 18C fatty acids that are transferred to milk fat

[4]Fatty acids (FA) <16 C originate from de novo synthesis in the mammary gland, 16 carbon FA originate both from de novo synthesis and uptake of preformed FA from plasma, and FA >16 C originate from plasma.

[5]Summation of odd and branch chain fatty acids (OBCFA), odd chain FA (OCFA), branch chain FA (BCFA), iso BCFA, and ante-iso BCFA.

[6]Prim - mean of primiparous cows; Mult - mean of multiparous cows.

Supplemental Table 3-1: Milk fatty acid production per day by source in cows fed a increasing levels of cottonseed inclusion.

	Cottonseed Inclusion					P-values[2]				
FA, g/day[3]	0	3.3	6.7	9.9	SE	Trt	Par	TxP	Linear	Quad
∑<16										
Total	399	336	370	334	45	0.07	<0.01	0.52	0.07	0.51
Prim	343	242	305	256	56				0.16	0.43
Mult	454	430	435	412	46				0.24	0.99
∑16										
Total	428	375	411	372	44	0.08	<0.01	0.53	0.10	0.70
Prim	374	288	350	297	56				0.19	0.56
Mult	482	463	472	447	46				0.31	0.88
∑>16										
Total	510	452	548	546	36	<0.01	0.12	<0.01	0.03	0.19
Prim	536	391	819	468	54				0.61	0.18
Mult	483	514	576	625	39				<0.01	0.71
∑OBCFA[4]										
Total	62	51	63	58	4	0.01	0.01	0.36	0.86	0.22
Prim	58	41	58	52	6				0.94	0.19
Mult	67	61	68	63	4				0.68	0.83

[1]Treatments were cottonseed included into the total mixed ration at 0% of DMI (0%), 3.4% of DMI (3.4%), 6.8% of DMI, (6.8%), and 9.9% of DMI (9.9%).
[2]Trt - main effect of treatment; Par - main effect of parity; TxP - interaction of treatment and parity; CON vs. FAT - preplanned contrast of CON diet compared to the supplemented diets; Linear - preplanned contrast of the linear effect of increased oleic acid concentration; Quad. - preplanned contrast for the quadratic effect of increased oleic acid concentration.
[3]Fatty acids (FA) <16 C originate from de novo synthesis in the mammary gland, 16 carbon FA originate both from de novo synthesis and uptake of preformed FA from plasma, and FA >16 C originate from plasma.
[4]Summation of odd and branch chain fatty acids (OBCFA)
[5]Prim - mean of primiparous cows; Mult - mean of multiparous cow

Supplemental Table 3-2 : Milk fatty acid profile for even-, straight-chain fatty acids ≤16C in cows fed increasing levels of cottonseed.

	Cottonseed Inclusion					P-values[2]				
FA, % FA	0%	3.4%	6.8%	9.9%	SE	Trt	Par	TxP	Linear	Quad
C4:0	4.68	4.53	4.65	4.58	0.3	0.86	0.37	0.29	0.79	0.76
Prim	4.75	4.21	4.50	4.41	0.4				0.49	0.35
Mult	4.62	4.85	4.81	4.76	0.3				0.62	0.40
C6:0	2.43	2.35	2.32	2.26	0.1	0.41	<0.01	0.75	0.11	0.85
Prim	2.28	2.11	2.12	2.02	0.2				0.14	0.75
Mult	2.58	2.59	2.51	2.50	0.1				0.43	0.90
C8:0	1.35	1.28	1.23	1.19	0.07	0.07	<0.001	0.92	<0.01	0.69
Prim	1.22	1.11	1.09	1.04	0.1				0.07	0.71
Mult	1.48	1.44	1.36	1.35	0.07				0.04	0.86
C10:0	3.20	3.00	2.88	2.73	0.2	0.02	<0.01	0.56	<0.01	0.83
Prim	2.70	2.55	2.54	2.40	0.3				0.22	0.98
Mult	3.70	3.45	3.22	3.05	0.2				<0.01	0.73
C10:1c9	0.26	0.26	0.22	0.20	0.02	<0.01	0.15	0.91	<0.01	0.42
Prim	0.23	0.24	0.20	0.17	0.03				<0.01	0.36
Mult	0.29	0.27	0.24	0.23	0.02				<0.01	0.94
C12:0	3.68	3.44	2.30	3.08	0.2	<0.01	0.01	0.06	<0.01	0.96
Prim	3.05	3.00	3.01	2.82	0.3				0.38	0.72
Mult	4.30	3.91	3.59	3.34	0.2				<0.01	0.54
C14:0	11.6	11.2	10.9	10.4	0.4	<0.01	0.04	0.17	<0.01	0.83
Prim	10.6	10.6	10.3	10.0	0.6				0.13	0.65
Mult	12.6	11.8	11.5	10.8	0.5				<0.01	0.78
C14:1	0.90	1.01	0.81	0.72	0.10	<0.01	0.50	0.10	<0.01	0.06
Prim	0.90	1.18	0.85	0.73	0.2				0.03	0.02
Mult	0.90	0.85	0.76	0.71	0.1				0.02	0.99
C16:0	29.0	29.0	28.0	27.1	0.8	<0.01	0.03	0.09	<0.01	0.15
Prim	26.9	28.6	26.8	26.3	1.2				0.24	0.12
Mult	31.1	30.1	29.2	28.0	0.9				<0.01	0.78
C16:1	1.31	1.44	1.21	1.14	0.1	<0.01	0.42	0.25	<0.01	0.10
Prim	1.33	1.60	1.29	1.15	0.2				0.05	0.05
Mult	1.30	1.29	1.13	1.14	0.1				0.05	0.94

[1]Treatments were cottonseed included into the total mixed ration at 0% of DMI (0%), 3.4% of DMI (3.4%), 6.8% of DMI, (6.8%), and 9.9% of DMI (9.9%).

[2]Trt - main effect of treatment; Par - main effect of parity; TxP - interaction of treatment and parity; Linear - preplanned contrast of the linear effect of increased cottonseed inclusion; Quad. - preplanned contrast for the quadratic effect of increased cottonseed inclusion.

[3]Prim - mean of primiparous cows; Mult - mean of multiparous cows.

Supplemental Table 3-3: Milk fatty acid profile for fatty acids ≥18-carbons in cows fed increasing levels of cottonseed.

	Cottonseed Inclusion					P-values				
FA, % FA	0%	3.4%	6.8%	9.9%	SE	Trt	Par	TxP	Linear	Quad
C18:0	10.40	10.40	11.30	12.10	0.7	<0.01	0.50	<0.001	<0.01	0.09
Prim	11.1	9.92	10.8	11.3	1.00				0.45	0.05
Mult	9.70	10.9	11.7	13.00	0.8				<0.01	0.91
C18:1t4	0.03	0.03	0.03	0.04	0.003	<0.01	0.01	0.93	<0.01	0.24
Prim	0.031	0.036	0.036	0.045	0.006				0.03	0.68
Mult	0.021	0.021	0.025	0.034	0.004				<0.01	0.15
C18:1t5	0.01	0.02	0.02	0.02	0.002	0.02	0.01	0.90	<0.01	0.51
Prim	0.016	0.019	0.021	0.027	0.004				0.02	0.60
Mult	0.011	0.012	0.014	0.018	0.003				0.04	0.68
C18:1t6-8	0.30	0.35	0.41	0.47	0.03	<0.01	<0.01	0.40	0.00	0.95
Prim	0.35	0.42	0.48	0.57	0.05				<0.01	0.90
Mult	0.26	0.28	0.34	0.36	0.03				0.01	0.94
C18:1t9	0.24	0.28	0.31	0.36	0.02	<0.01	<0.01	0.47	<0.01	0.88
Prim	0.28	0.34	0.36	0.43	0.03				0.00	0.86
Mult	0.20	0.22	0.26	0.29	0.02				<0.01	0.99
C18:1t10	0.56	0.75	1.08	1.24	0.2	0.03	<0.001	0.23	<0.01	0.93
Prim	0.77	1.10	1.53	1.91	0.3				<0.01	0.94
Mult	0.36	0.40	0.62	0.55	0.2				0.37	0.79
C18:1t11	1.01	1.18	1.25	1.67	0.2	<0.01	0.01	0.23	<0.01	0.29
Prim	1.21	1.48	1.42	2.14	0.2				<0.01	0.23
Mult	0.81	0.89	1.09	1.19	0.2				0.03	0.91
C18:1t12	0.48	0.59	0.64	0.74	0.03	<0.01	<0.01	0.35	<0.01	0.77
Prim	0.53	0.70	0.70	0.83	0.06				<0.01	0.68
Mult	0.43	0.48	0.58	0.64	0.04				<0.01	0.92
C18:1c9	19.1	19.3	20.0	20.2	0.8	0.16	<0.01	<0.01	0.03	0.94
Prim	21.9	21.0	22.0	21.0	1.2				0.48	0.95
Mult	16.2	17.6	18.0	19.4	0.8				<0.01	0.96
C18:1t15	0.24	0.28	0.30	0.35	0.03	<0.01	0.12	0.94	<0.01	0.63
Prim	0.27	0.30	0.32	0.38	0.03				<0.01	0.53
Mult	0.21	0.25	0.28	0.31	0.02				<0.01	0.94
C18:1c11	0.79	0.80	0.84	0.89	0.05	0.13	<0.01	0.66	0.02	0.57
Prim	0.90	0.93	0.98	1.05	0.07				0.06	0.93
Mult	0.68	0.64	0.71	0.73	0.05				0.19	0.39
C18:1c12	0.33	0.40	0.43	0.55	0.04	<0.01	0.03	0.35	<0.01	0.36
Prim	0.36	0.45	0.48	0.64	0.06				<0.01	0.38
Mult	0.30	0.34	0.39	0.45	0.04				<0.01	0.72
C18:2c9c12	2.20	2.06	2.17	2.19	0.06	0.22	0.04	0.55	0.72	0.15
Prim	2.32	2.10	2.30	2.29	0.1				0.76	0.23
Mult	2.08	2.03	2.04	2.09	0.07				0.85	0.39
C18:3c6c9c12	0.13	0.12	0.13	0.14	0.009	<0.01	0.80	0.14	0.03	0.01
Prim	0.49	0.43	0.44	0.45	0.04				0.36	0.25
Mult	0.49	0.42	0.39	0.25	0.03				<0.01	0.33

Supplemental Table 3-3 continued

C18:3c9c12c15		0.49	0.42	0.41	0.40	0.03	<0.01	0.01	0.24	<0.01	0.14
	Prim	0.49	0.43	0.44	0.45	0.04				0.36	0.25
	Mult	0.49	0.42	0.39	0.35	0.03				<0.01	0.33
C20:1c11		0.043	0.040	0.038	0.044	0.004	0.62	<0.01	0.21	0.96	0.20
	Prim	0.051	0.043	0.051	0.052	0.007				0.063	0.36
	Mult	0.035	0.038	0.026	0.035	0.005				0.046	0.33
CLAc9t11		0.48	0.58	0.59	0.71	0.06	<0.01	<0.01	0.15	<0.001	0.77
	Prim	0.58	0.77	.071	.093	0.1				<0.01	0.81
	Mult	0.38	0.39	0.47	0.49	0.07				0.07	0.86
C20:2n6		0.016	0.015	0.013	0.012	0.002					
	Prim	0.014	0.018	0.014	0.011	0.004					
	Mult	0.017	0.013	0.013	0.014	0.002					
C22:0		0.045	0.044	0.048	0.045	0.004	0.87	0.54	0.29	0.86	0.92
	Prim	0.047	0.041	0.051	0.048	0.006				0.48	0.76
	Mult	0.044	0.048	0.043	0.042	0.005				0.50	0.54
C20:3n6		0.10	0.10	0.10	0.10	0.007	0.97	0.25	0.95	0.93	0.86
	Prim	0.10	0.01	0.09	0.10	0.010				0.99	0.95
	Mult	0.11	0.11	0.11	0.11	0.008				0.90	0.81
C20:4n6		0.16	0.15	0.15	0.15	0.01	0.64	0.91	0.89	0.24	0.76
	Prim	0.15	0.15	0.15	0.15	0.01				0.68	0.95
	Mult	0.16	0.15	0.15	0.14	0.01				0.15	0.66
C20:5n3		0.025	0.025	0.021	0.020	0.002	<0.01	0.09	0.60	<0.01	0.38
	Prim	0.024	0.023	0.018	0.017	0.003				<0.01	0.93
	Mult	0.025	0.028	0.025	0.022	0.002				0.02	0.11

[1]Treatments were cottonseed included into the total mixed ration at 0% of DMI (0%), 3.4% of DMI (3.4%), 6.8% of DMI, (6.8%), and 9.9% of DMI (9.9%).

[2]Trt - main effect of treatment; Par - main effect of parity; TxP - interaction of treatment and parity; Linear - preplanned contrast of the linear effect of increased cottonseed inclusion; Quad. - preplanned contrast for the quadratic effect of increased cottonseed inclusion.

[3]Prim - mean of primiparous cows; Mult - mean of multiparous cows.

Supplemental Table 3-4: Milk fatty acid profile of odd- and branched-chain fatty acids in cows fed increasing levels of cottonseed.

	Cottonseed Inclusion					P-values				
FA, % FA	0	3.3	6.7	9.9	SE	Trt	Par	TxP	Linear	Quad
C11:0	0.07	0.07	0.08	0.07	0.008	0.90	0.70	0.06	0.80	0.62
Prim	0.058	0.071	0.077	0.078	0.01				0.08	0.51
Mult	0.087	0.074	0.078	0.067	0.01				0.06	0.92
iso C13:0	0.026	0.023	0.029	0.022	0.003	0.14	0.33	0.30	0.52	0.34
Prim	0.027	0.019	0.03	0.019	0.005				0.45	0.66
Mult	0.026	0.027	0.028	0.025	0.003				0.96	0.30
ante-iso C13:0	0.073	0.073	0.060	0.053	0.007	<0.001	0.51	0.26	<0.01	0.26
Prim	0.064	0.073	0.059	0.05	0.01				0.02	0.11
Mult	0.082	0.073	0.062	0.057	0.008				<0.01	0.69
C13:0	0.12	0.12	0.13	0.13	0.01	0.65	0.73	0.01	0.28	0.91
Prim	0.1	0.12	0.13	0.14	0.02				0.02	0.69
Mult	0.14	0.11	0.12	0.11	0.01				0.08	0.70
iso C14:0	0.071	0.080	0.069	0.071	0.01	0.65	0.11	0.74	0.66	0.60
Prim	0.059	0.060	0.051	0.059	0.02				0.84	0.74
Mult	0.083	0.10	0.087	0.082	0.01				0.64	0.17
iso C15:0	0.20	0.20	0.20	0.19	0.01	0.27	0.23	0.92	0.07	0.80
Prim	0.20	0.19	0.19	0.19	0.01				0.41	0.93
Mult	0.21	0.21	0.20	0.19	0.01				0.06	0.57
ante-iso C15:0	0.43	0.39	0.41	0.39	0.03	0.06	0.12	0.47	0.09	0.28
Prim	0.44	0.39	0.42	0.42	0.03				0.74	0.21
Mult	0.42	0.39	0.40	0.37	0.03				0.02	0.95
C15:0	1.10	1.10	1.14	1.11	0.07	0.88	0.26	0.03	0.68	0.69
Prim	1.05	1.20	1.21	1.26	0.10				0.06	0.57
Mult	1.14	1.00	1.09	0.96	0.08				0.06	0.89
iso C16:0	0.20	0.17	0.18	0.18	0.01	0.47	0.05	0.97	0.26	0.46
Prim	0.17	0.15	0.15	0.16	0.03				0.61	0.58
Mult	0.22	0.20	0.21	0.20	0.02				0.23	0.61
iso C17:0	0.055	0.056	0.063	0.080	0.008	<0.01	<0.01	0.26	<0.01	0.14
Prim	0.071	0.069	0.075	0.011	0.01				<0.01	0.08
Mult	0.040	0.044	0.050	0.054	0.009				0.09	0.98
ante-iso C17:0	0.44	0.38	0.41	0.38	0.04	0.01	<0.01	0.39	0.04	0.21
Prim	0.47	0.39	0.45	0.42	0.04				0.43	0.28
Mult	0.41	0.37	0.37	0.34	0.04				0.01	0.53
C17:0	0.56	0.52	0.57	0.56	0.04	0.15	<0.01	0.20	0.39	0.28
Prim	0.61	0.55	0.62	0.64	0.05				0.13	0.15
Mult	0.51	0.49	0.51	0.49	0.04				0.50	0.85
C17:1c9	0.20	0.20	0.19	0.18	0.02	0.56	<0.01	0.99	0.19	0.70
Prim	0.23	0.23	0.22	0.21	0.03				0.32	0.78
Mult	0.16	0.16	0.16	0.15	0.02				0.37	0.77

[1]Treatments were cottonseed included into the total mixed ration at 0% of DMI (0%), 3.4% of DMI (3.4%), 6.8% of DMI, (6.8%), and 9.9% of DMI (9.9%).

[2]Trt - main effect of treatment; Par - main effect of parity; TxP - interaction of treatment and parity; Linear - preplanned contrast of the linear effect of increased cottonseed inclusion; Quad. - preplanned contrast for the quadratic effect of increased cottonseed inclusion.
[3]Prim - mean of primiparous cows; Mult - mean of multiparous cows.

Chapter 4

Examination of the effects of increasing oleic acid in a saturated fatty acid prill fed to high-producing Holstein cows.

Abstract

Fat supplements are commonly included in rations to increase energy intake in support of milk and milk component yield of dairy cows. Recent research indicates fatty acid (FA) profile of fat supplements may change fatty acid digestibility and metabolism with a resulting impact on milk production. The hypothesis of this experiment was that increased oleic acid levels in a saturated fatty acid supplement reacted with magnesium would increase milk yield without induction of milk fat depression. Eight primiparous and eight multiparous cows (43.8 and 56.5kg milk and 74 and 84 DIM, respectively, at the start of experiment) were arranged in a 4x4 Latin square design with 21 day periods. Treatments were a control diet with no added fat and diets which included FA supplements at 1.1% of dry matter intake. The supplements contained either 5%, 10%, and 15% oleic acid (OA) at a percent of total FA. The fat supplement maintained a constant concentration of C18:0 but C16:0 was substituted for increasing levels of *cis*-9 C18:1. The FA were reacted with magnesium during prilling resulting in partial formation of magnesium salts. Data was analyzed using the random effect of cow and period and the fixed effect of treatment and preplanned contrasts tested the effects of the control diet vs fat addition and the linear effect of increasing OA level. Overall, there were no interactions between diet and parity. Milk yield, fat yield, protein yield, and fat percent were not affected by treatment. Fat percent was increased 0.18% ($P < 0.05$) with fat supplementation in primiparous cows but was not increased in multiparous cows. Protein percent was decreased 0.06% in primiparous cows ($P < 0.05$) fed the

diets with added fat and did not differ between OA levels. Dietary fat supplements tended to decrease milk protein in multiparous cows. Milk fatty acid profile was shifted toward higher concentrations of preformed fatty acids and lower concentrations of de novo fatty acids as OA concentration within the prill increased. The study suggests that replacing a constant ratio of C16:0 to C18:0 with OA does not negatively impact milk yield and milk fat yield.

Introduction

Delivery of energy to dairy cattle is paramount as average milk yield and thus, nutrient requirements, in dairy cattle continue to increase as genetic potential for milk and milk fat yield increase. Therefore, strategies to increase dietary energy content and increase digestibility of feedstuffs included in dairy cattle diets have been under more scrutiny in recent years. Dry fat supplements are an effective method of increasing energy density of TMR, but recent work suggests that the digestibilities of some saturated fat prill blends may be lower than optimal (de Souza et al., 2016; Boerman et al., 2017). Therefore, to maximize the efficiency of dry fat supplements fed to dairy cattle, factors affecting fat digestibility should be examined and quantified.

Fatty acid profile is one factor of a dry fat supplement that affects production responses in dairy cattle. PA is a common FA included in the rations of dairy cattle as it appears to have a high transfer rate from ingestion to secretion into milk fat as it readily increases milk fat concentration in dairy cattle (de Souza and Lock, 2018, 2019). SA is the other SFA commonly found in prilled fat supplements as SFAs pose a lesser risk for BH-induced MFD. However, Boerman et al. (2015) reported the digestibility of SA may decrease rapidly as duodenal flow of SA increases , especially when fed in supplements highly enriched in SFA. OA has received more attention recently as it has a lower melting temperature and has emulsifier properties that may help to improve FA digestibility.

Other recent work has led to the development of new methods for characterizing fat supplements. Shepardson (2018) used differential scanning calorimetry to examine the melting points of blends of FA to characterize the dissociation of FA in each sample. This research suggested that blends had lower melting points and were potentially more digestible than enriched

sources of FA. Western et al. (2020) saw no difference between an enriched PA supplement and a FA blend but found a lower digestibility for a high SA fat supplement. Shepardson (2018) fed a blend of FA that contained low levels of OA but did not see any significant changes in digestibility, but some commercial fat supplements contain much higher levels of OA than what was found in this trial. Some research does report positive effects of OA supplementation as de Souza et al. (2019) fed blends of OA and PA and reported linear increases in both total FA and 16-carbon FA and a tendency for increased 18-carbon digestibility suggesting that the increased OA concentration increased FA digestibility. In addition, Prom (2020) abomasally infused OA and observed similar results with increased 16-carbon, 18-carbon, and total FA digestibility in lactating cows. However, the prilled fat supplements used our research trial were reacted with magnesium oxide (**MgO**) during the manufacturing process rather than using calcium salts like those fed by de Souza et al. (2019) so the effects of increased OA on FA digestibility may be different. Mg-salts of FA have a higher melting point than Ca-salts of FA so digestibility of the prilled fats may be further decreased (Shepardson, 2018).

The purpose of the following experiment was to quantify the effects of increasing the level of OA in a prilled fat supplement containing a mixture of PA and SA on lactational performance of dairy cattle and whether OA would increase the energy supply to dairy cattle and how this would impact milk yield, milk fat yield, and milk fat concentration. Our hypothesis was that increased levels of OA in a prilled FA supplement would increase milk fat concentration and yield due to increased digestibility of the fat supplement without any effect on milk yield.

Materials and Methods

Eight multiparous and 8 primiparous (76 ± 58 dim and 64 ± 16 DIM, respectively) of The Pennsylvania State University Dairy Teaching and Research Center were arranged in a 4x4 balanced Latin Square design. All treatments and procedures were pre-approved by the Penn State University Institutional Animal Care and Use Committee (PRAMS 200946398). Cows were housed in a tie-stall barn and milked 2x/day (~0700 and 1830). The animals consumed a common covariate diet during for 10 d prior to the first period and baseline production was recorded. Experimental periods lasted 21 d and samples were collected throughout the last 4 days of each period. There were 4 treatments: a control diet without any fat supplement (**CON**) and three diets with a fat supplement added at 1.2% of DM to the TMR (**FAT**). The three fat supplements fed maintained a constant concentration of SA as OA was substituted for PA in the supplement across a titration curve. Oleic acid concentrations of supplement total FA were 5%, 10%, and 15%, respectively, as summarized by Table 4-2. Samples were analyzed for FA profile prior to the beginning of the trial and then again after the trial had finished. The prilled fats were not reacted with an antioxidant and it is expected that this is responsible for the change in the FA profile of the supplements. Fat supplements were formulated and blended specifically for this trial by Milk Specialties Global (Eden Prairie, MN) in a commercial manufacturing setting.

Feeding, Milk Sample Collection, and Milk Sample Analysis

Orts were weighed every day at 0530 as cows left the barn for the milking parlor. Cows were fed 1x/ day at 110% of the previous day's intake before the animals returning from morning milking. A common base diet was mixed in a stationary mixer (Electra-Mix 1062; I.H. Rissler Manufacturing, Mohnton, PA) and fat supplements were added to individual batches of the base

mix in a mobile apron-chain feed mixer at 1.2% of DMI (Rissler 1050 Feedcart; I.H. Rissler Manufacturing, Mohnton, PA) and mixed thoroughly before being delivered (control diet was the same as the base mix). Forages were sampled every week and dried in a 55°C oven for 48 hours and the base mix was updated accordingly.

Milk samples were collected every milking during the last 4 days of each experimental period and refrigerated until analysis for milk components (DairyOne Ithaca, NY). During the last 2 days of each experimental period, a second sample of milk was collected at each milking and refrigerated. This milk was then composited using a weighted average based off the milk weights from each milking, and spun at 1300 x g for 20 min at 4°C. The fat was then transferred to a separate vial and stored at -20°C until analysis. The fat cakes were analyzed as described by Rico and Harvatine (2013) with a 3:2 hexane: isopropanol mixture and transmethylated using sodium methoxide. The fatty acid methyl esters were determined by gas chromatography with a flame ionization detector and a capillary column [SP-2560; 100 m x 0.25 mm (i.d.) with a 0.2-μm film thickness; Supelco Inc., Bellefonte, PA].

Feed Sample Collection and Analysis

Feed ingredients were collected during the last 4 days of each experimental period and composited. Then, they were freeze dried (Ultra 35-XL; Virtis CO. Inc., Gardiner, NY) and ground using a Wiley mill (A.H. Thomas CO., Philadelphia, PA) with a 4-mm screen. Subsamples were collected and the main sample was reground with a 1-mm screen for analysis. Individual samples of treatment ingredients and forages were analyzed for CP, ADF, NDF, starch, 105°C DM, and FA concentration and profile. Book values for the urea and molasses were used (241% CP for urea (Optigen, Alltech Inc., Lexington, KY) and 6.3% CP, 0% NDF, and 11.0% ash for molasses).

AOAC (2019) protocols 990.03 and 973.18 were used for CP and ADF, respectively. NDF was calculated according to Van Soest et al. (1991) using sodium sulfite, heat stable amylase, and an acetone rinse. Starch was determined enzymatically according to (Hall, 2009) and iNDF was determined by batch culture in vitro incubation. These were completed by Cumberland Valley Analytical Services (Waynesboro, PA). Samples were analyzed for 105°C DM (overnight in a forced air oven) and FA profile and total FA concentration was determined using direct methylation as described by Sukhija and Palmquist (1988) including the modifications to the internal standard used by Rico and Harvatine (2013).

Statistical Analysis

Data was analyzed using the REML method of JMP Pro (version 14.0; SAS Institute Inc., Cary, NC) using the following model:

$$Y_{ijkl} = \mu + C_i + P_j + T_k + R_l + (T_k \times R_l) + e_{ijkl}$$

Where Y_{ijkl} represents the dependent variable of interest, μ is the overall mean, C_i is the random effect of cow (i = 1 to 16), P_j is the random effect of period (j = 1 to 4), T_k is the fixed effect of treatment (k = 1-4), R_l is the fixed effect of parity (j = primiparous or multiparous), T_k *x* R_l is the interaction of parity and treatment, and e_{ijkl} is the residual error. One multiparous cow was removed during period 2 due to severe mastitis. Studentized residuals outside of ± 3.0 were considered outliers and removed from analysis. Significance was claimed at $P \leq 0.05$ and trends at $P \leq 0.10$. A protected LSD was used for mean separation. Pre-planned contrasts comparing control to supplemented diets and linear and quadratic contrasts between levels of OA in the fat supplements were analyzed.

Results and Discussion

Production Responses

Table 4-3 summarizes the production responses of cows to fat supplementation and OA concentration in the prill. There was a treatment by parity interaction for DMI. Overall, DMI was reduced with fat supplementation when compared to CON diets in multiparous cows ($P < 0.05$), but not in primiparous cows. There were no linear or quadratic effects of level of OA on DMI. Treatment by parity interactions were not observed for milk production. Milk yield was unaffected by treatment but was higher in multiparous cows ($P < 0.05$). Milk fat concentration was unaffected by parity, but was increased in FAT diets compared to the CON ($P < 0.05$). Increasing OA concentration had no effect on milk fat concentration. Milk fat yield tended to be higher in multiparous cows compared to primiparous cows, but there was no effect from fat supplementation or OA concentration. Milk protein concentration was changed by diet and was greater in the CON compared to FAT ($P < 0.05$). Primiparous cows decreased in milk protein concentration ($P < 0.05$) but multiparous cows only tended to decrease in milk protein concentration. There was a tendency for a quadratic response in milk protein concentration as OA increased in the supplement. Milk protein yield differed by parity and was greater in multiparous cows ($P < 0.05$) but was unaffected by diet. No linear or quadratic effects of OA on milk protein concentration were observed.

Fatty Acid Profile in the Milk Fat

Concentrations of milk FA by source are summarized in Table 4-4. Diet and parity both had effects on de novo FA concentration in milk. Primiparous cows had a lesser de novo milk FA concentration than multiparous animals ($P < 0.05$). CON diets had greater concentrations of de novo FA concentration ($P < 0.05$), but no linear or quadratic effects were detected across OA

levels. Mixed FA were unaffected by parity, but affected by diet; they were increased in FAT diets compared to CON ($P < 0.05$) and linearly decreased as OA concentration increased ($P < 0.05$).

Preformed FA were also affected by parity and diet. Primiparous cows had greater concentrations of preformed FA than multiparous cows ($P < 0.05$). There was no difference in preformed FA concentration between the CON and FAT diets but were linearly increased ($P<0.05$) as OA concentration increased. No significant differences were observed for OBCFA. *trans*-10 C18:1 tended to be elevated in FAT diets compared to CON diets but no differences were observed between levels of OA. *cis*-9 C18:1 concentration in milk fat was unaffected by diet but was increased in primiparous cows compared to multiparous cows ($P < 0.05$). No observed differences in *cis*-9 C18:1 concentration in milk were detected across levels of OA.

C16:0 was affected by diets and linearly decreased as OA concentration increased ($P < 0.05$). This was observed only in primiparous cows and there was only a tendency for linear decrease in C16:0. C18:0 was linearly increased as OA concentration increased ($P < 0.05$). C18:0 was also increased in multiparous cows consuming the FAT diets when compared to the CON ($P < 0.05$).

Integrative Discussion

Results gathered from this trial did not completely support our hypothesis. First, there was a tendency for lower DMI in cows fed FAT treatments. However, the FAT diets had a higher milk fat concentration than the CON diet indicating that the fat supplementation did increase milk fat concentration. In addition, *trans*-10 C18:1 was not increased in any of the treatments or parities suggesting that increasing the level of *cis*-9 C18:1 in the fat supplement did not cause BH-induced MFD in any of the cows. Therefore, this data suggests that increasing the proportion of OA in a

primarily PA and SA blend can be reacted with magnesium oxide and safely fed to high-producing dairy cattle.

The reduction in DMI in multiparous cows did not agree with most previous research that supplemented dietary fat with a similar FA profile. Western et al. (2020) fed a blend of PA and SA with a low level of OA (just over 5%) that did not reduce DMI when compared to an unsupplemented diet. The multiparous cows in this trial had a similar daily milk yield but were earlier in their lactation (76 ± 58 DIM vs 146 ± 55 DIM, respectively) than those in Western et al. (2020). It is unclear as to whether the lower DIM coupled with fat supplementation affected DMI, but other trials have reported a decrease in DMI. Rabiee et al. (2012) reviewed 38 papers and found most fat supplements decrease DMI in cattle. Although there were only four studies included that fed prilled fats, there was a mean decrease of 1.2 kg per day in DMI. However, abomasal infusion of high doses of fat, especially unsaturated fat, have been shown to decrease DMI and the prilled fat treatments summarized by Rabiee et al. (2012) were fed at much higher rates than the 1.2% inclusion rate of our study (Drackley et al., 2007). Therefore, it is unclear if the decrease reported by Rabiee et al. (2012) is comparable to the results we observed. The lack of a change in DMI between OA levels does match previous research. de Souza et al. (2019) fed blends of PA and OA with PA substituted for increasing levels of OA but reported no change in DMI between levels of OA. They fed four blends that ranged from 10% up to 30% of FA concentration within the prill as OA, which exceeds the levels fed in our trial. Thus, it suggests that increasing levels of OA in a FA prill do not impact DMI. This may either be due to a limited effect of OA on intake or substantial BH of OA.

The tendency for a linear reduction in milk production in multiparous cows is not consistent with previous research. de Souza and Lock (2018), Shepardson (2018), de Souza et al. (2019), and

Western et al. (2020) did not report any reductions in daily milk yield. In addition, de Souza and Lock (2018) saw an increase in daily milk yield, but they fed a highly enriched PA supplement so the difference in milk yield may be partly due to the FA profile of the supplement. However, the decrease in milk yield in this study was only observed in multiparous cows and therefore it may be an anomaly. Similar to the primiparous cows in the current study, primiparous cows in previous trials showed no increase in daily milk yield when fed a diet supplemented with fat. The reduction in daily milk yield of multiparous cows in our trial may be the result of lower DMI, and thus a potential decrease in the production of glucogenic VFAs which have been linked to higher milk production (Seymour et al., 2005) . However, we observed a shift in the milk FA profile to higher concentrations of preformed FA and decreased de novo FAs. Higher concentrations of preformed FA without an increase of milk fat yield would suggest that substrates for de novo FA like glucose and acetate would be spared for other purposes and therefore milk yield could remain constant, if not increase. However, decreased DMI and milk production were only observed in multiparous cattle which are trends that have been noted in other papers where dietary fat is supplemented. Even with the tendency for lower milk yield as OA concentrations in the fat supplement increased, daily milk fat yields were unaffected by treatment and milk fat was not increased in FAT treatments compared to CON treatment. Therefore, the concurrent increase in milk fat concentration with the decrease in milk yield likely caused daily milk fat yield to remain unchanged.

The relatively unchanged preformed FA concentration in milk fat across levels of OA is surprising and contrasts with de Souza et al. (2019) who reported a linear increase in preformed FA as OA concentration in the prill increased and preformed FA increased 2% when cows were fed the 17% OA supplement compared to the 10% OA supplement. We had similar levels of OA in our fat supplements with the highest concentrations of OA at 15% and 10%, but did not observe

the same effect. We did observe a tendency for a linear increase in preformed FA in multiparous cattle and there was a numerical increase of 1.3% in the concentration of preformed FA between the 10% OA supplement and 15% OA supplement. There was a stronger trend for increased preformed FA in the primiparous cows in our trial compared to the multiparous cattle, but numerical changes in preformed FA concentrations were very similar between primiparous and multiparous cattle when comparing the 10% OA and 15% OA supplements (1.0% and 1.3% increases, respectively).

There was only a tendency for a linear increase in preformed FA as OA concentration increased but no difference was detected between FAT and CON. Our results in this study closely follow a FA blend (INT) of PA and SA fed in Shepardson (2018). The INT treatment of that trial fed a 45% PA, 49% SA, and 5% OA supplement at 1.95% of DMI. Our 5% OA treatment contained 42% PA and 37% SA, but in both trials, preformed FA concentration was not different from the CON. Shepardson (2018) only found increased preformed FA when feeding a highly enriched SA supplement (93% SA). Therefore, blends of PA and SA may not greatly affect preformed FA concentration in milk fat as supplemental fat would be transferred to milk as both mixed FA and preformed FA.

Mixed FA concentrations followed a different pattern than preformed FA. Overall concentrations of mixed FA were elevated by fat supplementation compared to CON but then linearly decreased as OA concentration increased. These changes were more pronounced in primiparous cows as multiparous cows showed no difference by fat supplementation or by OA concentration. The linear decrease of mixed FA concentration in primiparous cows as OA concentration follows the FA profile of fat supplements as de novo FA concentration did not change across the levels of OA. However, preformed FA increased as mixed FA decreased,

indicating a similar substitution of FA in the milk FA profile to the substitution of FA in the fat supplement, shifting from 16-carbon FA to 18 carbon-FA. In multiparous cows, changes in the FA profile were not the same as the changes in the primiparous cows. De novo FA concentration and mixed FA concentration in milk both numerically decreased as OA increased, suggesting a shift from all FA less than 18-carbon to higher concentrations of FA which are 18-carbons or longer.

Upon further investigation of specific FA, there are interesting trends which help explain the changes in de novo, mixed, and preformed FA concentrations. Generally, multiparous cows had higher levels of *cis*-9 unsaturated FA of all lengths and a numerically higher level of PA and numerically higher levels of C10:0 through C14:0. On the other hand, primiparous cows had higher levels of *trans*-11 C18:1 and LA, which suggests primiparous cows may have a lower capacity for ruminal BH than multiparous cows. The milk FA profile does match some prior research. Shepardson (2018) fed prilled fats reacted with MgO and observed no change in *cis*-9 C18:1 when increasing the OA concentration of fat supplements. The same trial fed prilled fat supplements comprised mostly of free fatty acids that increased in OA concentration by 2.5% but did not observe any increase in *cis*-9 C18:1 concentration in milk fat. Therefore, it is expected that increased dietary *cis*-9 C18:1 from a prilled FA supplement will not affect *cis*-9 C18:1 concentration in milk fat.

As noted previously, the shift of the FA profile leading to an increase in preformed FA is common with fat supplementation. However, differences in concentrations of de novo FA and preformed FA between primiparous and multiparous cows is a new observation. This disparity has not been well characterized in the literature as few studies include primiparous cattle in their analysis and requires further research. However, de Souza and Lock (2018) observed a tendency

for decreased de novo FA and a tendency for higher preformed FA in primiparous cows compared to multiparous in unsupplemented diets.

The decrease in milk protein concentration is not uncommon when adding a fat supplement to a diet for lactating dairy cattle and hypotheses focusing on decreased glucose availability or lower amino acid supply to the mammary gland exist as potential causes for the reduction in milk protein concentration (Rabiee et al., 2012). Western et al. (2020) reported a similar decrease in milk protein concentration when adding fat to a control diet and Rabiee et al. (2012) estimated a decrease in milk protein concentration for both prilled fats and Ca-salts of palm fats. However, it is unknown whether the decrease in milk protein concentration in primiparous cattle observed in this trial is expected. FAT treatments for multiparous cows only had a tendency for a reduction in milk protein concentration, which matches previous research data as not all diets examining fat supplementation in dairy cattle report a decrease in milk protein concentration. de Souza and Lock (2018) saw no treatment or parity effect of PA supplementation on milk protein concentration and reported an increase on milk protein yield. Wu et al. (1993) observed similar responses to those reported here when feeding prilled fats. Similar to Wu et al. (1993), milk protein yield was unaffected by treatment in this study, which does match experimental data in other studies that supplement calcium salts of FA or prilled fat supplements (Rabiee et al., 2012). However, our study utilized magnesium salts of FA, a relatively new reaction for FA supplements, so it is unclear if the production responses should mimic the effects of Ca-salt supplementation. The responses we observed were similar to those in Shepardson (2018) in regard to milk protein concentration and daily milk protein yield.

Table 4-1: Diet summary and the chemical composition for the four experimental diets.

	Diet			
Feed Ingredient. % of DM	CON	5%	10%	15%
Corn Silage[2]	40.9%	40.4%	40.4%	40.4%
Haylage[3]	22.0%	21.4%	21.4%	21.4%
Dry Hay[4]	2.5%	2.5%	2.5%	2.5%
Canola Meal[5]	11.8%	11.7%	11.7%	11.7%
Ground Corn[6]	14.0%	13.8%	13.8%	13.8%
Roasted Soybeans[7]	4.6%	4.5%	4.5%	4.5%
Molasses	2.9%	2.9%	2.9%	2.9%
Vit & Min[8]	1.3%	1.3%	1.3%	1.3%
NPN (Optigen)	0.3%	0.3%	0.3%	0.3%
5% OA Fat Supplement	--	1.2%	--	--
10% OA Fat Supplement	--	--	1.2%	--
15% OA Fat Supplement	--	--	--	1.2%
Nutrient, % of DM				
CP	17.5	17.3	17.3	17.3
NDF	30.9	30.6	30.6	30.6
ADF	21.3	21.0	21.0	21.0
Starch	24.8	24.5	24.5	24.5
Total FA	3.15	4.24	4.24	4.24

[1]Treatments were unsupplemented control (CON) and three fat supplements containing oleic acid at 5% of total FA in the prill (5%), 10% of total FA in the prill (10%), or 15% of the total FA in the prill (15%).
[2]Corn silage contained 9.3% CP, 39.2% NDF, 24.2% ADF, 32.5% starch, and 1.8% total FA on a DM basis.
[3]Haylage contained 20.6% CP, 37.2% NDF, 32.4% ADF, 0.7% starch, and 2.1% total FA.
[4]Dry Hay contained 8.1% CP, 68.1% NDF, 43.9% ADF, 0.8% starch, and 1.5% total FA.
[5]Sample analysis was not fully complete so it was assumed canola meal contained 42% CP, 27.3% NDF, 19.6% ADF, 5.1% starch, and 3.1% total FA.
[6]Sample analysis was not fully complete so it was assumed ground corn contained 8.2% CP, 10.2% NDF, 4.7% ADF, 74.9% starch, and 4.2% total FA.
[7]Sample analysis was not fully complete so it was assumed roasted soybeans contained 40.1% CP, 7.3% NDF, 4.7% ADF, 5.0% starch, and 21.7% total FA.
[8]The vitamin and mineral premix contained (as-fed basis) 37.234% calcium carbonate, 29.0% dried distillers grains, 24.85% salt, 4.15% MgO, 2.4521% phosphorus, 0.5% mineral oil, 0.8% zinc sulfate, 0.3685% Vitamin E (227M U/lb), 0.3672% manganese sulfate, 0.2575% copper sulfate, 0.1575% ferrous sulfate, 0.12671% selenium, 0.03% Vitamin A (650M U/g), 0.0125% Vitamin D3 (500M IU/g), 0.0085% calcium iodate, and 0.005% cobalt carbonate. Composition on a DM basis: 7.5% CP, 7.1% NDF, 3.7% ADF, 15.1% Ca, 0.75% P, 2.48% Mg, 0.28% K,

0.42% S, 9.8% Na, 23 ppm Co, 651 ppm Cu, 796 ppm Fe, 54 ppm I, 1190 ppm Mn, 13 ppm Se, 1721 ppm Zn, 195,290 IU/kg Vit A, 62,500 IU/kg Vit D, and 1,864 IU/kg Vitamin E.

Table 4-2: Fatty acid profile of the experimental fat supplements.

	Oleic Acid	FA Profile (g/100g)		
	Diet	C16:0	C18:0	C18:1c9
Fed[1]	5%	48.2	37.7	4.7
	10%	42.3	39.2	9.7
	15%	41.0	40.4	10.1
Expected[2]	5%	47.3	37.5	4.5
	10%	42.5	38.6	9.4
	15%	37.2	36.4	15.4

[1]Fed = Fatty acid profile of each supplement when analyzed 12 months after the trial started.
[2]Expected = Fatty acid profile of each supplement that was analyzed before the trial started.

Table 4-3: Summary of milk production components in cows fed a control diet or a fat supplement with increasing levels of oleic acid concentration.

	OA Concentration				P-values					
	CON	5%	10%	15%	SE	Trt	Par	TxP	CON vs FAT	Linear	Quad.
DMI											
Overall	29.0	27.2	27.9	27.7	0.9	0.03	<0.01	0.13	<0.01	0.38	0.41
Prim	25.6	25.0	24.4	24.6	1.2				0.16	0.64	0.58
Mult	32.5	29.5	31.4	30.9	1.3				0.01	0.11	0.11
Milk Yield (kg)											
Overall	44.0	42.3	42.0	42.2	1.7	0.22	0.03	0.34	0.04	0.9	0.78
Prim	40.3	38.3	39.6	39.5	2.2				0.32	0.41	0.54
Mult	47.6	46.3	44.3	44.8	2.3				0.06	0.34	0.35
Fat (kg)											
Overall	1.58	1.63	1.60	1.60	0.06	0.84	0.06	0.39	0.48	0.61	0.77
Prim	1.45	1.48	1.53	1.52	0.08				0.27	0.61	0.61
Mult	1.71	1.77	1.70	1.68	0.09				0.94	0.25	0.38
Fat (%)											
Overall	3.71	3.86	3.85	3.89	0.16	0.12	>0.99	0.98	0.02	0.66	0.79
Prim	3.70	3.86	3.85	3.90	0.20				0.05	0.72	0.75
Mult	3.74	3.85	3.86	3.88	0.22				0.17	0.79	0.95
Protein (kg)											
Overall	1.29	1.26	1.24	1.24	0.03	0.37	<0.01	0.36	0.11	0.57	0.76
Prim	1.19	1.15	1.17	1.17	0.04				0.49	0.6	0.59
Mult	1.40	1.38	1.31	1.31	0.04				0.11	0.21	0.36
Protein (%)											
Overall	3.05	3.00	3.97	3.01	0.09	0.02	0.8	0.97	<0.01	0.59	0.09
Prim	3.04	2.97	2.95	3.00	0.12				0.03	0.48	0.18
Mult	3.07	3.03	2.99	3.03	0.13				0.07	0.94	0.29
MUN (mg/dL)											
Overall	13.3	12.9	12.6	13.1	1.2	0.29	0.55	0.56	0.14	0.68	0.23
Prim	13.1	12.4	12.3	13.1	1.3				0.25	0.18	0.36
Mult	13.6	13.5	12.9	13.1	1.3				0.35	0.48	0.42

[1]Treatments were unsupplemented control (CON) and three fat supplements containing oleic acid at 5% of total FA in the prill (5%), 10% of total FA in the prill (10%), or 15% of the total FA in the prill (15%).

[2]Trt - main effect of treatment; Par - main effect of parity; TxP - interaction of treatment and parity; CON vs. FAT - preplanned contrast of CON diet compared to the supplemented diets; Linear - preplanned contrast of the linear effect of increased oleic acid concentration, does not include CON mean; Quad. - preplanned contrast for the quadratic effect of increased oleic acid concentration does not include CON mean.

[3]Prim - mean of primiparous cows, Mult - mean of multiparous cows.

Table 4-4: Relative concentration of milk fatty acids by source in cows fed a control diet or a fat supplement with increasing levels of oleic acid concentration.

		OA Concentration				P-values[2]					
Sum FA by source	CON	5%	10%	15%	SE	Trt	Par	TxP	CON vs FAT	Linear	Quad.
<16C[3]	28.4	26.7	26.2	25.8	0.7	<0.001	0.04	0.83	<0.001	0.13	0.97
Prim	27.7	25.9	25.2	25.3	0.8				<0.01	0.52	0.61
Mult	29.1	27.5	27.2	26.2	0.9				<0.01	0.14	0.67
16C	28.5	30.4	29.8	29.0	0.7	0.01	0.28	0.94	0.01	0.02	0.94
Prim	27.8	30.0	29.2	28.3	0.9				0.04	0.04	0.94
Mult	29.2	30.8	30.4	29.7	1				0.13	0.21	0.84
>16C	38.9	38.9	40.1	41.3	1	0.05	0.02	0.99	0.13	0.02	0.96
Prim	40.6	40.3	41.7	42.7	1.3				0.34	0.07	0.84
Mult	37.2	37.5	38.5	39.8	1.3				0.22	0.1	0.91
Sum of OBCFA[4]											
OBCFA	2.15	0.15	0.209	2.12	0.06	0.74	0.73	0.79	0.62	0.61	0.39
OCFA	1.8	1.74	1.73	1.64	0.09	<0.01	0.47	0.35	<0.01	0.04	0.31
BCFA	1.31	1.32	1.15	1.19	0.05	0.70	0.70	0.39	0.7	0.63	0.31
iso BCFA	0.31	0.3	0.29	0.3	0.01	0.52	0.86	<0.01	0.28	0.9	0.31
ante-iso BCFA	0.81	0.84	0.77	0.78	0.04	0.56	0.31	0.33	0.78	0.26	0.39

[1]Treatments were unsupplemented control (CON) and three fat supplements containing oleic acid at 5% of total FA in the prill (5%), 10% of total FA in the prill (10%), or 15% of the total FA in the prill (15%).
[2]Trt - main effect of treatment; Par - main effect of parity; TxP - interaction of treatment and parity; CON vs. FAT - preplanned contrast of CON diet compared to the supplemented diets; Linear - preplanned contrast of the linear effect of increased oleic acid concentration, does not include CON mean; Quad. - preplanned contrast for the quadratic effect of increased oleic acid concentration, does not include CON mean.
[3]Fatty acids (FA) <16 C originate from de novo synthesis in the mammary gland, 16 carbon FA originate both from de novo synthesis and uptake of preformed FA from plasma, and FA >16 C originate from plasma.
[4]Summation of odd and branch chain fatty acids (OBCFA), odd chain FA (OCFA), branch chain FA (BCFA), iso BCFA, and ante-iso BCFA.
[5]Prim - mean of primiparous cows; Mult - mean of multiparous cows

Supplemental Table 4-1: Milk fatty acid production per day by source in cows fed a control diet or a fat supplement with increasing levels of oleic acid concentration.

			OA Concentration				P-values[2]					
Sum FA by source[3]		CON	5%	10%	15%	SE	Trt	Par	TxP	CON vs FAT	Linear	Quad
<16C		448	437	420	414	21	0.23	0.01	0.54	0.11	0.21	0.75
	Prim	402	384	387	385	27				0.41	0.99	0.92
	Mult	495	490	454	402	28				0.15	0.09	0.59
16C		453	496	479	468	24	0.07	0.07	0.44	0.04	0.09	0.82
	Prim	403	442	448	431	33				0.04	0.63	0.56
	Mult	503	551	511	506	35				0.32	0.06	0.39
>16C		612	628	640	655	23	0.25	0.4	0.58	0.11	0.22	0.94
	Prim	588	602	640	648	30				0.09	0.13	0.57
	Mult	635	654	641	662	32				0.52	0.8	0.54
OBCFA[4]		45	45	43	42	2	0.14	0.02	0.35	0.36	0.03	0.78
	Prim	41	40	40	40	3				0.75	0.74	0.93
	Mult	49	50	46	44	3				0.34	0.01	0.64

[1]Treatments were unsupplemented control (CON) and three fat supplements containing oleic acid at 5% of total FA in the prill (5%), 10% of total FA in the prill (10%), or 15% of the total FA in the prill (15%).
[2]Trt - main effect of treatment; Par - main effect of parity; TxP - interaction of treatment and parity; CON vs. FAT - preplanned contrast of CON diet compared to the supplemented diets; Linear - preplanned contrast of the linear effect of increased oleic acid concentration, does not include CON mean; Quad. - preplanned contrast for the quadratic effect of increased oleic acid concentration, does not include CON mean.
[3]Fatty acids (FA) <16 C originate from de novo synthesis in the mammary gland, 16 carbon FA originate both from de novo synthesis and uptake of preformed FA from plasma, and FA >16 C originate from plasma.
[4]Summation of odd and branch chain fatty acids (OBCFA), odd chain FA (OCFA), branch chain FA (BCFA), iso BCFA, and ante-iso BCFA.
[5]Prim - mean of primiparous cows; Mult - mean of multiparous cow

Supplemental Table 4-2: Milk fatty acid profile for even, straight-chain fatty acids ≤16-carbons in cows fed a control diet or a fat supplement with increasing levels of oleic acid concentration.

		OA Concentration				P-values					
FA, % FA	CON	5%	10%	15%	SE	Trt	Par	TxP	CON vs FAT	Linear	Quad.
C4:0	4.76	4.83	4.94	5.08	0.2	0.17	0.37	0.99	0.13	0.09	0.89
Prim	4.86	4.91	5.04	5.21	0.3				0.23	0.16	0.94
Mult	4.67	4.74	4.83	4.96	0.3				0.33	0.34	0.91
C6:0	2.62	2.50	2.52	2.46	0.08	0.08	0.29	0.83	0.01	0.57	0.47
Prim	2.57	2.43	2.45	2.44	0.1				0.06	0.87	0.85
Mult	2.67	2.57	2.59	2.48	0.1				0.11	0.35	0.42
C8:0	1.45	1.33	1.31	1.27	0.05	<0.001	0.09	0.65	0.0001	0.18	0.72
Prim	1.4	1.27	1.23	1.24	0.06				<0.01	0.66	0.63
Mult	1.51	1.38	1.39	1.29	0.06				<0.01	0.15	0.35
C10:0	3.27	2.87	2.78	2.67	0.1	<0.0001	0.90	0.66	<0.0001	0.05	0.94
Prim	3.11	2.73	2.58	2.59	0.2				0.0001	0.32	0.48
Mult	3.43	3.01	2.98	2.74	0.2				0.0001	0.08	0.45
cis-9 C10:1	0.30	0.28	0.27	0.25	0.02	<0.0001	0.01	0.28	0.0001	<0.001	0.86
Prim	0.27	0.25	0.24	0.23	0.02				0.01	0.14	0.369
Mult	0.33	0.31	0.3	0.26	0.2				<0.01	<0.001	0.54
C12:0	3.65	3.21	3.06	2.95	0.1	<0.0001	0.05	0.66	<0.0001	0.02	0.79
Prim	3.42	3.02	2.82	2.84	0.2				0.0001	0.23	0.42
Mult	3.87	3.41	3.29	3.06	0.2				<0.0001	0.03	0.7
C14:0	11.4	10.7	10.5	10.3	0.3	<0.0001	0.17	0.87	<0.0001	0.07	0.85
Prim	11.3	10.4	10.1	10.1	0.3				0.0001	0.23	0.66
Mult	11.5	11.0	10.8	10.5	0.4				<0.01	0.16	0.87
cis-9 C14:1	0.94	0.95	0.88	0.8	0.05	<0.001	<0.01	0.31	0.02	0.0001	0.87
Prim	0.80	0.81	0.74	0.71	0.07				0.23	0.04	0.66
Mult	1.1	1.1	1.0	0.9	0.07				0.04	<0.001	0.53
C16:0	27.3	29.1	28.6	27.9	0.7	0.02	0.34	0.95	0.01	0.05	0.85
Prim	26.6	28.8	28.1	27.3	0.9				0.04	0.07	0.94
Mult	27.9	29.4	29.1	28.5	1.0				0.11	0.31	0.85
cis-9 C16:1	1.22	1.33	1.23	1.11	0.07	<0.01	0.08	0.89	0.94	<0.001	0.83
Prim	1.12	1.27	1.16	1.05	0.08				0.55	<0.01	0.99
Mult	1.32	1.40	1.30	1.16	0.09				0.65	<0.01	0.77

[1]Treatments were unsupplemented control (CON) and three fat supplements containing oleic acid at 5% of total FA in the prill (5%), 10% of total FA in the prill (10%), or 15% of the total FA in the prill (15%).

[2]Trt - main effect of treatment; Par - main effect of parity; TxP - interaction of treatment and parity; CON vs. FAT - preplanned contrast of CON diet compared to the supplemented diets; Linear - preplanned contrast of the linear effect of increased oleic acid concentration, does not include CON mean; Quad. - preplanned contrast for the quadratic effect of increased oleic acid concentration, does not include CON mean.

[3]Prim - mean of primiparous cows; Mult - mean of multiparous cows

Supplemental Table 4-3: Milk fatty acid profile for all fatty acid ≥18-carbons in length in cows fed a control diet or a fat supplement with increasing levels of oleic acid concentration.

		OA Concentration				P-values					
FA, % FA	CON	5%	10%	15%	SE	Trt	Par	TxP	CON vs FAT	Linear	Quad.
C18:0	10.5	10.3	11.1	12.2	0.4	<0.001	0.32	0.60	0.01	<0.0001	0.57
Prim	10.8	10.5	11.6	12.2	0.5				0.11	<0.001	0.62
Mult	10.2	10.1	10.6	12.1	0.5				0.04	<0.001	0.22
trans-4 C18:1	0.032	0.031	0.032	0.036	0.003	0.27	0.73	0.21	0.77	0.07	0.52
Prim	0.034	0.027	0.03	0.035	0.004				0.27	0.03	0.75
Mult	0.03	0.034	0.033	0.036	0.004				0.16	0.64	0.56
trans-5 C18:1	0.015	0.016	0.014	0.019	0.004	0.24	0.67	0.06	0.54	0.28	0.11
Prim	0.019	0.017	0.011	0.016	0.004				0.21	0.84	0.09
Mult	0.011	0.016	0.017	0.023	0.004				0.05	0.10	0.52
trans-6-8 C18:1	0.34	0.35	0.37	0.37	0.02	0.08	0.93	0.16	0.06	0.13	0.29
Prim	0.36	0.35	0.36	0.37	0.03				0.88	0.28	0.86
Mult	0.32	0.35	0.39	0.37	0.03				<0.01	0.29	0.2
trans-9 C18:1	0.28	0.30	0.31	0.36	0.02	<0.0001	0.34	0.26	<0.0001	<0.0001	<0.01
Prim	0.29	0.31	0.31	0.38	0.02				<0.01	<0.0001	<0.01
Mult	0.26	0.29	0.31	0.35	0.02				<0.0001	<0.01	0.32
trans-10 C18:1	0.47	0.56	0.64	0.62	0.1	0.27	0.5	0.28	0.09	0.43	0.51
Prim	0.50	0.48	0.49	0.52	0.2				0.95	0.75	0.94
Mult	0.47	0.63	0.79	0.73	0.2				0.02	0.44	0.34
trans-11 C18:1	1.00	0.96	1.00	0.95	0.05	0.51	0.03	0.25	0.43	0.91	0.20
Prim	1.15	1.03	1.07	1.11	0.08				0.24	0.34	0.96
Mult	0.88	0.88	0.97	0.79	0.08				0.99	0.30	0.09
trans-12 C18:1	0.53	0.53	0.56	0.54	0.03	0.16	0.65	0.03	0.31	0.37	0.07
Prim	0.56	0.53	0.55	0.57	0.03				0.52	0.10	0.85
Mult	0.51	0.53	0.58	0.52	0.03				0.06	0.72	<0.01
cis-9 C18:1	20.0	20.5	20.7	21.0	0.8	0.56	0.01	0.99	0.22	0.47	0.93
Prim	21	21.5	21.8	21.9	0.9				0.28	0.63	0.89
Mult	19.1	19.4	19.5	20	1.0				0.50	0.59	0.81
cis-11 C18:1	1.18	1.11	1.12	1.08	0.06	<0.01	0.47	0.53	<0.01	0.24	0.36
Prim	1.2	1.15	1.12	1.12	0.07				0.03	0.55	0.65
Mult	1.15	1.08	1.11	1.03	0.07				0.02	0.30	0.10
cis-12 C18:1	0.39	0.34	0.36	0.33	0.03	0.01	0.51	0.36	<0.01	0.43	0.18
Prim	0.4	0.36	0.36	0.36	0.04				0.06	0.99	0.89
Mult	0.38	0.32	0.36	0.29	0.04				0.02	0.28	0.06
cis-9,cis-12 C18:2	2.51	2.37	2.39	2.33	0.09	<0.01	0.14	0.30	<0.01	0.41	0.27
Prim	2.58	2.42	2.47	2.47	0.1				0.01	0.48	0.67
Mult	2.44	2.32	2.32	2.2	0.1				<0.01	0.08	0.26
C18:3 n6	0.13	0.13	0.13	0.15	0.007	<0.01	0.63	0.73	0.09	<0.01	0.31
Prim	0.13	0.13	0.13	0.15	0.009				0.59	0.03	0.25
Mult	0.12	0.13	0.14	0.15	0.009				0.07	0.03	0.75

Supplemental Table 4-3 continued

C20:0		0.020	0.018	0.018	0.016	0.005	0.82	0.28	0.16	0.46	0.58	0.84
	Prim	0.021	0.018	0.011	0.01	0.006				0.11	0.17	0.13
	Mult	0.019	0.018	0.024	0.021	0.006				0.62	0.58	0.36
C18:3 *n*3		0.57	0.55	0.56	0.55	0.04	0.16	0.06	0.27	0.06	>0.99	0.22
	Prim	0.59	0.57	0.58	0.58	0.04				0.52	0.62	0.83
	Mult	0.56	0.52	0.55	0.51	0.04				0.04	0.65	0.06
C20:1		0.051	0.046	0.043	0.049	0.006	0.68	0.60	0.49	0.39	0.72	0.44
	Prim	0.057	0.05	0.039	0.048	0.007				0.16	0.86	0.20
	Mult	0.045	0.042	0.047	0.049	0.008				0.89	0.81	0.90
cis-9,*trans*-11 CLA		0.5	0.5	0.5	0.44	0.03	0.04	0.07	0.19	0.23	0.03	0.14
	Prim	0.58	0.54	0.53	0.51	0.04				0.07	0.45	0.91
	Mult	0.43	0.45	0.47	0.37	0.05				0.97	0.02	0.06
C20:2		0.0052	0.006	0.0068	0.0046	0.005	0.61	0.27	0.40	0.68	0.44	0.31
	Prim	0.0045	0.0074	0.0090	0.0051	0.005				0.18	0.35	0.18
	Mult	0.0059	0.0046	0.0047	0.0041	0.005				0.48	0.85	0.88
C22:0		0.12	0.11	0.12	0.12	0.04	0.64	0.57	0.53	0.57	0.40	0.43
	Prim	0.12	0.12	0.11	0.12	0.04				0.86	0.69	0.65
	Mult	0.12	0.11	0.12	0.11	0.04				0.54	0.44	0.14
C20:3 n6		0.004	0.017	0.016	0.016	0.02	0.94	0.01	0.75	0.56	0.83	0.95
	Prim	0.015	0.023	0.02	0.02	0.02				0.28	0.61	0.76
	Mult	0.013	0.011	0.013	0.012	0.02				0.83	0.85	0.85
C20:4n6		0.15	0.14	0.13	0.13	0.006	<0.01	0.99	0.66	<0.01	0.03	0.87
	Prim	0.14	0.14	0.14	0.13	0.008				0.05	0.19	0.57
	Mult	0.15	0.14	0.13	0.13	0.008				<0.01	0.07	0.76
C20:5n3		0.036	0.032	0.033	0.04	0.004	0.32	0.15	0.58	0.78	0.09	0.45
	Prim	0.031	0.033	0.029	0.037	0.005				0.75	0.61	0.3
	Mult	0.041	0.03	0.037	0.044	0.005				0.5	0.07	0.94

[1]Treatments were unsupplemented control (CON) and three fat supplements containing oleic acid at 5% of total FA in the prill (5%), 10% of total FA in the prill (10%), or 15% of the total FA in the prill (15%).

[2]Trt - main effect of treatment; Par - main effect of parity; TxP - interaction of treatment and parity; CON vs. FAT - preplanned contrast of CON diet compared to the supplemented diets; Linear - preplanned contrast of the linear effect of increased oleic acid concentration, does not include CON mean; Quad. - preplanned contrast for the quadratic effect of increased oleic acid concentration, does not include CON mean.

[3]Prim - mean of primiparous cows; Mult - mean of multiparous cows.

Supplemental Table 4-4: Milk fatty acid profile for odd- and branched-chain fatty acids in cows fed a control diet or a fat supplement with increasing levels of oleic acid concentration.

		OA Concentration				P-values					
FA, % FA	CON	5%	10%	15%	SE	Trt	Par	TxP	CON vs FAT	Linear	Quad.
C11:0	0.06	0.05	0.04	0.03	0.005	<0.001	0.02	0.35	<0.001	<0.01	0.97
Prim	0.05	0.04	0.03	0.03	0.007				<0.01	0.02	0.18
Mult	0.07	0.05	0.05	0.04	0.004				<0.01	0.08	0.23
iso C13:0	0.017	0.013	0.011	0.014	0.003	0.14	0.96	0.25	0.04	0.60	0.39
Prim	0.02	0.014	0.009	0.012	0.004				<0.01	0.72	0.25
Mult	0.015	0.012	0.013	0.016	0.004				0.73	0.30	0.92
C13:0	0.10	0.089	0.088	0.075	0.007	<0.001	0.09	0.53	<0.001	0.01	0.21
Prim	0.094	0.083	0.076	0.07	0.009				0.01	0.06	0.91
Mult	0.11	0.094	0.1	0.08	0.009				<0.01	0.08	0.07
ante-iso C13:0	0.078	0.072	0.65	0.58	0.004	<0.001	<0.01	0.12	<0.001	0.0001	0.95
Prim	0.065	0.06	0.054	0.053	0.005				0.02	0.07	0.56
Mult	0.091	0.083	0.075	0.064	0.006				0.0001	<0.001	0.65
iso C14:0	0.064	0.067	0.073	0.07	0.007	0.27	0.95	0.54	0.14	0.53	0.25
Prim	0.067	0.068	0.072	0.066	0.009				0.75	0.79	0.36
Mult	0.061	0.066	0.074	0.074	0.01				0.08	0.27	0.47
iso C15:0	0.19	0.18	0.18	0.18	0.005	0.02	0.41	0.75	0.01	0.63	0.07
Prim	0.19	0.18	0.18	0.18	0.006				0.06	0.72	0.56
Mult	0.19	0.18	0.17	0.18	0.006				0.10	0.75	0.05
ante-iso C15:0	0.36	0.34	0.34	0.34	0.01	0.07	0.65	0.24	0.01	0.93	0.73
Prim	0.37	0.34	0.34	0.34	0.02				<0.01	0.68	0.64
Mult	0.35	0.34	0.35	0.33	0.02				0.50	0.61	0.37
C15:0	0.97	0.91	0.89	0.81	0.05	<0.001	0.22	0.67	<0.01	<0.01	0.27
Prim	0.93	0.88	0.84	0.8	0.06				0.03	0.07	0.96
Mult	1.01	0.93	0.95	0.83	0.06				0.01	0.06	0.12
iso C16:0	0.19	0.18	0.19	0.21	0.02	0.27	0.21	0.39	0.81	0.06	0.54
Prim	0.21	0.2	0.21	0.2	0.02				0.67	0.87	0.71
Mult	0.17	0.17	0.17	0.22	0.02				0.47	0.02	0.24
iso C17:0	0.038	0.041	0.031	0.033	0.008	0.53	0.56	<0.01	0.64	0.31	0.32
Prim	0.048	0.044	0.024	0.017	0.01				0.03	0.01	0.45
Mult	0.028	0.038	0.037	0.05	0.01				0.14	0.29	0.52
ante-iso C17:0	0.37	0.43	0.36	0.38	0.04	0.56	0.57	0.37	0.60	0.37	0.33
Prim	0.39	0.37	0.37	0.38	0.05				0.74	0.80	0.92
Mult	0.35	0.48	0.36	0.38	0.05				0.31	0.15	0.22
C17:0	0.49	0.51	0.53	0.54	0.02	<0.01	0.32	0.96	<0.001	<0.01	0.78
Prim	0.5	0.52	0.54	0.56	0.03				<0.01	0.06	0.96
Mult	0.48	0.49	0.52	0.54	0.03				<0.01	0.03	0.67
cis-9 C17:1	0.19	0.19	0.18	0.17	0.02	0.63	0.92	0.24	0.29	0.44	0.96
Prim	0.17	0.2	0.18	0.18	0.03				0.48	0.45	0.73
Mult	0.21	0.17	0.18	0.17	0.03				0.04	0.73	0.80

[1]Treatments were unsupplemented control (CON) and three fat supplements containing oleic acid at 5% of total FA in the prill (5%), 10% of total FA in the prill (10%), or 15% of the total FA in the prill (15%).
[2]Trt - main effect of treatment; Par - main effect of parity; TxP - interaction of treatment and parity; CON vs. FAT - preplanned contrast of CON diet compared to the supplemented diets; Linear - preplanned contrast of the linear effect of increased oleic acid concentration, does not include CON mean; Quad. - preplanned contrast for the quadratic effect of increased oleic acid concentration, does not include CON mean.
[3]Prim - mean of primiparous cows; Mult - mean of multiparous cows.

Chapter 5

Integrative discussion

Both experiments indicate cottonseed and Mg-salts of FA can be safely fed to multiparous cows. Induction of BH-induced MFD when feeding whole cottonseed to primiparous cows should be researched further to determine whether this is repeatable or if there were other underlying factors causing this response. Nevertheless, other sources of fat can be safely fed to primiparous cows as indicated by the results of the fat supplementation trial. Milk fat concentration increased in these animals and there was a numerical increase in milk fat yield. For the multiparous cows in both trials, milk protein concentration and milk protein yield were generally unaffected. The more pronounced decrease in milk protein concentration in primiparous cows matches previous research which explored fat supplementation, although no meta-analysis examining milk protein concentration and yield has been conducted in primiparous cattle.

Differences in milk FA by source in primiparous cows is a new observation. The consistent higher concentrations of preformed FA and lower concentrations of de novo FA has not been well characterized as only one previous study was found that reported concentrations of FA by source in both primiparous and multiparous cows and they noticed a tendency for the same results found in both of the current trials (de Souza and Lock, 2018). One possible explanation for the differences could be focused around nutrient utilization for growth in primiparous cattle. Primiparous cattle may partition more carbohydrates and ruminal fermentation products toward growth and limit their availability for de novo FA synthesis while simultaneously allowing greater quantities of preformed FA to be used for milk fat synthesis.

Another difference we noted between different parities of dairy cattle was their average DMI. Primiparous cows did not decrease DMI with increasing levels of fat in the diet whereas multiparous cows' DMI decreased when fed additional dietary fat. This may also be due to partitioning of energy toward growth in cattle, as the primiparous cows can use additional energy from fat for milk fat synthesis and milk yield, and shuttle energy from other sources toward growth or body reserves without compromising milk production or composition.

Although the data in these trials was very interesting, it would have been very interesting to characterize the rumen conditions and duodenal flow of primiparous cows in the cottonseed experiment to determine if it was the additional unsaturated FA from cottonseed that caused induction of MFD or if there were other factors that also increased the risk of the primiparous cattle for MFD.

Finally, there are several questions that remain regarding these experiments. It appears cottonseed should not be fed to primiparous cattle as it can readily cause MFD but primiparous cows may be able to utilize additional energy from dietary fat supplementation as cows in these trials did not increase in milk output but also did not decrease DMI. It seems primiparous cattle need to consume extra energy but are limited on the volume of feed that they can consume and therefore dietary fat supplementation is necessary to maximize growth and production in primiparous cattle. However, balancing rations for primiparous cows should focus on lower concentrations of dietary unsaturated FA than for multiparous cows, and therefore prilled fat supplements are more important in the diets for primiparous cows.

Digestibility of additional fat in these experiments will be calculated but it is expected that increasing cottonseed inclusion up to 10% of DMI will maintain or only slightly decrease fat digestibility in lactating cows, as apparent transfer rates in multiparous cattle did not decrease, milkfat yield remained constant, and dry matter decreased so any decrease in digestibility is not expected to be large. Digestibility of FA is expected to decrease between CON and FAT diets of the OA experiment but increase between OA concentrations in the prills in primiparous cows as milk fat concentration and yield increased with higher levels of OA. Fat digestibility in multiparous cows may decrease as milk fat yield numerically decreased as the milk fat concentration did not increase proportionally to the decrease in milk yield.

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Vita

Reilly Benjamin Pierce was born on August 6, 1995 in Hammond, New York. He was raised on a hobby farm by his parents who also owned a large animal veterinary practice. He grew up raising sheep, chickens, and various other animals depending on the year. After graduating from Heuvelton Central School, Reilly enrolled at Cornell University with a major in Animal Science and a minor in Crop Management where he graduated with a Bachelor of Science in May of 2018. During his academic studies, he accepted farm management internships in Vermont and California, worked as an intern for Phibro Animal Health Corporation in New York, and spent a summer as the assistant herd manager for a large dairy in Northern New York. In August of 2018, Reilly started his graduate work in Dr. Kevin Harvatine's lab at The Pennsylvania State University. Reilly's work focused on feeding trials examining the inclusion of sources of fat into the diets of dairy cattle and their respective digestibilities. Upon completing his degree, Reilly will be joining Poulin Grain Inc. as a Dairy Nutrition Consultant.