HARNESSING THE POTENTIAL OF OLEIC ACID WITHIN FATTY ACID SUPPLEMENTS AND OILSEEDS TO IMPROVE MILK PRODUCTION PERFORMANCES IN HIGH-PRODUCING, MID-LACTATION DAIRY COWS

By

Alycia Marie Bales

A DISSERTATION

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

Animal Science – Doctor of Philosophy

2024

ABSTRACT

The addition of fatty acid (**FA**) supplements and oilseeds are a common practice in the dairy industry to increase yields of fluid milk and milk components. FA supplements contain >85% total FA while oilseeds are closer to 15-20% FA. While both contain differing amounts of individual FA, the level of oleic acid (***cis*-9 C18:1**) within these fat sources are vastly different. Previously, supplementation of *cis*-9 C18:1 to dairy cows has been found to increase nutrient digestibility and body weight reserves. Additionally, varying amounts of *cis*-9 C18:1 can impact production responses differently depending on production level of the cow. In order to explore the impacts of *cis*-9 C18:1, the inclusion of FA supplements and oilseeds containing *cis*-9 C18:1 were evaluated to improve cow performance and efficiency. The first two experiments used FA supplements containing different amounts of *cis*-9 C18:1. First, the effectiveness of a *cis*-9 C18:1-enriched Ca-salt was assessed to increase flow of *cis*-9 C18:1 past the rumen and second, FA supplements were evaluated to test if the FA profile is more important than the form of a supplement. Results showed that a small dose of a *cis*-9 C18:1-enriched Ca-salt can be an effective tool to increase flow of *cis*-9 C18:1 to the small intestine and the FA profile of the supplement is more critical than the form. Next, we determined doses of oilseeds varying in *cis*-9 C18:1 level on cow performance. Whole cottonseed can increase nutrient digestibility and milk production up to 16% dry matter inclusion, whereas a high *cis*-9 C18:1 soybean had continued positive production effects from 0 -24% DM inclusion. Additionally, heat-treatment impacted ground high *cis*-9 C18:1 soybeans, as roasting improved milk production responses compared with raw.

To myself, for beating the imposter syndrome and doing something
I never thought I would accomplish.

ACKNOWLEDGEMENTS

First and foremost, I have immense gratitude for my advisor, Dr. Adam Lock. For several years now, he has encouraged and supported me, which has shaped me into the scientist I have become today. Dr. Lock has never been afraid to give me nudge in the right direction, nor send me a dad joke to boost my mood, and he helped me to build up my self-confidence. Through all of the ups and downs I have experienced during my time in his lab, I always felt supported and that he had a sincere interest in my well-being, happiness, and progress in my degree. I thank him for his willingness to allow me to conduct research that I felt was important to the dairy industry and the many discussions regarding experiments and overall life. I will miss our daily conversations, most of which were telling dad jokes and laughing, and I look forward to being able to work with him in the future as I leave MSU.

I would also like to thank my committee members, Dr. Mike Vandehaar, Dr. Barry Bradford, Dr. Andres Contreras, and Dr. Janice Siegford for their support and suggestions throughout my PhD. I have learned a lot through many lunch discussions, and I am grateful for their time and dedication to my program.

I truly owe one of the biggest thank you to my lab manager, Lynn Worden. I owe much of my success in my program to her, as Lynn has guided me through protocols involving lab analysis and experimental design and set-up. I have once joked that Lynn is our “matriarch” of the lab, and she truly is the glue that holds us all together. Because I have been in the Lock Lab since my senior year of undergrad, I have had the privilege to know Lynn for the past 7 years and I truly consider her one of my friends. Outside of work-related topics, Lynn and I have bonded over books, ice-cream, and so much more. She has been someone I can turn to when I need support, both for work and personal issues, and knowing I will no longer be seeing her on a daily

basis will be saddening. Additionally, Jair Esteban Parales-Girón holds a special place in my heart, as he has had to put up with my shenanigans for over 5 years now, and I am so grateful to have him as a co-worker. Through working at all hours of the day together, to Jair refusing to laugh at my great dad jokes, to sharing coffee and treats, Jair and I have been through a lot, and he has kept me sane over the years. I will miss sitting back-to-back in our corner of 2280, bickering like siblings, and tending to the jungle on our windowsill. Lynn and Jair have been such bright spots in my time during my program and have truly brought such joy to my life. They say that it is always hard to leave a good thing, and I can attest that is absolutely true.

Additionally, there are others in the Lock Lab that deserve my gratitude. Jose Mauricio dos Santos Neto joined the lab at a similar time that I started my Master's degree, and since then we have become travel buddies and someone that I can rely on. Jose has helped with many of my experiments, both on farm and statistically, and I have enjoyed our many movie discussions and debate on whether he is actually the crazy uncle. Also, I would like to thank Alex Benoit for her friendship and love of Taylor Swift and true crime. Although Alex was only in our lab group for 2 years, I had numerous belly laughs and days full of fun with her, and she had an impact on my life and program. If there is one thing that I have accomplished during my program, it was the fact that I was a part of getting Alex addicted to coffee. Thank you to Maggie Miller and the several undergraduate students in the Lock Lab that have helped with my numerous experiments over the years, and of course the many laughs along the way.

Next, the MSU dairy farm staff deserves a large thank you for all of the handwork they have bestowed in caring for the cows that were on my research trials. Their attention to animal care and thoughtfulness to always keep me updated was greatly appreciated. Additionally, I have

enjoyed my time of laughing and joking with many of the staff members, and they definitely made performing experiments more enjoyable.

Last, but certainly not least, I extend the biggest thank you to my family and friends. Without their support, I believe I wouldn't be completing this document. My husband, Scott Bales, has been my biggest supporter, and I appreciate all of his patience, support, and sacrifice to help get me to this point. My dad, brother, grandparents, Jenna Beeker, Jordyn Ruggles, Jakelyn Spencer, and Lauren Kriewall constantly reassured and believed in me, and helped me to persevere when the program felt like too much. Of course, thank you to the rest of my family for cheering me on as well. My family might not have understood my desire to continue on with my education, but they never stopped supporting me.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ABO	Abomasal infusion of *cis*-9 C18:1 treatment
BCS	Body condition score
BH	Biohydrogenation
BUN	Blood urea nitrogen
BW	Body weight
C16:0	Palmitic acid
C18:0	Stearic acid
C18:2	Linoleic acid
C18:3	Linolenic acid
CCK	Cholecystokinin
cis-9 C18:1	Oleic acid
CLA	Conjugated linoleic acid
CP	Crude protein
CON	Control treatment
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
ECM	Energy-corrected milk
ECM/DMI	Feed efficiency
EE	Ether extract
FA	Fatty acids
FABP	Fatty acid binding protein

FAME	Fatty acid methyl ester
FAS	Fatty acid synthase
FAYR-INT	Preformed milk FA yield response to additional 18-carbon intake
FAYR-ABS	Preformed milk FA yield response to absorbed 18-carbon FA
FCM	3.5% Fat-corrected milk
FFA	Free fatty acids
GLC	Gas liquid chromatography
GLUT	Glucose transporter
G3P	Glycerol-3-phosphate
HOSB	High oleic acid soybean
ME	Metabolizable energy
MFD	Milk fat depression
MUFA	Monounsaturated fatty acids
MUN	Milk urea nitrogen
NADPH	Nicotinamide adenine dinucleotide phosphate
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acids
PFAD	Palm FA distillate
PUFA	Polyunsaturated fatty acids
RAW-D	16% DM Raw HOSB treatment
RAW-U	16% DM Raw HOSB + rumen by-pass protein treatment
RDP	Rumen degradable protein
RST	16% DM Roasted HOSB treatment

RUM	Ruminal infusion of *cis*-9 C18:1 treatment
RUP	Rumen undegradable protein
SALT	*cis*-9 C18:1-enriched Ca-salt treatment
SCD	Stearoyl-CoA desaturase
SEM	Standard error of the mean
SD	Standard deviation
SFA	Saturated fatty acids
TAG	Triacylglyceride
TG	Triglycerides
TMR	Total mixed ration
WCS	Whole cottonseed
UFA	Unsaturated fatty acids
45CS	45% C16:0 + 35% *cis*-9 C18:1 Ca-salt treatment
70CS	70% C16:0 + 20% *cis*-9 C18:1 Ca-salt treatment
70FB	70% C16:0 + 20% *cis*-9 C18:1 FA blend treatment

CHAPTER 1: INTRODUCTION

Milk fat and protein are the current major contributors to producer income in the dairy industry, with milk fat being easier to manipulate than milk protein. Thus, nutritional strategies that will increase milk fat and protein, and in turn milk income, are important to investigate. Additionally, the dairy industry continues to improve genetics and average milk production per cow, thus increasing the nutrient requirements for milk production. Meeting these nutrient demands is crucial for optimal milk production and animal health, and feed ingredients enriched in fatty acids (**FA**), such as fat supplements and oilseeds, can increase the energy density of the diet to meet these needs.

Fat supplementation has been studied for many years, and a recent 100-year review highlighted the positive benefits of including fat supplements in lactating dairy cow diets (Palmquist and Jenkins, 2017) and multiple meta-analyses have been conducted to evaluate FA supplementation. Over a decade ago, Rabiee et al. (2012) reported increased milk production that varied amongst different types of supplemental fat and recently, dos Santos Neto et al. (2021a; b) reiterated that not all FA supplements are the same and that FA profile of the supplement alters milk production responses. Our lab has given focus to three specific FA, palmitic acid (**C16:0**), stearic acid (**C18:0**), and oleic acid (***cis-9* C18:1**), as they are the most abundant FA present in commercial fat supplements and milk fat and adipose tissue of dairy cows (Palmquist, 2006). Through our previous research, we have shown beneficial effects of *cis-9* C18:1 supplementation on FA digestibility, regardless of milk production level (Burch et al., 2021; Prom et al., 2021) and increased milk production responses in higher-producing dairy cows (de Souza et al., 2019; Western et al., 2020a). Calcium salts of palm fat are a commercial fat supplement that contains an average of ~35-38% *cis-9* C18:1 (de Souza et al., 2019; dos Santos Neto et al., 2021b) and is

the most common rumen-inert FA supplement used to minimize negative effects of unsaturated FA (**UFA**) on rumen fermentation (Palmquist, 1991). Our research group has recently manipulated the FA profile of supplemental FA by utilizing multiple commercial fat supplements, including calcium salts of palm fat, to create FA blends that allowed for the delivery of specific desired FA profiles (Western et al., 2020b; Burch et al., 2021; Prom and Lock, 2021). Even though differences in cow performance were observed due to the specific FA profiles of the blends (de Souza et al., 2018a, 2019; Bales et al., 2024), these experiments were not designed to test if production responses were due to the type of FA supplement within the FA blend itself. Additionally, to our knowledge, there is limited research on calcium salts of palm fat that contain higher or lower levels of *cis-9* C18:1 than the average commercial supplement. Due to the continuing manufacture of FA supplements, continual research of calcium salts with differing content of *cis-9* C18:1 is crucial to maximize both cow performance and producer income.

Oilseeds are another feed ingredient containing high levels of FA, and similar to FA supplements, oilseeds contain a range of FA profile (Glasser et al., 2008a). Oilseeds are unique as they contain both high amounts of FA and crude protein (**CP**), making them an excellent feedstuff to increase the energy density of the diet. Two common oilseeds that are fed in the Midwest are whole cottonseed and soybeans. Whole cottonseed (**WCS**) and conventional soybeans contain predominantly linoleic acid (**C18:2**) and lower levels of *cis-9* C18:1 (~15% and 25%, repectively; Smith et al., 1981; Weld and Armentano, 2018). A new variety of soybean (**HOSB** [Plenish; Dupont-Pioneer, Johnston, IA]) has been bred to contain 73% *cis-9* C18:1 content (Weld and Armentano, 2018). Inclusion of both WCS and conventional soybeans has been common practice, but research has shown variability in responses to WCS (Arieli, 1998;

Johnson et al., 2002; Rico et al., 2017) and conventional soybeans differing in processing method (Scott et al., 1991; Grummer et al., 1994; Tice et al., 1994), as well as conventional versus HOSB (Weld and Armentano, 2018). Differences in results could be due to production level of the cow, interactions with other dietary nutrients and ingredients, as well as inclusion rate and processing method of the oilseed. Due to the improvements in milk production in the dairy industry today, it is important to evaluate optimal feeding rates of both WCS and HOSB, as well as processing methods of HOSB.

To our knowledge, the research presented in this dissertation is novel and timely. It is critical to understand how *cis-9* C18:1 can increase milk production responses of dairy cattle, specifically in high-producing cows. This will advance our understanding of the utilization and functionality of FA supplements and oilseeds in order to improve dairy cattle nutrition and thus increasing farm profitability. Therefore, the main objective of this dissertation was to examine different levels of *cis-9* C18:1 in both FA supplements and oilseeds and their effects on nutrient digestibility and milk production responses of high-producing, mid-lactation dairy cows.

CHAPTER 2: LITERATURE REVIEW

Importance of Milk Production and Milk Components

The Federal Milk Order Program uses milk fat and protein yields as the major price influencers when they establish milk prices. Thus, an increase in the yield of these milk components will increase gross income for dairy producers. When evaluating the economic value of milk components, St-Pierre (2017) found that a 5% increase in the yields of milk, milk fat, and milk protein increased net farm income by 2%, 13%, and 15% respectively. This emphasizes the importance of increasing milk components, and not necessarily milk yield, to maximize milk profit. Milk fat is the most variable component of milk and can be positively and negatively impacted through nutritional strategies. Therefore, strategies to increase milk fat and protein yield, and as a result increase farm profitability, is a topic that is becoming increasingly examined by researchers.

Fatty Acid Content of Feedstuffs in Dairy Cow Diets

The diet of lactating dairy cattle is relatively low in dietary fatty acids (**FA**), especially high forage-based diets due to forages containing approximately 2-3% total FA as a percent of DM (Drackley, 2004). Additionally, lactating dairy rations predominantly contain unsaturated FA (**UFA**), as grass and legume forages contain high levels of linolenic acid (**C18:3**) while corn silage is rich in linoleic acid (**C18:2**). Grains are also higher in C18:2 and range from 1 to 4% total FA content, although distiller grains can reach 10% (NRC, 2001). Total FA content and FA profile of common feedstuffs typically used in the Midwest are presented in Table 1. Formulation of dairy cow diets can vary in total FA content, depending on the type and inclusion

rate of forages and concentrates. Inclusion of oilseeds and FA supplements will result in rations higher in total FA content and are often used to increase the energy density of the diet.

Fatty Acid Supplements

The most common FA in FA supplements are palmitic (**C16:0**), stearic (**C18:0**), and oleic acid (***cis*-9 C18:1**). Commercial FA supplements are typically either Ca-salts or prills, as dry fats are easier to handle. Ca-salts produced from palm FA are comprised of both saturated FA (**SFA**) and UFA, with a FA profile of ~45% C16:0 and 35% *cis*-9 C18:1 and total FA content of 80 - 85% (dos Santos Neto et al., 2021b). The manufacture of Ca-salts allowed for the utilization of FA supplements with higher levels of UFA in a dry product, and allowed the cow to effectively digest this product and reduce the negative effects of UFA on rumen fermentation (Palmquist and Jenkins, 2017). Prilled fats are comprised of SFA with FA profiles of mainly C16:0 (≥ 80%) or a combination of C16:0 + C18:0 (~38% + ~45%) and are ~95% total FA (dos Santos Neto et al., 2021a). It was previously thought that all FA supplements were similar and used simply as energy sources, but recently we have shown that the profile of FA supplements can impact cow performance (e.g. de Souza et al., 2018a, 2019; Bales et al., 2023a).

Oilseeds

In general, whole oilseeds typically range from 15 to 20% total FA, while some can be as high as 40%, e.g. sunflower seeds (NRC, 2001; Walker, 2006). Oilseeds, such as whole cottonseed (**WCS**) and conventional soybeans, are also high in UFA and predominantly contain C18:2. New varieties of soybeans have recently been developed that are high in *cis*-9 C18:1 (>70%; **HOSB**). These have an inverse FA profile to that of the conventional soybean, containing a higher level of *cis*-9 C18:1 and very little C18:2 (Table 1).

WCS is a by-product of the cotton-gin industry and is a feedstuff generally fed to dairy cattle due to its high level of fat and crude protein (Coppock et al., 1987; Moreira et al., 2004). Additionally, WCS contains digestible fiber that has been proposed to be able to replace forages, if needed (Arieli, 1998). The FA profile of WCS is comprised of ~62% C18:2 and 15% oleic acid (Smith et al., 1981). Within the rumen, the FA in WCS may be released slowly or even leave the rumen still partially enclosed within the seed (Sklan et al., 1992), indicated by increased amount of SFA in digesta entering the small intestine with inclusion of WCS in the diet (Arieli, 1998). This will result in differing FA profile of the digesta leaving the rumen that will be absorbed by the cow for utilization for milk production and other metabolic purposes. Digestibility and milk production responses vary when feeding WCS as some studies have reported increases in milk fat yield while others have reported negative effects on milk production and milk protein content, although these responses could happen simultaneously (Arieli, 1998). A recent meta-regression found that for every 1% increases of WCS, milk fat production increased whereas nutrient digestibility decreased (dos Santos Neto et al., 2022).

Whole soybeans are mostly produced for oil and animal feed, where they can be fed as the whole seed or processed into pellets and meals, generally with the oil removed for cooking oil for human consumption. Soybeans can be heat-treated to increase rumen undegradable protein content (Grummer et al., 1994) and to denature a trypsin inhibitor that could otherwise reduce protein digestibility in the small intestine (NASEM, 2021). Additionally, it is common to crack, roll, and grind soybeans to decrease particle size. Conventional soybeans contain ~47 – 54% C18:2 and ~22 – 26% *cis*-9 C18:1 (Glasser et al., 2008a; Weld and Armentano, 2018) and are included in lactating dairy cow rations at low feeding rates due to the higher content of C18:2, a FA known to increase the risk of milk fat depression (He et al., 2012; Dorea and

Armentano, 2017). Both nutrient digestibility and milk production responses are likely impacted by variety of soybean and processing methods and needs further investigation. Increasing nutrient digestibility will increase availability of nutrients for use by the mammary gland and other tissues.

Rumen Metabolism of Dietary Fats

Although ruminant diets are high in UFA, they are toxic to rumen bacteria and undergo rumen biohydrogenation (**BH**), a process that ultimately results in mostly SFA reaching the small intestine (Harfoot and Hazlewood, 1997). Due to this extensive metabolism on dietary lipids, there is major impact on the profile of FA available for absorption and utilization and is a reason why ruminant meat and milk contain a much higher amount of SFA than UFA (Palmquist et al., 2005).

Lipolysis and BH are the two main processes that contribute to the metabolism of FA in the rumen (Figure 1; Buccioni et al., 2012), with lipolysis being the first step. Lipolysis is the process of microbial lipases hydrolyzing the ester linkages in lipids in order to release FA from their glycerol backbone, thus exposing the UFA to ruminal microbes (Jenkins et al., 2008). This is a critical step, especially with esters, salts, and other modifications of UFA, as a free carboxyl group is a requirement for BH to occur (Harfoot and Hazlewood, 1997). The process of BH results in the reduction of the double bonds on the carbon chain of an UFA to produce a SFA (Buccioni et al., 2012), with the main end product being C18:0 (Harfoot and Hazlewood, 1997). Since UFA are more toxic to rumen bacteria than SFA, BH is a detoxification mechanism against UFA that would otherwise negatively impact bacterial growth (Maia et al., 2010). This toxicity could be due to the double bonds in the structure of the UFA and the level of toxicity differs

depending on the individual FA and bacterial species (Maia et al., 2010). Two groups of bacteria are responsible for BH of UFA to SFA, with Group A bacteria converting UFA to *trans* C18:1 isomers and Group B bacteria completing the process of *trans*- and *cis*-monounsaturated FA to C18:0. Group B are the only species known to BH *cis*-9 C18:1 to C18:0 (Harfoot and Hazlewood, 1997; Palmquist et al., 2005). Both groups must be present to fully complete the process of BH to C18:0 (Figure 2). Rates of BH differ amongst UFA, with ruminal loss for *cis*-9 C18:1, C18:2, and C18:3 ranging between 30-75%, 70-90%, and 85-100%, respectively (Jenkins and Bridges, 2007; Jenkins et al., 2008). Apparent ruminal loss of *cis*-9 C18:1 has previously been estimated to be 62% for protected fat supplements and 30% for oilseeds (Jenkins and Bridges, 2007). Due to continual passage of digesta leaving the rumen, some UFA and BH intermediates escape the rumen and are absorbed in the small intestine.

There are numerous factors that can affect the extent of BH, such as increased rumen concentration of UFA and decreased rumen pH (Jenkins and Harvatine, 2014). Rumen bacteria are sensitive and can be altered by changes in pH, thus a low ruminal pH can result in differences in growth of microbial populations, thus producing altered BH pathway intermediates, such as converting *cis*-9 C18:1 to *trans*-10 C18:1 instead of *trans*-11 C18:1 (Jenkins et al., 2008). Alterations in BH pathways can produce other intermediates that are potent inhibitors of milk fat synthesis, such as *trans*-10, *cis*-12 CLA (Figure 3; Bauman et al., 2011).

Effects of Dietary Fatty Acids on Rumen Fermentation

Rumen protozoa, as well as cellulolytic bacteria, are highly sensitive to FA (Hino and Nagatake, 1993) and FA supplementation can lead to a shift in the rumen microbial population (Lourenço et al., 2010). Since UFA are toxic to rumen bacteria, they can inhibit rumen fermentation (Jenkins, 1993). Rumen fermentation processes, such as digestibility and microbial

cell synthesis and sites of digestion, are known to be affected by the amount of FA entering the rumen (Jenkins and Palmquist, 1984; Boggs et al., 1987). Jenkins (1993) proposed a theory that FA potentially coat microorganisms with a hydrophobic film that disrupts the adherence of bacteria to cellulose fibers, thus decreasing cellulose digestion (Jenkins, 1993). A free carboxyl group was suggested as being required to disrupt rumen fermentation and supplying the FA as Ca-salts has been found to reduce this inhibitory effect (Jenkins and Palmquist, 1994).

Effects of Dietary Fatty Acids on Nutrient Digestibility

A common misconception is that dietary FA consistently decrease fiber digestion, as several studies in the 1950s using vegetable oils reported negative effects on cellulose digestibility (Palmquist and Jenkins, 2017). In more recent years, research has shown that fiber digestibility can be influenced depending on the FA profile and the form of the FA entering the rumen. Although vegetable oils were found to decrease neutral detergent fiber (**NDF**) digestibility (Weld and Armentano, 2017), C18:0 supplementation had no effect on NDF digestibility (Piantoni et al., 2015; Boerman et al., 2017) while C16:0 has been observed to consistently increase NDF digestibility (Piantoni et al., 2013; de Souza et al., 2018a). In a recent meta-analysis, dos Santos Neto et al. (2021a) reported that a C16:0-enriched prill increased NDF digestibility compared with a combination of C16:0 + C18:0 and diets with no FA supplementation. In an in vitro study using pure FA products, Sears et al. (2023) observed that compared to a control treatment, C16:0 increased NDF digestion while C18:0 had no effect and *cis*-9 C18:1 decreased NDF digestion. The decrease in NDF digestibility when pure *cis*-9 C18:1 was included in the diet is most likely due UFA impacting fibrolytic bacteria, as there was no protection due to the FA being a free FA. Inclusion of *cis*-9 C18:1 within a FA supplement has shown positive impacts, as combining commercial FA products to create blends of C16:0 + *cis*-9

C18:1 increased NDF digestibility compared with a blend of C16:0 + C18:0 (de Souza et al., 2018). But multiple studies with differing ratios of C16:0 + *cis*-9 C18:1 have observed varying effects on fiber digestibility (de Souza et al., 2019; Western et al., 2020b). Furthermore, inclusion of oilseeds has also been observed to have variable effects on fiber digestion. In one study evaluating cottonseed and soybean products, both cottonseed and soybean oil decreased total tract digestion compared with whole seeds (Mohamed et al., 1988). There have been many studies evaluating inclusion of WCS to lactating diets, all with different feeding rates, and have found either no effect (Smith et al., 1981) or a decrease in NDF digestibility (Coppock et al., 1985; Rico et al., 2017; de Souza et al., 2018a). Additionally, in a meta-regression, for every 1% increase of WCS in diet DM, digestibility of DM and NDF decreased (dos Santos et al., 2022) Conventional soybeans, regardless of processing method, increased NDF digestion compared with a control treatment (Tice et al., 1993). Chapters 3, 4, and 5 examine the impacts of FA profile and the inclusion of FA supplements and WCS on nutrient digestion.

Differences in effects on nutrient digestion across experiments utilizing FA supplements and oilseeds may also be partially attributed to changes in dry matter intake (**DMI**) as increased DMI can decrease NDF digestion (de Souza et al., 2018b). Although, decreases in DMI due to gut peptides associated with satiety can slow rumen retention time and increase NDF digestion (Choi and Palmquist, 1996; Piantoni et al., 2013). A hypothesis suggested by dos Santos Neto et al. (2021a) proposed that dietary C16:0 could spare ATP when it was incorporated into rumen bacterial membranes (Hackmann and Firkins, 2015), possibly sparing energy that favors bacteria growth by incorporation of C16:0 into the cell membranes and positively affecting fibrolytic bacteria (Hauser et al., 1979; Mackie et al., 1991). This was tested in vitro and C16:0 was observed to alter the bacterial community by enhancing bacteria groups responsible for fiber

digestion (Sears et al., 2023). The differences observed in NDF digestibility for FA supplements and oilseeds could be attributed to DMI, interactions with other dietary nutrients and specific effects of individual FA on bacteria growth. Chapters 3 – 5 will further investigate the effects of FA supplements and WCS on nutrient digestibility.

Digestibility and Absorption of Dietary FA

Lipids that leave the rumen are predominantly free FA (85 to 90%) and phospholipids (10 to 15%). As mentioned previously, the major FA leaving the rumen is C18:0 due to BH of UFA. Due to the acidic conditions (pH ~ 2.0) in the abomasum, FA will be adsorbed to the surface of feed particles that pass through (Noble, 1978; Drackley, 2004). Upon entry to the duodenum, secretions of bile and pancreatic juice are added to the digesta and are essential for FA digestion and absorption (Scarlet and Drackley, 2013; Figure 4). Bile supplies bile salts and lecithin while pancreatic secretions provide enzymes to convert lecithin to lysolecithin, thus allowing for bile salts and lysolecithin to dissociate FA from feed particles to enable micelle formation (Lock et al., 2005). In ruminants, lysolecithin acts as an amphiphile, containing both hydrophobic and hydrophilic molecules that help aid formation of micelles for FA absorption. Lysolecithin is the most effective amphiphile at increasing the micellar solubility of C18:0 (Freeman, 1969) and is a requirement for FA absorption to occur (Moore and Christie, l984). Micelles consist of water-insoluble lipids surrounded by bile salts and phospholipids that transport the lipids across the unstirred water layer of intestinal epithelial cells of the jejunum, where the FA and lysolecithin are absorbed (Lock et al., 2006). After absorption, FA are re-esterified into triglycerides in the endoplasmic reticulum of the enterocyte and then combined into lipoprotein particles, such as chylomicrons or VLDL (Drackley, 2004; Cifarelli and Abumrad, 2018).

Effects of Dietary Fat on FA Digestibility

Rumen outflow of total FA is similar to dietary intake of total FA (Lock et al., 2006), although the profile of the digesta leaving the rumen is vastly different due to BH. Additionally, apparent digestibility of FA can decline as the supply of FA increases (Palmquist, 1991). In a meta-analysis evaluating intestinal digestibility, Boerman et al. (2015) concluded that total FA digestibility was negatively affected by the flow of total FA in the duodenum. This decrease in digestibility could be attributed to limitations in secretion and activity of bile salts and pancreatic lipases that can affect absorption at elevated FA intakes (Bauchart, 1993). Additionally, Boerman et al. (2015) reported that increased flow of C18:0 not only negatively affected C18:0 digestibility, it also negatively impacted the digestion of other FA. These results indicate that profile of the FA reaching the duodenum is an important factor affecting FA digestibility.

In two recent meta-analyses, FA profile of supplemental fats impacted FA digestion, as a Ca-salt (C16:0+ *cis*-9 C18:1) increased FA digestion compared with a control (dos Santos Neto et al., 2021b), and a highly enriched C16:0 prill increased FA digestibility compared with a prill containing a combination of C16:0 + C18:0 (dos Santos Neto et al., 2021a). Ca-salts of FA were found to be more digestible than an animal-vegetable FA blend, most likely due to the *cis*-9 C18:1 in the Ca-salt (Wu et al., 1991). Supplementation of C16:0 and C18:0 have both been observed to decrease FA digestibility as the amount of FA reaching the duodenum increased, but this decrease was more pronounced for C18:0 (Boerman et al., 2017; Rico et al., 2017).

Whole oilseeds were found to be less digestible compared with Ca-salts and vegetable oils (Boerman et al., 2015) while a review by Coppock et al. (1987) reported that increasing the amount of WCS increased digestibility of ether extract. It is important to note that ether extract also contains nonnutritive waxes and pigments that are extracted (Sukhija and Palmquist, 1988)

and thus cannot give an accurate estimation of FA digestibility. WCS was found to either increase or decrease total FA digestion compared with other dietary feed ingredients (Rico et al., 2017; de Souza et al., 2018a), and addition of conventional soybeans (raw, roasted, and roasted and ground) decreased FA digestibility compared with a control treatment (Tice et al., 1993). These observed differences with oilseeds could be attributed to feeding rate and FA intake, fermentation of the diet, and BH rates. Since WCS and conventional soybeans contain high levels of C18:2, and BH converts C18:2 to C18:0, FA absorption could have been decreased due to increased flow of C18:0 leaving the rumen. There is currently no digestibility data for HOSB, but it could be expected to increase FA digestion with the higher levels of *cis*-9 C18:1 it contains, as discussed in the following section.

The impact that FA supplements and oilseeds have on FA digestion is important to consider, as increasing FA digestibility increases the amount of FA available for absorption and utilization. Chapters 3 – 5 will continue to evaluate the impacts of dietary FA on FA digestibility.

Effects of Oleic Acid on FA Digestibility

Oleic acid has improved FA digestibility, compared with other FA, in studies with FA supplements differing in *cis*-9 C18:1 content (de Souza et al., 2018a; Burch et al., 2021; Prom and Lock, 2021) and when infusing *cis*-9 C18:1 directly into the abomasum (Prom et al., 2021, 2022; Figure 5). Thus, the FA profile of the digesta reaching the small intestine impacts FA digestibility. Boerman et al. (2015) reported that UFA had a higher digestibility compared with SFA, with values for individual FA being 76.5, 73.7, 80.8, 79.9, and 78.8% for C16:0, C18:0, *cis*-9 C18:1, C18:2, and C18:3, respectively. The effect of *cis*-9 C18:1 on FA digestibility is likely due to the amphiphilic properties of *cis*-9 C18:1, thus improving micelle formation and function and increasing solubility of other FA (Freeman, 1969). Improvements in micelle

formation can lead to increases in the amount of FA absorbed, thus more FA can be available for utilization for milk production and other metabolisms.

Increasing the amount of *cis*-9 C18:1 flowing to the small intestine should improve micelle formation and thus FA digestion and absorption. It is crucial to continue to find ways to provide more *cis*-9 C18:1 to the small intestine, but with limited BH of *cis*-9 C18:1 to C18:0, to maximize digestion that will support and increase milk production. Chapter 3 will investigate the effects of a high *cis*-9 C18:1 Ca-salt on FA digestibility, Chapter 4 will evaluate FA supplements that differ in form and FA profile and their effects on FA digestion, and Chapter 5 will examine the effects of increasing WCS, thus increasing *cis*-9 C18:1 and overall UFA content and the impacts on overall FA digestibility.

Milk Fat Synthesis in the Mammary Gland

Milk fat is a major energy and nutrient expense to the cow (Emery, 1973) and is also the most variable component of milk, both in concentration and composition. Diet composition and stage of lactation are a few factors that influence milk fat (Palmquist, 2006). Lipid globules are emulsified in the aqueous phase of milk, with bovine milk fat concentration ranging from 3 to 5% and mostly comprised of triglycerides (**TAG**; 98%), along with phospholipids, diglycerides, and cholesterol (Jensen, 2002). Bovine milk fat is complex, with more than 400 different FA that differ in chain length, structure, and configuration of double bonds (Jensen, 2002). The three stages of milk fat synthesis are: 1) synthesis of short-chain FA and absorption of long-chain FA, 2) TAG synthesis, and 3) formation of the fat globule for secretion into milk (Shorten et al., 2004). Sources of milk FA can be categorized into two groups: de novo and preformed FA. Short-chain FA are derived from de novo synthesis and the uptake of long-chain FA from

circulation are preformed (Palmquist, 2006). Additionally, there are mixed source FA (C16:0 and *cis*-9 C16:1) that are derived from both de novo synthesis in the mammary gland and long-chain FA extraction from plasma.

De Novo FA Synthesis

In ruminants, de novo synthesis accounts for approximately half of the FA in milk (Bauman and Griinari, 2003). A carbon source (acetate and beta-hydroxybutyrate) and reducing equivalents in the form of NADPH (glucose and acetate) are requirements for ruminant milk FA synthesis (Palmquist, 2006). Acetate is produced during rumen fermentation and is the main carbon source for FA synthesis (Moore and Christie, 1979). For initiation of de novo FA synthesis, approximately 50% of the initial 4-carbon skeleton comes from condensation of 2-carbon units from acetate and the other 50% comes from beta-hydroxybutyrate that is metabolized from butyrate (Moore and Christie, 1979; Palmquist, 2006). Acetate and beta-hydroxybutyrate account for all carbons in C4:0 to C12:0 milk FA, 75% of C14:0, and 50% of C16:0 (Smith et al., 1974). The reductive steps of fatty acid synthase (**FAS**) require NADPH, which come from glucose or acetate, although the demand for these reducing equivalents can be decreased when cows are fed high-fat diets that reduce the synthesis of de novo FA (Palmquist, 2006), which can spare glucose for lactose synthesis (Cant et al., 1993). The chain length of de novo FA is determined by a series of decarboxylative condensation reactions that add 2-carbon units in a continuous cycle until the FA reaches C12:0 to C16:0 (Smith et al., 2003).

Acetate is activated to acetyl-CoA and converted to malonyl-CoA by acetyl-CoA carboxylase in an irreversible reaction (Bauman and Davis, 1974; Bauman and Griinari, 2003), making this the rate-limiting step for de novo FA synthesis. Malonyl-CoA enters the FAS complex to provide the 2-carbon units needed for chain elongation until terminated by a

thioesterase enzyme for a specific chain length (Smith et al., 2003). Both acetyl and malonyl substrates are loaded by the same acyl transferase in a random process (Palmquist, 2006) and are exchanged rapidly until a combination occurs that permits chain initiation or a continuation of elongation (Smith et al., 2003). This acyl transferase performs thioesterase activity and terminates FA synthesis to produce short- and medium-chain FA in ruminants (Yao et al., 2022) and intermediates released by the acyl transferase result in short-chain FA incorporated into milk fat (Palmquist, 2006). Under optimal concentrations of acetyl-CoA, malonyl-CoA, and NADPH in the bovine mammary gland, C16:0 is the main FA produced by FAS, suggesting that C16:0 production or supply is important for milk fat synthesis (Kinsella et al., 1975). Additionally, butyryl-CoA can be synthesized from acetyl-CoA in a reversal of beta-oxidation in both the liver and mammary gland of rabbits, rats, and cows, and was observed to be a more efficient "primer" for milk FA synthesis than acetyl-CoA, but only in ruminants (Lin and Kumar, 1972; Knudsen and Grunnet, 1980). The synthetic pathway responsible for the reversal of beta-oxidation, and thus utilization of butyryl-CoA, is independent of malonyl-CoA and therefore is not subject to regulation by acetyl-CoA carboxylase (Palmquist, 2006). This pathway explains the utilization of beta-hydroxybutyrate as the methyl terminal C_4 moiety of ~50-60% of de novo FA (Palmquist et al., 1969; Smith et al., 1974) and small quantities of C6:0, C8:0, and C10:0 (Palmquist, 2006).

Uptake of Preformed FA

Long-chain FA, >16-carbons in length, that are used for preformed milk FA synthesis mainly come from absorption of dietary FA, with these FA mostly being saturated 18-carbon FA. The primary sources of these FA is from TAG-rich lipoproteins from blood and accounts for greater than 95% of the 18-carbon and long-chain FA in milk fat (Palmquist, 2006). Therefore, the mammary gland is supplied with long-chain FA that are used exclusively for fat synthesis. A

lipoprotein lipase hydrolyzes the TAG to release FA for absorption and utilization by the mammary gland. Non-esterified FA (**NEFA**), derived from the mobilization of body reserves and lipolysis, usually account for only a small fraction of the FA in milk fat (Bauman and Griinari, 2003), except when cows are experiencing negative energy balance, the contribution from NEFA is higher due to greater mobilization of body reserves (Palmquist, 2006). Although transport of FA into the mammary gland is not well understood, research suggests FA translocator CD36 and FA binding proteins, as these proteins have a higher affinity for long-chain FA, mainly UFA (Moore and Christie, 1979; Barber et al., 1997). CD36 is a crucial protein involved in transport of long-chain FA (Spitsberg et al., 1995) and FA binding proteins are involved in the uptake and metabolism of long-chain FA (Liang et al., 2014). As stated earlier, 16-carbon FA come from blood lipids and de novo synthesis. C16:0 can be synthesized de novo in large quantities when dietary fat intake is low, but as C16:0 intake and uptake from circulation increases, the amount of C16:0 synthesized from de novo synthesis will decrease (Palmquist, 2006).

Triglyceride Synthesis

Both de novo and preformed FA are utilized for TAG synthesis in the mammary gland. The primary path for this synthesis is through the *sn*-glycerol-3 phosphate pathway and incorporated on the glycerol-3 phosphate backbone (Dils, 1983). Glycerol-3 phosphate (**G3P**) is required for esterification of FA, generated through glycolysis or phosphorylation of free glycerol by glycerol kinase, and acylation of G3P is the first step in TAG synthesis (Palmquist, 2006). G3P acyl transferase adds the first fatty-acyl CoA to the *sn*-1 position of the G3P, then acyl glycerol phosphate acyl transferase adds the second fatty-acyl CoA to the *sn*-2 position and ends with diglycerol acyl transferase adding the final fatty-acyl CoA to the *sn*-3 position to form the TAG (Palmquist, 2006). Placement of individual FA to the 3 positions of the glycerol

backbone are not random (Jensen, 2002) as short-chain FA are esterified at *sn*-3, medium-chain FA at *sn*-2, and long-chain FA at *sn*-1 (Jensen, 2002; Lindmark Månsson, 2008; Table 2). Even though long-chain FA are predominantly at *sn*-1, C16:0, C18:0, and *cis*-9 C18:1 can be found at other positions. C16:0 can be found equally at *sn*-1 and 2-positions, while C18:0 is mostly found at the *sn*-1 position but can be acylated at *sn*-3, and *cis*-9 C18:1 is located at *sn*-1 and 3-positions (Jenson, 2002).

In a recent meta-analysis, Glasser et al. (2008) discussed the interdependence between short-/medium-chain FA and long-chain FA and postulated that milk fat synthesis is dependent on the simultaneous supply of these FA for milk fat synthesis. Diglycerol acyl transferase (**DGAT**) esterifies both short-chain and long-chain FA at the sn-3 position and is up-regulated with increasing amounts of FA (Palmquist, 2006). As a result, increases in the supply of exogenous long-chain FA can reduce de novo synthesis due to the competition for DGAT between short- and long-chain FA (Palmquist, 2006). The location and control of FA placement within the TAG manages milk fat fluidity and allows for the secretion of TAG droplets to be incorporated into milk, as well as being fluid at body temperature (Dils, 1986; Jensen, 2002). There are mechanisms in place that help control the melting point of milk fat, including increasing unsaturated FA via desaturation of SFA and synthesizing a larger supply of short-chain FA at the sn-3 position (Dils, 1986).

Effects of Dietary FA on Milk FA Sources

It is important to understand the influence that dietary FA have on milk FA sources and thus, overall milk fat yield. In several studies, milk FA sources have been impacted by increased inclusion of dietary FA to dairy cow diets. Glasser et al. (2008b) described differences in yields

between FA sources as a substitution effect, as the decrease in de novo FA are compensated for by an increase in preformed FA when supplementing FA in the diet.

Dietary C16:0 is a predictor for 16-carbon milk FA (mixed) while dietary 18-carbon FA are predictors of preformed milk FA (Dorea and Armentano, 2017). As stated earlier, fat supplements range in FA profile and in two meta-analyses it was found that FA profile of the supplement impacted milk FA sources (dos Santos Neto et al., 2021a; b). Compared to diets without FA supplementation, Ca-salts (C16:0 + *cis*-9 C18:1) were observed to decrease yields of de novo milk FA but increased mixed and preformed milk FA (dos Santos Neto et al., 2021b). Additionally, compared to no FA supplementation, saturated FA prills also negatively impacted de novo milk FA yield regardless of FA profile, but a C16:0-enriched prill increased 16-carbon FA yield while supplementation of C16:0 + C18:0 prill increased preformed milk FA yield (dos Santos Neto et al., 2021a). Increasing the amount of *cis*-9 C18:1 in FA blends interacted with production level of mid-lactation cows, as high-producing dairy cows increased preformed milk FA yield (de Souza et al., 2019; Burch et al., 2021) while lower-producing cows decreased both de novo and mixed FA yields (de Souza et al., 2019; Western et al., 2020b). Increasing C16:0 supplementation, at the expense of C18:0, in FA blends did not impact yields of de novo milk FA, increased 16-carbon milk FA, and decreased preformed milk FA (Burch et al., 2021). This would be expected, as increasing C16:0 supplementation would supply more exogenous 16-carbon FA and less 18-carbon FA. Overall, increased supplementation of *cis*-9 C18:1, compared with C16:0 and C18:0, decreased de novo milk FA yield but positively influence preformed FA yield (de Souza et al., 2018a; Burch et al., 2021; Prom and Lock, 2021). Shifts in milk FA sources are likely a result of changes in FA supply to the mammary gland and highlights the

importance of maintaining milk fat fluidity, indicative of efficient utilization of supplied versus synthesized FA.

Dietary oilseeds can also effect milk FA sources, as observed in a meta-analysis by Glasser et al. (2008a) where supplementation of different oilseeds increased preformed milk FA. Addition of oilseeds containing high levels of C18:2 can negatively impact milk fat due to the potential of altered BH of C18:2 resulting in *trans*-10, *cis*-12 C18:2 that will depress FA synthesis and lower transfer of long-chain FA into the mammary gland (Palmquist et al., 2005; He et al., 2012; Dorea and Armentano, 2017). In studies evaluating increasing dietary FA content of basal diets by utilizing WCS, both de novo and mixed FA yields were not impacted but preformed FA yield was increased with inclusion of WCS (Rico et al., 2017; de Souza et al., 2018a; Bales et al., 2023). When evaluating soybean variety (conventional versus HOSB), Weld and Armentano (2018) reported that overall soybean inclusion, regardless of particle size, decreased yields of de novo and mixed milk FA but increased preformed FA. Additionally, HOSB increased incorporation of *cis*-9 C18:1 into milk fat compared with conventional soybeans (Weld and Armentano, 2018).

In general, increasing dietary FA content with the addition of FA supplements and oilseeds will alter milk FA sources, with FA profile potentially having a greater influence on milk FA sources. This could be due to nutrient utilization in the mammary gland, potential influence of other dietary ingredients, and rumen BH of UFA. Chapter 4 will examine the impact of the form and FA profile of fat supplements on milk FA sources, and Chapters 5 – 7 evaluate how different oilseeds alter milk FA.

Effects of Oleic Acid

The mammary gland utilizes UFA to help maintain milk fat fluidity, where the UFA are derived directly from circulation or from the desaturation of SFA. The stearoyl-CoA desaturase (**SCD**) enzyme adds a *cis*-double bond between the 9th and 10th carbon, which converts SFA to UFA (Palmquist, 2006). The main substrates for SCD are C14:0, C16:0, and C18:0 being converted to *cis*-9 C14:1, *cis*-9 C16:1, and *cis*-9 C18:1, respectively. Increased C18:0 content of milk fat has been observed due to BH of *cis*-9 C18:1, thus not all the *cis*-9 C18:1 present in milk fat is due to dietary supplementation, as ~50% would be due to desaturation via SCD (Palmquist, 2006). Analysis of milk FA of cows receiving supplemental *cis*-9 C18:1 has shown increases in the level of *cis*-9 C18:1 in milk fat, most likely due to a greater absorption and incorporation of dietary *cis*-9 C18:1 into milk fat (de Souza et al., 2019; Burch et al., 2021; Prom and Lock, 2021). Additionally, *cis*-9 C18:1 can affect esterification on the triglyceride due to having high affinities for all *sn*-positions of the glycerol backbone, thus creating competition with short- and medium-chain FA for the *sn*-3 and *sn*-2 and positions, respectively (Jensen et al., 1991). This is highlighted in a meta-analysis by Dorea and Armentano (2017), as they found a negative relationship between dietary *cis*-9 C18:1 intake and de novo milk FA yield.

When evaluating bovine mammary epithelial cells, *cis*-9 C18:1 has been found to play key roles in mechanisms and proteins involved with milk fat synthesis. Liang et al. (2014) proposed that fatty acid binding protein (**FABP**) 3 has an important role in the signaling pathway of milk fat synthesis, and expression of FABP3 was enhanced with addition of *cis*-9 C18:1 in vitro. Additionally, they observed that *cis*-9 C18:1 increased acetyl-CoA carboxylase and fatty acid synthase mRNA expression, two key enzymes involved in de novo FA synthesis. This could partially explain why C4:0 has been found in greater quantities when *cis*-9 C18:1 is

supplemented to dairy cows (de Souza et al., 2019; Burch et al., 2021; Prom and Lock, 2021), further aiding fluidity of milk fat. In another study, Cohen et al. (2015) observed culture mediums of bovine mammary epithelial cells were affected by FA treatments, with *cis*-9 C18:1 increasing both mitochondrial quantity and TAG secretion. Mitochondrial physiology is vital to support the mammary gland, as the mitochondria fuels production precursors, such as amino acids and FA for milk biosynthesis (Favorit et al., 2021), and has a key role in mammary lipogenesis (Cohen et al., 2015).

Extent of BH is an important consideration when supplementing fat sources higher in *cis*-9 C18:1, as *trans*-10 C18:1 can be produced from *cis*-9 C18:1 under unfavorable rumen conditions (Jenkins et al., 2008), but is unlikely to produce *trans*-10-, *cis*-12 CLA (Mosley et al., 2002; Dewanckele et al., 2020). When normal BH pathways are overwhelmed or during a shift in bacteria profile (Weimer et al., 2010), C18:2 can produce *trans*-10-, *cis*-12 CLA, a bioactive FA that has negative effects on milk fat, which can be further biohydrogenated to produce *trans*-10 C18:1, a FA that is less potent than *trans*-10-, *cis*-12 CLA (Lock et al., 2007). Kadegowda et al. (2009) observed *trans*-10 C18:1 to decrease expression of fatty acid synthetase and SCD, both of which are crucial for de novo FA synthesis and desaturation of *cis*-9 C18:1 for milk fat fluidity and TAG formation. Weld and Armentano (2018) reported increased levels of *trans*-10 C18:1 in milk fat of cows fed conventional soybeans (~55% C18:2) when compared with HOSB (~73% *cis*-9 C18:1), and postulated the lower milk fat yield with conventional soybean could be due to *trans*-10 C18:1 altering regulation of milk fat synthesis. During abomasal infusions of *trans*-10 C18:1, Lock et al. (2007) did not observe milk fat depression whereas Shingfield et al. (2009) reduced milk fat secretion. Differences between studies could be due to the infusate used by Shingfield et al. (2009) contained other FA while Lock et al. (2007) infused almost pure *trans*-10

C18:1. Additionally, Shingfield et al. (2009) infused almost twice the amount of Lock et al. (2007) and reported greater levels *trans*-10 C18:1 in milk (4.4 g/100g), whereas Lock et al. (2007) observed lower concentrations in milk (1.1 g/100g of *trans*-10 C18:1), suggesting potentially lower amounts of *trans*-10 C18:1 are not as detrimental to milk fat synthesis. A recent review performed a regression analysis which concluded an association between increased proportion of *trans*-10 C18:1 and lower milk fat concentration in dairy cows (Dewanckele et al., 2020), although a meta-analysis evaluating dairy ewes observed shifts in *trans*-10 C18:1 milk FA, regardless of ewes that did or did not experience milk fat depression (Toral et al., 2020). Therefore, the impact *of trans*-10 C18:1 on milk fat is varied and may be a synergistic effect with other diet aspects and biological mechanisms, but certainly much less potent than *trans*-10-, *cis*-12 CLA. This is likely important when considering feeding oilseeds rich in either *cis*-9 C18:1 or C18:2.

Milk Production Responses to Dietary Fatty Acids

With the steady increase in milk production of today's high producing dairy cow, there is also a greater energy and nutrient demand to sustain these levels of production. Thus, exploring ways to increase the energy and nutrient density of the diet is of importance. Sources rich in FA are often included in dairy cattle diets to increase the energy density of the diet and help support milk production and milk fat yield (Rabiee et al., 2012). Fat supplements and oilseeds are commonly used as a source of energy, and have a range in FA profile and FA content. Banks et al. (1976) concluded that low-fat diets could limit the yields of milk and milk fat and increasing dietary FA supply increased these yields when different oils and oilseeds were fed (Virtanen, 1966; Banks et al., 1976). These low-fat diets potentially restricted energy supply to the cow and

increasing dietary FA content resulted in improved energy balance. Further research into the impact of FA profile, specifically the impact of *cis*-9 C18:1, and type of fat source (i.e. fat supplements and oilseeds) is needed.

Effects of Dietary FA on DMI

There is variability in the literature regarding the effects of fat supplements and oilseeds on DMI. Evidence across the literature suggests that the FA profile of the fat source can impact DMI (Rabiee et al., 2012; dos Santos Neto et al., 2021a; b). Other factors, such as the amount of UFA in the diet, the profile of the FA leaving the rumen, and a hypophagic effect of UFA on gut peptides that signal satiety (Allen, 2000) could also effect DMI.

In a recent meta-analysis, Ca-salts of palm FA decreased DMI compared with a control diet with no FA supplementation (dos Santos Neto et al., 2021b) whereas SFA prills had no effect (dos Santos Neto et al., 2021a). Diets higher in UFA have been found to depress DMI greater than SFA and non-FA control treatments (Harvatine and Allen, 2005; Relling and Reynolds, 2007; de Souza et al., 2018a). In two studies utilizing SFA prills and a Ca-salt of palm FA in FA blends to alter the ratio of C16:0 + *cis*-9 C18:1, there was no difference in DMI as level of *cis*-9 C18:1 increased up to 30% (de Souza et al., 2019; Western et al., 2020b). Although, Western et al. (2020b) observed an interaction with production level, as high producing cows increased DMI with more *cis*-9 C18:1 and less C16:0 in the FA blend. Fat supplements could trigger signals that affect gut motility and induce satiety, as increased unsaturation level in the FA supplement decreased DMI and increased secretion of gut peptides associated with satiety (i.e., cholecystokinin; Relling and Reynolds, 2007; Bradford et al., 2008). As the degree of unsaturation increases, this hypophagic effect becomes more pronounced, and can result in a reduction of milk yield (Christensen et al., 1994; Relling and Reynolds, 2007).

Studies that have utilized WCS in the diet did not observe changes in DMI (Bernard et al., 1997; Smith et al., 1980; Johnson et al., 2002) or reported a decrease (Hawkins et al., 1985; Rico et al., 2017). Inclusion level of WCS should be considered, as Coppock et al. (1985) found a linear decrease with 0, 15, and 30% DM inclusion, whereas Smith et al. (1981) reported no effect on DMI of WCS when included at 0, 5, 15, and 25% DM. Additionally, Knapp et al. (1991) found no effect on DMI when increasing whole, roasted, conventional soybeans from 0 to 24% inclusion. Evaluating other oilseeds, crushed canola seed, an oilseed naturally high in *cis*-9 C18:1, increased DMI compared with other seeds containing high levels of C18:2 and C18:3 (linolenic acid; Beauchemin et al., 2009). Particle size can potentially impact DMI, as Tice et al. (1993) observed increased DMI with a smaller soybean particle size, whereas Dhiman et al. (1997) observed no difference between soybean treatments of different grind size.

The effect that different fat sources have on DMI could also be due to milk production level of the cow, fermentability of the diet, and the effects of satiety induced gut peptides. Fiber digestion potentially could have an impact on DMI (Allen, 2000; Weld and Armentano, 2017), although, as discussed earlier in this literature review, FA impacts on fiber digestion is FA profile dependent and most likely a result of overall diet fermentability and interactions with other dietary feed ingredients.

Effects of Fat Supplements on Milk Production Responses

In the past century, the utilization of fat sources has advanced significantly, resulting in extensive research on fat supplements (Palmquist and Jenkins, 2017), but in the past decade there has been increased emphasis on how FA profile of FA supplements impacts milk production responses. Although Rabiee et al. (2012) reported an increase in yields of milk and milk fat, the FA supplements were not separated by FA profile or type of supplement, and two recent meta-

analyses investigated fat supplements based on the manufacturing process, as well as FA profile (dos Santos Neto et al., 2021a; b).

Historically, the manufacture of fat supplements was not developed with the dairy cow in mind and were simply by-products of other industries. Although the use of tallow and vegetable oils were popular early on, the development of dry fat supplements (Ca-salts and prills) allowed for easier handling and were less disruptive to the rumen environment then oils (Palmquist and Jenkins, 2017). The main FA found in commercial supplements are C16:0, C18:0, and *cis*-9 C18:1 and their respective melting points need to be considered, as C16:0, C18:0, and *cis*-9 C18:1 have melting points of 71°C, 63°C, and 7°C, respectively, (NASEM, 2021). Prills, enriched in SFA, are a high melting point fat supplement and current prill technologies do not allow for inclusion of UFA >10%. Although salts of FA typically contain higher amounts of UFA, they will have a high milting point dependent on the respective cation (i.e. calcium) used to manufacture the product (NASEM, 2021). Therefore, Ca-salts of palm FA contain ~ 46% C16:0 and ~38% *cis*-9 C18:1 while SFA prills contain mainly C16:0 (≥ 80%) or a combination of ~38% C16:0 + ~45% C18:0 (Table 1; dos Santos Neto et al., 2021a; b).

Inclusion of a Ca-salt of palm FA (~ 46% C16:0 + ~38% *cis*-9 C18:1) in dairy cow diets has been reported to increase milk production responses compared with no FA supplementation (dos Santos Neto et al., 2021b). Recent work has evaluated different ratios of C16:0+ *cis*-9 C18:1 to determine if different proportions affect cow performance. Although decreases in yields of milk and milk fat yield have been observed with increases in the degree of unsaturation of the FA supplement (Harvatine and Allen, 2006; Relling and Reynolds, 2007), this response could be due to BH of UFA producing intermediates that reduce milk fat synthesis in the mammary gland (Bauman et al., 2011). In contrast, studies directly comparing prilled SFA supplements and Ca-

salts reported no difference in yields of milk production between treatments (Harvatine and Allen, 2005; Rico et al., 2014a). Previous research has determined that cows at different milk production levels can have differing production and metabolic responses to FA supplementation (Harvatine and Allen, 2005; Piantoni et al., 2014; Rico et al., 2014b). When evaluating production level, high producing cows increased ECM when increasing *cis*-9 C18:1, at the expense of C16:0, in FA blends (de Souza et al., 2019; Western et al., 2020b) and when C18:0 was replaced with *cis*-9 C18:1 (Burch et al., 2021). The differences observed for milk production responses when utilizing FA supplements is most likely related to the FA profile and the production level of the cow. All of these variables should be considered when supplementing FA supplements, as well as consideration of overall diet and rumen fermentation when including FA supplements higher in UFA. Chapter 4 continues to investigate differences amongst FA profile, as well as evaluating if production responses could be influenced by the form of the FA supplement (prill vs. Ca-salt), as well as the FA profile.

Effects of Oilseeds on Milk Production Responses

The use of oilseeds has been reported to increase the yield of milk and milk fat (Rabiee et al., 2012) while low-fat diets reduced these yields (Maynard and McCay 1929; Banks et al., 1976). Utilization of WCS was found to increase milk fat content and yield, and FCM (Smith et al., 1981; Sklan et al., 1992) compared with low levels or no WCS. In a recent meta-regression, dos Santos Neto et al. (2022) reviewed the available literature with WCS inclusion of up to 17% DM and found that for every 1-percentage increase in inclusion level, yields of milk fat, milk protein, and ECM were increased. These results could be due to the greater intake of UFA and incorporation of preformed FA into milk fat. The addition of canola and WCS to a diet increased yield of milk and milk fat, resulting in increased FCM compared with a low-fat basal diet

(Johnson et al, 2002). Similarly, de Souza et al. (2018) observed that a WCS diet increased milk fat yield, although milk yield was not impacted (de Souza et al., 2018). Inclusion of 16.7% WCS did not impact milk production responses (Rico et al., 2017) and inclusion of WCS to high corn silage based diets reduced milk fat yield (Smith et al., 1993; Adams et al., 1995). A review by Arieli (1998) discussed that feeding WCS, in many cases, did not affect milk protein concentration and yield, although in cases where milk protein was impacted could be related to microbial protein synthesis, as Adams et al. (1995) reported milk protein production was affected by forage source.

There is also variability among other oilseeds, as Lopes et al. (2017) and Weld and Armentano (2018) observed no difference or a decrease in milk yield, respectively, when comparing HOSB and conventional soybeans, although Plenish soybeans increased milk fat yield compared with conventional soybean. Additionally, Beauchemin et al. (2009) reported no difference between sunflower seeds, canola seeds, and flax seed for milk production responses. Oilseeds and oils containing more *cis*-9 C18:1, compared with other 18-carbon FA, either did not impact milk yield (Casper et al., 1988; Kelly et al., 1998) or increased milk yield (Dai et al., 2011) and 4% FCM (Casper et al., 1988; Dai et al., 2011). Knapp et al. (1991) increased milk yield when conventional soybeans were included up to 25% DM. When comparing whole raw soybeans and WCS, the WCS diet increased milk yield but milk fat production did not differ between the two treatments (Abel-Caines et al., 1997), most likely due to both oilseeds containing similar levels of C18:2. Processing method may impact milk production responses, as roasting conventional soybeans increased yields of milk, ECM, and 4% FCM when compared with raw soybeans (Tice et al., 1993), although some studies reported no difference between roasted or raw whole soybeans (Bernard, 1990; Grummer et al., 1994).

Overall, inclusion of oilseeds to the diet is beneficial, as it can increase yields of milk and milk fat. Further research is required to evaluate different oilseeds in high-producing dairy cows, as most studies in the literature examining oilseed supplementation used lower producing cows (<45 kg/d milk yield). Due to the lack of established feeding rates, Chapters 5 and 6 examine increasing inclusion rates of WCS and HOSB, respectively, and the impacts of these oilseeds on milk production responses. Additionally, because there is limited research utilizing HOSB, Chapters 6 and 7 investigate inclusion of HOSB and processing method of HOSB on milk production of high-producing dairy cows.

Effects of Oleic Acid on Milk Production Responses

As stated previously, oilseeds with higher content of *cis*-9 C18:1, compared with 18:2 and 18:3, have increased yields of milk and milk fat (Casper et al., 1988; Dai et al., 2011; Weld and Armentano, 2018). Additionally, recent research has shown that high producing dairy cows (> 50 kg/d of milk yield) respond best to a ratio of 60% C16:0 + 30% *cis*-9 C18:1 (de Souza et al., 2019; Western et al., 2020b; Burch et al., 2021), whereas lower producing cows (< 50 kg/d of milk yield) increased milk production with a ratio of 80% C16:0 + 10% *cis*-9 C18:1 (de Souza et al., 2019; Western et al., 2020b; Bales et al., 2024). As discussed earlier in this literature review, the increase in milk production responses observed when supplementing with *cis*-9 C18:1 could be attributed to the positive effects of *cis*-9 C18:1 on FA digestibility and absorption (Boerman et al., 2015a; Prom and Lock, 2021; Prom et al., 2021), milk fat synthesis and mitochondrial activity in the mammary epithelial cell (Liang et al., 2014; Cohen et al., 2015), and positioning of *cis*-9 C18:1 on milk fat TAG (Jensen, 2002). Additionally, abomasal infusion of *cis*-9 C18:1 increased mitochondrion biogenesis in adipose tissue of lactating dairy cows (Abou-Rjeileh et al., 2023), further supporting the positive impact that *cis*-9 C18:1 can have on mitochondrial

activity. Even though the majority of UFA in oilseeds and FA supplements will undergo BH, the response of cows to *cis*-9 C18:1 shows positive impacts on milk production responses, especially in high-producing dairy cows.

Conclusion

Considerable research has examined the impacts of FA supplementation to dairy cows and how different individual FA and blends of FA are utilized for production and metabolic needs. Although recent research indicates that the FA profile of the supplement is a major factor affecting production responses, there remains gaps in our understanding. Higher producing cows benefit from increased levels of *cis*-9 C18:1 in a FA supplement, although it is unknown what the potential of a *cis*-9 C18:1-enriched Ca-salt could be that would continue to increase milk production responses. Additionally, there are gaps in knowledge on how best to utilize oilseeds, especially when feeding to high producing dairy cows, and if the FA profile of an oilseed will impact production responses differently. Overall, future research is needed to better understand the utilization of *cis*-9 C18:1 in FA supplements and oilseeds. Therefore, this dissertation will focus on *cis*-9 C18:1 across fat sources. Chapters 3 and 4 will focus on *cis*-9 C18:1 content of FA supplements while Chapters 5-7 will focus on oilseeds containing different levels of *cis*-9 C18:1.

The objective for Chapter 3 is to determine the efficacy of a high *cis*-9 C18:1 Ca-salt, with the use of rumen and abomasal infusions of *cis*-9 C18:1 as markers to examine the impacts of this Ca-salt. The results of Chapter 3 will be important, as it will help better determine the next steps on how to utilize FA supplements higher in *cis*-9 C18:1 in diets for higher producing dairy cows. The objective for Chapter 4 is to determine if cow performance is impacted by FA

profile or the form of the FA supplement. Due to previous research in our lab blending multiple FA supplements to achieve our desired FA profiles, results from this chapter are important to understand if the form of the FA blend is more impactful than the FA profile. Both chapters will aid nutritionists and producers with decisions based upon feeding FA supplements.

In Chapter 5, the objective is to assess nutrient digestibility and milk production performance to increasing dietary inclusion of WCS fed to high producing dairy cows. For Chapter 6, the objective is to evaluate milk production responses of high producing dairy cows to increasing dietary inclusion of roasted and ground HOSB. Establishing suitable feeding rates for oilseeds will help to improve milk production responses and producer income. The objective for Chapter 7 is to investigate the effect of processing method of HOSB on milk production responses of high producing dairy cows. Due to the limited research regarding HOSB, the results from this chapter are important, as there are additional costs associated with roasting HOSB, thus understanding if raw HOSB will increase milk production responses will aid future nutritional decisions.

Results from the research presented in these chapters will advance overall knowledge regarding *cis*-9 C18:1 metabolism in high-producing dairy cows. Furthermore, the results will allow for more strategic decision-making for dairy producers and nutritionists.

CHAPTER 3: POTENTIAL FOR AN OLEIC ACID ENRICHED CA-SALT TO POST-REUMINALLY SUPPLY OLEIC ACID AND IMPROVE DIGESTIBILITY OF DAIRY COWS

Abstract

We determined the effects of a high oleic acid (*cis*-9 C18:1) Ca-Salt (23% C16:0 and 64% C18:1) alongside ruminal and abomasal infusion of *cis*-9 C18:1 on digestibility and production responses of lactating dairy cows. Eight multiparous cows (46.2±5.96 kg/d of milk; 161±11 DIM) were assigned to treatment sequences in a replicated 4x4 Latin square design with 18-d periods, consisting of 7-d of washout and 11-d of infusion. Treatments were: water infusions (CON), abomasal infusion of *cis*-9 C18:1 (ABO), ruminal infusion of *cis*-9 C18:1 (RUM), or rumen supplementation through the cannula of a *cis*-9 C18:1-enriched Ca-salt (SALT). Treatments delivered 50 g/d of *cis*-9 C18:1. All cows were fed a diet that contained (%DM) 30% NDF, 16% CP, 30% starch, and 3.8% FA. The statistical model included the random effect of cow within square and the fixed effects of period, treatment, and their interaction. Pre-planned contrasts were: CON vs the average of the three *cis*-9 C18:1 treatments (OA), ABO vs RUM and SALT vs RUM. There was no effect of treatment on DMI or NDF intake, but compared with CON, OA tended to decrease 16-carbon FA digestibility. ABO increased digestibility of NDF, 16-carbon, 18-carbon FA, and total, compared with RUM. SALT increased digestibility of DM, NDF, 16-carbon FA, 18-carbon FA, and total FA compared with RUM. Treatments did not affect milk production responses. In summary, both *cis*-9 C18:1 infused into the abomasum and a *cis*-9 C18:1-enriched Ca-salt in the rumen improved the digestibility of NDF and FA compared with rumen infusion of OA in mid-lactation dairy cows. Although there was a lack of production responses observed, the increase in NDF and FA

digestibility with a *cis*-9 C18:1-enriched Ca-salt highlights the potential for this FA supplement to positively impact production responses of high-producing dairy cows.

Introduction

Nutrient demand and milk production of the modern dairy cow are simultaneously increasing, thus investigating ways to meet these requirements are critical. A common practice to increase the energy density of the diet is the utilization of fat supplements, with the majority of supplements primarily containing varying amounts of three fatty acids (**FA**): palmitic (**C16:0**), stearic (**C18:0**), and oleic acid (***cis-9* C18:1**). Additionally, C16:0, C18:0, and *cis*-9 C18:1 are the most common FA found in bovine milk (Palmquist, 2006) and adipose tissue (Douglas et al., 2007). Fatty acid supplements have been found to improve milk production (dos Santos Neto et al., 2021b; a), with yields of milk and milk components differing depending on the FA profile of the supplement (de Souza et al., 2018a, 2019; Bales et al., 2024). Recently, our group has focused on different ratios of C16:0 and *cis*-9 C18:1 in FA supplements and observed higher-producing cows (>55 kg/d of milk yield) produce more ECM with more *cis-9* C18:1 while lower-producing cows (<45 kg/d of milk yield) respond better to higher levels of C16:0 (de Souza et al., 2019; Western et al., 2020b). Additionally, *cis-9* C18:1 has been observed to improve FA digestibility compared with C16:0 and C18:0 (Boerman et al., 2015a; Prom et al., 2022). The impact of *cis*-9 C18:1 on FA digestibility and milk production responses has led to an interest in the targeted feeding of *cis*-9 C18:1.

Ca-salts of FA allow for unsaturated FA (**UFA**) to be fed in a dry form that the dairy cow can efficiently digest and can reduce the negative effects of UFA on rumen fermentation (Palmquist and Jenkins, 2017). The majority of Ca-salts are produced from palm FA distillate

(**PFAD**) and have a FA profile consisting of C16:0 (~46%) and *cis*-9 C18:1 (~38%) with lesser amount of C18:0 (~4%) and linolenic acid (~8%; dos Santos Neto et al., 2021b). At present, Ca-salts of PFAD are the main source of supplemental *cis*-9 C18:1 in diets. Ca-salts are not completely rumen-inert as they do have some degree of dissociation in the rumen (Chalupa et al., 1986). At normal rumen pH levels, dissociation of the Ca-salt has been reported to not surpass 50% (Sukhija and Palmquist, 1990), thus a portion of the *cis-9* C18:1 leaves the rumen and can be utilized by the cow for milk production and other physiological needs. In a recent meta-analysis evaluating the effects of Ca-salts of PFAD compared with non-FA supplemented control diets, Ca-salts decreased DMI but yields of milk and milk fat increased (dos Santos Neto et al., 2021b). The average dietary inclusion of Ca-salts was 2.20% DM of the diet with a FA profile averaging 46% C16:0 and 38% *cis-9* C18:1, reflective of a feeding rate and FA profile used in the industry.

Utilization of abomasal infusions allows for evaluation of UFA that will by-pass the rumen, thus results can be attributed to the direct effect of the UFA on nutrient digestibility and metabolism. In an abomasal infusion experiment increasing dosage of *cis*-9 C18:1 from 0 to 60 g/d improved 16-carbon, 18-carbon, and total FA digestibility and increased the yields of milk and milk fat without a negative effect on DMI (Prom et al., 2021). These results suggest that even small levels of *cis*-9 C18:1 that by-pass the rumen will improve FA digestibility and absorption and thus increase milk production responses. Abomasal infusion of 500 g/d of *cis*-9 C18:1 increased arterial concentrations of plasma non-esterified FA and triglycerides (Enjalbert et al., 1998) and up to 400 g of infusion of canola and high *cis*-9 C18:1 sunflower oil (63 and 86% *cis*-9 C18:1, respectively) increased plasma triglycerides, resulting in an increase in milk fat (LaCount et al., 1994). The increase in plasma triglycerides in the aforementioned studies is most

likely a result of improved FA digestion from *cis*-9 C18:1. dos Santos Neto et al. (2023a) infused 30 g/d of an exogenous emulsifier (~5 g/d of *cis*-9 C18:1) into either the rumen or abomasum, and observed some differences in digestion.

Currently available Ca-salts of PFAD typically contain up to 45% *cis*-9 C18:1, resulting in a limitation in the supply of dietary *cis*-9 C18:1. Due to the positive impacts of *cis-9* C18:1 on digestion and milk production, there is interest in the targeted supply of *cis-9* C18:1. Considering the aforementioned results with Ca-salts and increasing *cis*-9 C18:1 in both FA blends and abomasal infusion doses, can we utilize a low inclusion rate of a Ca-salt enriched in *cis*-9 C18:1 to deliver an targeted amount of *cis*-9 C18:1 available for absorption, thus increasing both FA digestibility and production responses? Our objective of this study is to evaluate the effects of a *cis*-9 C18:1-enriched Ca-salt on nutrient digestibility and milk production responses. We infused *cis*-9 C18:1 directly into the rumen and abomasum to compare these to the *cis*-9 C18:1-enriched Ca-salt to determine the potential of the Ca-salt to supply *cis*-9 C18:1 past the rumen.

Material and Methods

Design and Treatments

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University (East Lansing, MI). Eight mid-lactation, ruminal cannulated multiparous Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center were randomly assigned to a treatment sequence in a replicated 4 × 4 Latin square design. Cows were blocked by milk yield and balanced for BCS. All animals received a common diet with no FA supplementation during a 7-d preliminary period to obtain baseline values. The starting average for all animals, with mean ± standard deviation, were 46.2 ± 5.96

kg/d of milk yield and 161 ± 11 DIM. Each 18-d treatment period consisted of a 7-d washout period followed by an 11-d treatment infusion period, with sampling during the last 4-d (de Souza et al., 2020; Prom et al., 2021).

Treatments consisted of: water infusions (**CON**), abomasal infusion of 55 ± 0.5 g/d of *cis-9* C18:1 (O1008, Sigma-Aldrich; **ABO**), ruminal infusion of 55 ± 0.5 g/d of *cis-9* C18:1 (O1008, Sigma-Aldrich; **RUM**), and rumen supplementation via the cannula of 78 ± 0.5 g/d of a *cis-9* C18:1 enriched Ca-salt (**SALT**). Treatments were designed to deliver 50 g/d of *cis*-9 C18:1 and FA content and profiles are shown in Table 3. Our dose was based upon *cis*-9 C18:1 doses in a previous infusion experiment (Prom et al., 2021), as well as consideration of biohydrogenation estimates (Jenkins and Bridges, 2007) that represent realistic flows of *cis*-9 C18:1 from FA supplements to the abomasum. Daily doses of *cis*-9 C18:1 for infusions were suspended in ~45 ± 5.0 g of ethanol in individual glass jars. The infusate solution was divided into 4 equal infusions per day occurring every 6 h, based on previous studies (de Souza et al., 2020; Prom et al., 2021). Infusate solutions were delivered into infusion lines using 60-mL plastic syringes. The SALT treatment was also divided into 4 equal amounts and delivered every 6 h to coincide with timing of the infusion treatments. The SALT treatment was administered by the removal of the fistula plug, then pouring the Ca-salt into the rumen and mixing with the rumen contents, and then placing the plug back into the fistula. This ensured cows received the same amount of product to ensure the delivery of 50 g/d of *cis*-9 C18:1.

Stainless steel abomasal infusion devices as described by Westreicher-Kristen and Susenbeth (2017), with the addition of a circular, flexible rubber flange, were inserted into the abomasum 5 d before the beginning of the study. Infusion lines were made using 0.5-cm diameter polyvinyl chloride tubing and passed through the rumen fistula. The abomasum

infusion line was then passed through the sulcus omasi into the abomasum (Lock et al., 2007) and rumen infusion lines were cut to be ~12 inches into the rumen. The abomasal and rumen infusion lines stayed in the cows for the duration of the study. All lines were checked daily and flushed with water throughout the study to ensure proper placement and to check for blockages.

Access to feed was blocked from 0700 to 0800 h for orts collection and offering of new feed and cows were fed 115% expected intake at 0800 h daily. Cows were housed in individual tie-stalls throughout the experiment with water available ab libitum in each stall that were bedded with sawdust and cleaned twice daily. Cows were milked 3 times per day at 0400, 1200, and 2000 h. All animals received a common diet formulated to meet the nutrient requirements of a mid-lactation dairy cow (Table 4; NASEM, 2021). The DM concentration of forages was determined twice weekly, and diets adjusted when necessary. The diet included a commercially available C16:0 + C18:0 FA supplement (Energy Booster 100; Table 4) fed at 2% of diet DM to increase the saturated FA supply and lower FA digestibility in order to assess potential improvements in FA digestibility from the *cis*-9 C18:1 treatments.

Data and Sample Collection

Samples and production data were collected during the last 4 d of each treatment period (d 15-18). Samples of all diet ingredients (0.5 kg) and orts (12.5%) were collected daily and composited by cow/period for analysis. Milk yield was recorded, and 2 milk samples were collected at each milking. One sample was collected in a sealed tube with preservative and stored at 4^{o}C for milk component analysis. The second sample was stored without preservative at -20^{o}C until analyzed for FA composition. Blood (~15 mL) samples were collected every 9 h resulting in 8 samples/cow/period and stored on ice until centrifugation at 2,000 × *g* for 15 min at 4^{o}C. Plasma was transferred into microcentrifuge tubes and stored at -20^{o}C until composited by

cow/period. Fecal (~400 g) samples were collected every 9 h resulting in 8 samples/cow/period and stored at -20°C until composited by cow/period. BW was measured on the last 2 d of each period following the afternoon milking. On the last day of each period, BCS was determined by three trained investigators on a 5-point scale in 0.25 increments (Wildman et al., 1982).

Sample Analysis

Dietary ingredients, orts, and feces were dried at 55°C in a forced-air oven for 72 h for DM determination. Dried fecal samples for each cow were composited by period and dried feces, orts, forages, and diet samples were ground in a Wiley mill (1 mm screen; Arthur H. Thomas, Philadelphia, PA). Feed ingredients were analyzed for absolute DM, ash, NDF, indigestible NDF, CP, and starch, and orts and feces for absolute DM, ash, NDF, and indigestible NDF by Cumberland Valley Analytical Services (Waynesboro, PA) as described by Boerman et al. (2017). Absolute DM was determined by drying samples in an oven at 105°C using the National Forage Testing Association reference method (Shreve et al., 2006). Indigestible NDF was estimated as NDF after 240-h in vitro fermentation (Goering and Van Soest, 1970) and was used as an internal marker to estimate fecal output to determine apparent total-tract digestibility of nutrients (Cochran et al., 1986). The FA content of feed ingredients, orts, and feces were determined as described by Bales et al. (2024). Milk samples were analyzed for fat, true protein, and lactose concentrations by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan Dairy Herd Improvement Association (Central Star DHI, Grand Ledge, MI). Yields of milk components, 3.5% FCM, and ECM were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each period. Milk samples used for analysis of FA composition were composited based on milk fat yield (d 15-18 per period). Milk lipids were extracted, FAME prepared, and analyzed by gas chromatography as

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

Plasma samples from each cow were composited by period before analysis. Samples were analyzed in duplicate with a coefficient of variation of <5% between duplicates. Plasma samples were analyzed at the Michigan State University Veterinary Diagnostic Laboratory (Lansing, MI) for insulin, which was quantified with a bovine insulin ELISA using a solid phase 2-site enzyme immunoassay (Mercodia).

Statistical Analysis

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

$$Y_{ikl} = \mu + C(S)_{i(j)} + S_j + P_k + T_l + P_k \times T_l + e_{ikl}$$

Where Y_{ijkl} = the dependent variable, μ = the overall mean, $C(S)_{i(j)}$ = random effect of cow nested within square (j = 1 to 4), S_j = fixed effect of square (i = 1 to 2), P_k = fixed effect of period (k = 1 to 4), T_l = fixed effect of treatment (l = 1 to 4), $P_k \times T_l$ = the interaction of period and treatment, and e_{ijkl} = residual error. Two squares, each one with 4 cows, were formed based on lower (milk yield [mean ± SD] = 42.4 ± 2.66 kg/d) and higher (milk yield = 50.0 ± 6.08 kg/d) production cows. The interactions between period and treatment, period and square, and square and treatment were initially included in the model and removed when $P > 0.20$ (de Souza et al., 2020). Main effects were declared significant at $P \leq 0.05$ and tendencies $P \leq 0.10$. Three

orthogonal contrasts were evaluated: (1) the overall effect of *cis*-9 C18:1 (CON vs. the average of the *cis*-9 C18:1 treatments (**OA**) [1/3 {ABO + RUM + SALT}]), (2) the effect of infusing *cis*-9 C18:1 into the abomasum vs the rumen (ABO vs RUM), and (3) the effect of infusing *cis*-9 C18:1 vs supplementation of the *cis*-9 C18:1-enriched Ca-salt in the rumen (RUM vs SALT).

Results

Nutrient Intake and Total-Tract Digestibility

Overall effect of OA treatments

Overall, OA did not affect intake of DM ($P = 0.39$; Table 5), NDF ($P = 0.53$), or 16-carbon FA ($P = 0.28$) but increased intake of 18-carbon and total FA (both $P < 0.01$). Overall OA did not affect digestibility of DM ($P = 0.97$), NDF ($P = 0.77$), 18-carbon FA ($P = 0.13$), or total FA ($P = 0.12$), and absorption of 16-carbon ($P = 0.11$), 18-carbon ($P = 0.60$), or total FA ($P = 0.98$) but tended to decrease 16-carbon FA digestibility ($P = 0.09$).

ABO vs RUM infusions of cis-9 C18:1

Infusions of *cis*-9 C18:1 into either the abomasum or the rumen did not affect intakes of DM ($P = 0.52$; Table 5), NDF ($P = 0.39$), 16-carbon FA ($P = 0.64$), 18-carbon FA ($P = 0.86$), or total FA ($P = 0.79$). Compared to RUM, ABO tended to increase DM digestibility ($P = 0.06$) and increased digestibility of NDF ($P = 0.04$), 16-carbon FA ($P < 0.01$), 18-carbon FA ($P = 0.01$), and total FA ($P < 0.01$). Due to the increase in FA digestibility, compared with RUM, ABO increased absorption of 16-carbon FA ($P < 0.01$), 18-carbon ($P = 0.01$), and total FA ($P < 0.01$).

SALT vs RUM

We did not observe any difference between SALT and RUM for intakes of DM ($P = 0.95$; Table 5), NDF ($P = 0.80$), or total FA ($P = 0.60$), but compared with RUM, SALT

increased 16-carbon FA intake ($P = 0.01$) and tended to decrease 18-carbon FA intake ($P = 0.09$). Compared with RUM, SALT increased digestibility of DM ($P = 0.04$), NDF ($P = 0.01$), 16-carbon FA ($P < 0.01$), 18-carbon FA ($P = 0.04$), and total FA ($P = 0.03$). SALT increased absorption of 16-carbon FA ($P < 0.01$) and total FA ($P = 0.05$) but did not affect 18-carbon FA ($P = 0.15$).

Production Responses

Overall effect of OA treatments

Overall OA had no effect on yields of milk, 3.5% FCM, ECM, milk fat, milk protein, or milk lactose (all $P \geq 0.19$; Table 6), content of milk fat (P=0.58) or lactose ($P = 0.15$), BW ($P = 0.89$), BCS ($P = 0.37$) or plasma insulin concentration ($P = 0.12$). Overall OA increased milk protein content ($P = 0.05$) and feed efficiency (ECM/DMI; $P = 0.05$).

ABO vs RUM infusions of cis-9 C18:1

We did not observe any differences between ABO and RUM for yields of milk, 3.5% FCM, and ECM, milk fat yield and content, milk protein yield and content, milk lactose yield and content, ECM/DMI, BW, BCS, and plasma insulin (all $P \geq 0.15$; Table 6).

SALT vs RUM

Compared with RUM, SALT increased milk protein content ($P = 0.04$; Table 6) and tended to decrease plasma insulin ($P = 0.06$). There was no effect of treatment for yield of milk, 3.5% FCM, ECM, and milk protein, milk fat yield and content, milk lactose yield and content, ECM/DMI, BW, and BCS (all $P \geq 0.33$).

Milk FA Yields

Milk FA are derived from two sources: <16 carbon FA (de novo) from de novo synthesis in the mammary gland and >16 carbon FA (preformed) originating from extraction from plasma.

Mixed source 16-carbon FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis in the mammary gland and extraction from plasma.

Overall effect of OA treatments

Overall OA did not affect the yields of de novo (P=0.47; Table 7) or preformed milk FA (P=0.68) but decreased the yield of mixed milk FA (P=0.04). For select individual milk FA, compared with CON, overall OA did not affect the yields of C4:0 (P=0.75) or *cis*-9 C18:1 (P=0.11), decreased yields of C16:0 (P=0.04) and C18:0 (P=0.05) and tended to decrease the yield of *trans*-11 C18:1 (P=0.09), and increased the yields of *trans-9* C18:1 (P=0.05) and *trans*-10 C18:1 (P=0.02).

ABO vs RUM infusions of cis-9 C18:1

Compared with RUM, ABO did not affect the yields of de novo (P=0.49; Table 7) or mixed milk FA (P=0.26) but tended to increase the yield of preformed milk FA (P=0.08). For select individual milk FA, compared with RUM, ABO did not affect the yields of C4:0 (P=0.59), C16:0 (P=0.22), C18:0 (P=0.61), and *trans*-10 C18:1 (P=0.27), increased the yield of *trans*-9 C18:1 (P=0.03) and *cis*-9 C18:1 (P<0.01), and decreased the yield of *trans*-11 C18:1 (P=0.03).

SALT vs RUM

There was no effect of treatment between RUM and SALT for yields of de novo (P=0.33; Table 7), mixed (P=0.94), or preformed milk FA (P=0.77). For select individual milk FA, compared with RUM, SALT did not affect the yields of C4:0 (P=0.32), C16:0 (P=0.95), C18:0 (P=0.25), *trans*-10 C18:1 (P=0.54), *trans*-11 C18:1 (P=0.13), and *cis*-9 C18:1 (P=0.94), but decreased the yield of *trans-9* C18:1 (P=0.05).

Discussion

Improving FA digestibility and absorption is important for improving nutrient absorption and utilization. Our previous studies have observed increases in FA digestion when infusing *cis*-9 C18:1 up to 60 g/d into the abomasum (Prom et al., 2021) and in feeding trials utilizing FA blends containing up to 35% *cis*-9 C18:1 (de Souza et al., 2018a, 2019; Burch et al., 2021). Currently, the majority of Ca-salts are based on palm oil which contains ~35% *cis*-9 C18:1. To our knowledge, there has been no research examining the potential of *cis*-9 C18:1-enriched (>50% *cis*-9 C18:1) Ca-salts on nutrient digestion and milk production. This will potentially allow for a more targeted delivery of *cis*-9 C18:1 at specific times in small doses for improved digestion and nutrient utilization. Therefore, we designed a study to evaluate the effects of a *cis*-9 C18:1-enriched Ca-salt alongside abomasal and ruminal infusions of *cis*-9 C18:1 and to our knowledge, it is the first to do so. Although other experiments have examined ruminal versus abomasal infusions of FA (Kazama et al., 2010; dos Santos Neto et al., 2023a; Litherland et al., 2023), our study is the first to do so with > 90% *cis*-9 C18:1 as a free FA. We expected OA treatments to increase nutrient digestion and absorption. Although we did not observe overall improvement with *cis*-9 C18:1 treatments, we did observe differences between abomasal and ruminal infusions of *cis*-9 C18:1 and between ruminal infusions of *cis*-9 C18:1 and the *cis*-9 C18:1-enriched Ca-salt. To evaluate the efficacy of the *cis*-9 C18:1-enriched Ca-salt, we utilized abomasal infusion of *cis*-9 C18:1 to by-pass the rumen while the ruminal infusion of *cis*-9 C18:1 allowed for an unprotected source of *cis*-9 C18:1 into the rumen. Due the increases observed in nutrient digestion, our study provides evidence that a *cis*-9 C18:1-enriched Ca-salt can adequately supply *cis*-9 C18:1 to the small intestine and improve nutrient digestion.

We did not observe any treatment effect on intake of DM or NDF, similar to results observed when infusing polysorbates containing *cis*-9 C18:1 (Prom et al., 2022; dos Santos Neto et al., 2023b; a). Oleic acid has been observed to decrease DMI in FA blends (de Souza et al., 2018a; Burch et al., 2021) and a recent meta-analysis reported reduced DMI when Ca-salts of PFAD (~38% *cis*-9 C18:1) were fed at ≤ 3% of diet DM (dos Santos Neto et al., 2021b). Although our treatments were designed to deliver 50 g/d of OA, it is likely that studies that observed decreased DMI delivered more *cis*-9 C18:1 to the small intestine that stimulated secretion of gut peptides associated with satiety promoting hypophagic effects (Choi et al., 2000; Relling and Reynolds, 2007; Bradford et al., 2008). This hypophagic effect was most likely observed by Drackley et al. (2007), as DMI was reduced from 22.0 to 5.8 kg/d with abomasal infusions of up to 1,000 g/d of a high *cis*-9 C18:1 sunflower oil. Although we did not observe any effects on DMI, there is potential that in a feeding trial, cows supplemented with the *cis*-9 C18:1-enriched Ca-salt could decrease DMI which would most likely be dependent on inclusion rate and the amount of *cis*-9 C18:1 delivered post ruminally.

We observed that the SALT treatment increased nutrient digestibility compared with RUM, indicating that a *cis*-9-enriched Ca-salt can be utilized to increase *cis*-9 C18:1 supply to the small intestine and not negatively impact rumen fermentation. Ca-salts have been observed to improve NDF digestion compared with oils (Palmquist and Jenkins, 2017; Weld and Armentano, 2017), potentially explaining the increase in NDF digestibility with the SALT treatment. Additionally, the SALT treatment contained 22% C16:0, therefore increasing C16:0 intake by 10 g/d compared with RUM. Palmitic acid has been observed to increase fiber digestion compared with other FA (dos Santos Neto et al., 2021a; Sears et al., 2023), thus the increase in nutrient digestion due to SALT could be attributed to the form (Ca-salt) and the C16:0 content compared

with RUM. The observed differences between SALT and RUM for FA digestibility are most likely due to differences in biohydrogenation rates and post rumen supply of *cis*-9 C18:1 between the SALT and RUM treatments, with the RUM treatment potentially having greater biohydrogenation of *cis*-9 C18:1. Oleic acid is an unsaturated FA that is extensively biohydrogenated in the rumen, with C18:0 being the major end product (Jenkins, 1993). Stearic acid is a FA known to decrease FA digestibility compared with other FA (Boerman et al., 2015a; Prom and Lock, 2021). Ca-salts are not completely rumen-inert, since there is partial dissociation within the rumen (Chalupa et al., 1986). Under normal rumen pH levels, dissociation of a Ca-salt does not surpass 50% (Sukhija and Palmquist, 1990) and using 62% rumen loss for *cis*-9 C18:1 in a protected fat supplement (Jenkins and Bridges, 2007) we can estimate that 20 of the 50 g of *cis*-9 C18:1 in the SALT treatment reached the small intestine. The free FA used for the RUM treatment was not protected, and using a rumen loss of 75% for *cis*-9 C18:1 in an unprotected form (Jenkins and Bridges, 2007), we would expect ~13 g/d of *cis*-9 C18:1 flowing to the small intestine. Therefore, the increase in FA digestibility for SALT would likely be due to the increase in *cis*-9 C18:1 reaching the small intestine that will increase FA digestion and absorption (Prom and Lock, 2021; Prom et al., 2021).

As expected, *cis*-9 C18:1 directly infused in the abomasum increased FA absorption compared to the ruminal infusion of *cis*-9 C18:1, similar to experiments infusing flax oil and polysorbates into the rumen and abomasum (Kazama et al., 2010; dos Santos Neto et al., 2023a). As discussed earlier, *cis*-9 C18:1 increases FA digestion compared with other FA (Boerman et al., 2015a), which is likely associated with its emulsification properties that can improve micelle formation (Freeman, 1969). When studying canola oil (62% *cis*-9 C18:1), Chelikani et al. (2004) observed that feeding canola oil decreased duodenal flow of *cis*-9 C18:1 but increased flow of

C18:0 and *trans* C18:1 biohydrogenation intermediates compared with abomasal infusion of canola oil. Thus, we expected the ABO treatment to increase FA digestibility compared with RUM, due to the 50 g/d flow of *cis*-9 C18:1 directly to the abomasum whereas we estimate ~13 g/d of *cis*-9 C18:1 reaching the abomasum with RUM. Similarly, dos Santos Neto et al. (2023b) infused 30 g/d of an exogenous emulsifier (polysorbates-*cis*-9 C18:1; providing ~5 g/d of *cis*-9 C18:1) either into the abomasum or the rumen and observed that the abomasal infusion increased total FA digestion and absorption, although to a lesser extent than our current observations (2.7 versus 6.1%, respectively). Additionally, we have observed a consistent positive effect on FA digestibility and absorption in feeding trials when replacing C18:0 with *cis*-9 C18:1 in FA blends (Burch et al., 2021; Prom and Lock, 2021) and when increasing *cis*-9 C18:1 at the expense of C16:0 (de Souza et al., 2019). These results highlight the importance of improving FA digestibility to increase absorbed FA that can be utilized by the mammary gland and other tissues. The potential for *cis*-9 C18:1 to increase FA digestion offers a practical way that could be used to improve FA digestion and absorption.

Overall, we did not observe an increase in nutrient digestibility with the OA treatments compared with CON. However, this was mostly driven by the RUM treatment negatively impacting the average for overall OA treatments. In previous infusion studies with *cis*-9 C18:1, nutrient digestibility was increased compared with a control treatment (Prom et al., 2021; dos Santos Neto et al., 2023b; a). It has been demonstrated that increasing the amount of FA reaching the small intestine decreases FA digestibility (Boerman et al., 2015a), although this has not been previously observed with small increases in *cis*-9 C18:1 supply in our previous infusion trials. The increase in total FA intake of ~30-40 g/d for overall OA treatments potentially impacted FA digestion and consequently, the lack of difference between CON and OA treatments. Unlike dos

Santos Neto et al. (2023b), our CON and SALT treatment did not receive additional ethanol to match the amount used in the infusate for the ABO and RUM treatments, but previously, ethanol was not observed to impact rumen fermentation when infused into the abomasum (Chalupa et al., 1964) or in a semicontinuous culture system (Durix et al., 1991). Differences may be related to individual animal variation, stage of lactation, differences in diet composition, and DMI.

Additionally, there was a lack of difference for production responses, regardless of treatment. However, it is important to note that our study was primarily designed to examine nutrient digestibility and may not have had adequate power to properly evaluate production responses (Prom et al., 2021), although *cis*-9 C18:1 has improved production responses in other infusion trials (Romo et al., 1996; Prom et al., 2021; dos Santos Neto et al., 2023b). Additionally, previous feeding trials supplementing *cis*-9 C18:1 up to 30% in FA supplements observed increased production responses in higher-producing (≥ 50 kg/d of milk yield), whereas our cows were producing ~45 kg/d, and this lower milk production could also explain the lack of response. The lack of production responses between CON and overall OA treatments are also likely related to the absence of differences in digestibility. Although we observed differences in digestion when comparing ABO vs RUM and SALT vs RUM, lactation stage potentially influenced energy partitioning and the additional absorbed FA may have been partitioned to body reserves. However, there is high variability in assessing BW change in short-term studies, mostly due to gut fill (Prom et al., 2021) and we observed no difference in BW-related variables. There is potential that the DIM for our cows (161 ± 11 d) influenced our results, as they were later in lactation than previous studies infusing *cis*-9 C18:1 (138 ± 52 DIM, Prom et al., 2021; 96 ± 23 DIM, dos Santos Neto, 2023b) as well as feeding trials supplementing *cis*-9 C18:1 (115 ± 42 DIM; de Souza et al., 2019). Our observations are similar to those of dos Santos Neto et al.

(2023b), who also did not observe differences in production responses when infusing a polysorbate-*cis*-9 C18:1 in to the abomasum and rumen in cows that were 170 ± 14 DIM. This study was designed mainly to evaluate the effectiveness of a *cis*-9 C18:1-enriched Ca-salt. The differences in digestibility demonstrate the potential for this Ca-salt to positively impact dairy cattle, but a feeding trial designed to examine production responses should be the next approach to the test the efficacy of a Ca-salt enriched in *cis*-9 C18:1.

Conclusion

Overall, *cis*-9 C18:1infused directly into the abomasum and a *cis*-9 C18:1-enriched Ca-salt added to the rumen increased nutrient digestibility compared with *cis*-9 C18:1 infused into the rumen. Although treatments did not affect yields of milk and milk components, the increase in nutrient and FA digestibility with a *cis*-9 C18:1-enriched Ca-salt highlights the potential for this FA supplement to positively impact production responses of high-producing dairy cows. Future research is needed to test this FA supplement in a feeding trial designed to evaluate production responses.

CHAPTER 4: PRODUCTION RESPONSES ARE IMPACTED BY FATTY ACID PROFILE RATHER THAN FORM OF FAT SUPPLEMENTS IN MID-LACTATION DAIRY COWS

Abstract

We determined the effects of fatty acid (**FA**) profile versus the form of a FA supplement on milk production and nutrient digestibility responses of post-peak dairy cows. Twenty multiparous Holstein cows (mean ± standard deviation; 44.3 ± 3.00 kg/d of milk; 99 ± 23 DIM) were randomly assigned to treatment sequences in a replicated 4 × 4 Latin square design with 21-d periods. Treatments were a non-FA supplemented control diet (**CON**) and 3 diets incorporating FA supplements at 2.0% dry matter (**DM**) of total FA of 1) blend of FA supplements to achieve a ratio of 70% palmitic (**C16:0**) + 20% oleic (*cis*-9 C18:1) using a FA prill and a Ca-salt (**70FB**), 2) a Ca-salt containing 70% C16:0 + 20% *cis*-9 C18:1 (**70CS**), and 3) a Ca-salt of palm FA distillate containing 55% C16:0 + 35% *cis*-9 C18:1 (**45CS**). The 3 FA treatments replaced soyhulls in the CON diet. Diets contained similar (%DM) neutral detergent fiber (NDF; 29.8%), forage NDF (18.4%), starch (28.6%), and crude protein (17.3%). The statistical model included the random effect of cow within square and the fixed effects of treatment, period, and square. Pre-planned contrasts included CON vs. the average of the 3 FA treatments (**FAS**), the form of the FA supplement (70FB vs. 70CS), and the FA profile of the Ca-salt (70CS vs. 45CS). Compared with CON, FAS decreased nutrient intake, increased intakes of 16-carbon, 18-carbon, and total FA, and increased nutrient digestibility, and digestibility of 16-carbon, 18-carbon, total FA. There was no difference in nutrient intake detected for the form of the supplement, but 70FB increased DM and 18-carbon FA digestibility but decreased NDF and 16-carbon FA digestibility, with no effect on total FA digestibility, compared with 70CS. When considering the FA profile

of a Ca-salt, 70CS increased both DM and NDF intake but did not affect the digestibility of DM or NDF and decreased 16-carbon and total FA digestibility. Overall, FAS increased 3.5% fat corrected milk and milk fat yield but decreased milk protein yield. There were no differences observed for production responses when comparing the form of the supplement. When comparing the FA profile of a Ca-salt, 70CS increased milk fat yield but decreased yields of milk and milk lactose. In conclusion, FAS increased milk fat production through increases in FA digestibility and absorption. Additionally, there were differences observed for the form of the supplement for digestibility measures although there were no differences detected for production variables. However, the FA profile difference between the traditional Ca-salt and the 70% C16:0 + 20% *cis*-9 C18:1 impacted both nutrient digestibility and production responses, indicating that the FA profile of a FA supplement is more important than the form.

Introduction

Fatty acid (**FA**) supplementation to dairy cow diets is a common practice for improving the yields of milk and milk fat (Rabiee et al., 2012; dos Santos Neto et al., 2021a; b). Commercial FA supplements predominantly contain different ratios of palmitic (**C16:0**), stearic (**C18:0**), and oleic (***cis*-9 C18:1**) acids and are available in different physical dry formulations that make them easier to handle. Prilled free FA supplements are comprised of saturated FA (**SFA**), mainly C16:0 (≥ 80%) or a combination of C16:0 + C18:0 (~38% + ~45%; dos Santos Neto et al., 2021a). Development of Ca-salts provided for the ability to feed unsaturated FA (**UFA**) in a dry form and reduce the negative effects of UFA on rumen fermentation (Palmquist and Jenkins, 2017). Most Ca-salts are produced from palm FA containing mostly C16:0 (~46%) and *cis*-9 C18:1 (~38%), with lesser amounts of C18:0 (~4%) and linoleic acid (C18:2, ~8%; dos

Santos Neto et al., 2021b). Recent research has highlighted that different FA combinations have different effects on nutrient digestibility and production responses(de Souza et al., 2018a, 2019; Burch et al., 2021). It is crucial to consider when feeding FA supplements that cow performance could potentially be heavily impacted by the product formulation (i.e. Ca-salts vs. prills), the differences in FA profile between prilled SFA and Ca-salts, or both.

Two recent meta-analyses evaluated nutrient digestibility and production responses to supplementation of free SFA supplements (dos Santos Neto et al., 2021a) and Ca-salts of palm FA (dos Santos Neto et al., 2021b). A C16:0 enriched prilled FA increased yields of milk fat and ECM compared with a non-FA control treatment and a C16:0+C18:0 prill. The authors concluded that lactating dairy cows responded best to a SFA supplement containing more C16:0 and less C18:0, indicating that FA profile of the prilled fat is an important consideration. Supplementation of Ca-salts of palm FA decreased DMI but increased yields of milk, milk fat, and 3.5% fat corrected milk compared with a non-FA control diet. Our lab has focused on different combinations of FA in recent years, mixing multiple FA products differing in form and FA profile to achieve our desired ratios. We have shown that blending different commercial FA supplements to alter the FA profile of FA blends and treatments impacts production responses dependent on FA profile (de Souza et al., 2019; Burch et al., 2021; Bales et al., 2024). Within each FA blend, the proportions of the supplements used differed, resulting in some FA blends having a higher percentage of prilled SFA supplements or Ca-salt of palm FA. When blending a C16:0 enriched FA supplement and Ca-salt of palm FA at differing proportions to achieve different ratios of C16:0 + *cis*-9 C18:1, overall DMI was not affected by treatment (de Souza et al., 2019; Western et al., 2020b) but milk production responses differed due to interactions between FA profile of the blend and production level of the cow. In another FA blend study,

DMI was not impacted by overall FA supplementation, but DMI and milk fat yield were increased as the proportion of C16:0, at the expense of C18:0, increased in FA blends (Bales et al., 2024).

Considering the results across the aforementioned experiments and with continued research utilizing FA supplements and the production of new fat products, it is important to examine if the form of the supplement (prilled FA vs. Ca-salt of palm FA) has any influence on dairy cow performance or if the FA profile of a supplement is the main driver that impacts production responses. With our continued FA blend work, there have been inquiries if there would be differences in milk production responses between a FA blend and a Ca-salt with the same ratio of C16:0 + *cis*-9 C18:1. To our knowledge, there is only one published study evaluating FA profile versus the form of a FA supplement (Shpirer et al., 2023), but they did not utilize commercial products to achieve their desired blends of FA. Therefore, our objective was to determine the effects of form vs. FA profile of supplements on nutrient digestibility and milk production of mid-lactation dairy cows. We hypothesized that there would be no difference between a Ca-salt and FA blend with the same ratio FA, whereas we would observe differences between two Ca-salts with different FA profiles (70% C16:0 + 20% *cis*-9 C18:1 versus 45% C16:0 + 35% *cis*-9 C18:1).

Material and Methods

Design and Treatments

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University, East Lansing. Twenty mid-lactation, multiparous Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center were randomly assigned to a treatment sequence in a replicated 4 × 4 Latin square design

balanced for carryover effects in four 21-d periods. All animals received a common diet with no fat supplementation during a 14-d preliminary period to obtain baseline values. The baseline averages for all animals, with mean ± standard deviation, were 44.3 ± 3.0 kg of milk yield, 99 ± 23 d DIM, and 690 ± 67 kg of BW.

The treatments consisted of 1) control (**CON**; diet with no supplemental FA), 2) a FA blend utilizing a C16:0-enriched prill and a Ca-salt of palm FA to achieve a ratio of 70% C16:0 and 20% *cis*-9 C18:1 (**70FB**; Table 9), 3) a Ca-salt containing 70% C16:0 and 20% *cis*-9 C18:1 (**70CS**; Table 8) and 4) a traditional Ca-salt of palm FA containing 45% C16:0 and 35% *cis*-9 C18:1 (**45CS**; Table 8). The FA blend and Ca-salts were fed at 2.0% FA (DM basis) of the diet and replaced soyhulls from the control diet. All experimental diets were formulated to meet the nutrient requirements of the average cow (Table 10; NASEM, 2021). The DM concentration of forages was determined twice weekly, and diets adjusted when necessary. A base diet, containing corn silage, alfalfa silage, corn grain, soybean meal, mineral mixes, whole cottonseed, and soybean meal, were mixed in a wagon daily. Then, soyhulls, FA blends, and base diet were mixed in a tumble-mixer for each experimental diet. Cows were fed 115% expected intake at 8000 h daily. Feed access was blocked from 0600 to 8000 h for orts collection and offering of new feed. Cows were milked 3x/d and housed in individual tie-stalls throughout the experiment with water available ab libitum in each stall which were bedded with sawdust and cleaned twice daily.

Data and Sample Collection

Samples and production data were collected during the last 5 d of each treatment period (d 17-21). Samples of all diet ingredients (0.5 kg) and orts (12.5%) were collected daily and composited by cow/period for analysis. Milk yield was recorded, and two milk samples were

collected at each milking. One aliquot was collected in a sealed tube with preservative and stored at 4ºC for milk component analysis. The second aliquot was stored without preservative at -20ºC until analyzed for FA composition. Fecal samples were taken every 15 h, resulting in 8 samples/cow per period and stored in a sealed plastic cup at −20°C. Fecal samples were later dried and composited by cow per period for analysis. BW was measured 3 times per week following the afternoon milking with changes in BW determined according to (Boerman et al., 2015b). On the last day of each period, BCS was determined by three trained investigators on a 5-point scale in 0.25 increments (Wildman et al., 1982).

Sample Analysis

Dietary ingredients, orts and feces were dried at 55ºC in a forced-air oven for 72 h for DM determination. Dried samples were ground in a Wiley mill (1 mm screen; Arthur H. Thomas, Philadelphia, PA). Samples of feed ingredients, orts and feces were analyzed for NDF, indigestible NDF (iNDF), starch, and CP according to Boerman et al. (2017) and FA according to Bales et al. (2024). Milk samples were analyzed for fat, true protein, and lactose concentrations by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan Dairy Herd Improvement Association (Central Star DHI, Grand Ledge, MI). Yields of milk components, 3.5% FCM, and ECM were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each period. Milk samples used for analysis of FA composition were composited based on milk fat yield (d 17-21 per period). Milk lipids were extracted, FAME prepared, and analyzed by gas chromatography as described previously (Lock et al., 2013). Yields of individual FA (g/d) in milk fat was calculated using milk fat yield and FA concentration to determine yield on a mass basis using the molecular

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

Statistical Analysis

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

$$Y_{ijkl} = \mu + C_i(S_j) + P_k + T_l + S_j + e_{ijkl},$$

Where Y_{ijkl} = the dependent variable, μ = the overall mean, $C_i(S_j)$ = random effect of cow nested within square (i = 1 to 4), P_k = fixed effect of period (k = 1 to 4), T_l = fixed effect of treatment (l = 1 to 4), S_j = fixed effect of square (j = 1 to 5), and e_{ijkl} = residual error. The interaction of $P_k \times T_l$ was tested, but results were not significant ($P > 0.20$) and were removed from the model. Main effects were declared significant at $P \leq 0.05$ and tendencies $P \leq 0.10$. Three contrasts were evaluated: (1) the overall effect of FA supplements {CON vs. the average of the FA treatments (**FAS**) [1/3 (70FB + 70CS + 45CS)]}, (2) the effect of form of the FA supplement (70FB vs. 70CS), and (3) the effect of FA profile of the Ca-salt (70CS vs. 45CS).

Results

Nutrient Intake and Digestibility

Overall FAS decreased DM (P=0.02; Table 11) and NDF intake (P<0.01), and increased intake of 16-carbon, 18-carbon, and total FA (all P<0.01), compared with CON. Compared with CON, FAS increased DM and NDF digestibility (P<0.01), increased 18-carbon (P<0.001) and

total FA digestibility (P<0.01), tended to increase 16-carbon FA digestibility (P=0.07), and increased the amount of absorbed 16-carbon, 18-carbon, and total FA (all P<0.001).

For FA form, there was no difference between 70FB and 70CS for intakes of DM, NDF, 18-carbon, and total FA (all P ≥0.15; Table 11) but 70FB decreased 16-carbon intake by 20 g/d (P<0.01). Compared with 70CS, 70FB increased digestibility of DM, NDF, and 18-carbon FA (all P<0.01), decreased 16-carbon FA digestibility (P<0.01), but had no effect on total FA digestibility (P=0.36). 70FB decreased absorbed 16-carbon FA (P<0.001) and increased absorbed 18-carbon FA (P<0.01), but there was no difference in absorbed total FA (P=0.55) compared with 70CS.

For FA profile, compared with 45CS, 70CS increased intake of DM (P=0.02l; Table 11), NDF (P=0.01), 16-carbon FA (P<0.01), and total FA (P<0.01), and decreased 18-carbon FA intake (P<0.01). 70CS decreased 16-carbon (P<0.01) and total FA digestibility (P=0.01) compared with 45CS, but there were no differences between treatments for digestibility of DM, NDF, and 18-carbon FA (all P ≥0.12). Compared with 45CS, 70CS increased the absorption of 16-carbon FA but decreased absorption of 18-carbon FA (both P<0.001), but there was no difference in absorption of total FA (P=0.90).

Production Responses

Compared with CON, FAS increased yields of 3.5% FCM (P =0.01; Table 12) and milk fat (P <0.01) and feed efficiency (ECM/DMI; P <0.01) but decreased milk protein yield (P <0.001), BW change (P =0.04), BCS (P =0.03), and BCS change (P <0.01). FAS did not affect yields of milk, ECM, or milk lactose, or BW (all P ≥ 0.33) compared with CON.

For the form of the FA supplements, there were no differences between 70FB and 70CS for yields of milk and milk components, ECM/DMI, BW, BW change, BCS, or BCS change (all $P \geq 0.13$; Table 12).

For FA profile, compared with 45CS, 70CS decreased yields of milk and milk lactose (both $P < 0.01$; Table 12) and tended to decrease milk protein yield ($P = 0.08$), but increased milk fat yield ($P = 0.04$). There was no difference between 70CS and 45CS for yields of 3.5% FCM and ECM, ECM/DMI, BW, BW change, BCS, or BCS change (all $P \geq 0.18$).

Milk FA Content and Yield

Milk FA are derived from two sources: <16 carbon FA (de novo) from de novo synthesis in the mammary gland and >16 carbon FA (preformed) originating from extraction from plasma. Mixed source 16-carbon FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis in the mammary gland and extraction from plasma.

Overall, FAS decreased the yield of de novo milk FA and increased yields of mixed and preformed milk FA (all P <0.001; Table 13), compared with CON. For individual milk FA, FAS increased the yields of C4:0, C16:0, C18:0, *trans*-6 to 8 C18:1, *trans*-9 C18:1, *trans*-10 C18:1, *trans*-11 C18:1, *cis*-9 C18:1, and decreased C8:0-C14:0 and C18:3 (all P<0.01) compared with CON. On a content basis, FAS decreased de novo milk FA and increased both mixed and preformed milk FA (all P <0.001; Table 4.7) when compared with CON.

For FA form, 70FB decreased yield of mixed FA (P<0.001; Table 13) but there was no difference in the yields of de novo or preformed FA compared with 70CS (both P >0.50). For individual milk FA, compared with 70CS, 70FB decreased the yields of C16:0 (P<0.001) and *cis*-9 C16:1 (P=0.01), and tended to decrease the yield of C18:0 (P=0.09), but there was no difference between treatments for the yield of *cis*-9 C18:1 (P=0.88). 70FB increased de novo and

preformed FA content (P<0.01; Table 14) but decreased mixed milk FA content (P<0.001) compared with 70CS.

For FA profile, there was no effect of treatment for the yield of de novo FA (P=0.20; Table 13), but compared with 45CS, 70CS increased the yields of mixed but decreased preformed FA (both P<0.001). For individual milk FA, 70CS increased yields of C12:0 and C14:0 (both P=0.01), C16:0 and *cis*-9 C16:1 (both P<0.001), and decreased C18:0, *trans*-6 to 8 C18:1, *trans*-9 C18:1, *trans*-10 C18:1, *trans*-11 C18:1, *cis*-9 C18:1 (all P<0.001), and C18:2 (P<0.01) compared with 45CS. On a content basis, 70CS tended to increase de novo (P=0.09; Table 14), increased mixed (P<0.001), and decreased preformed milk FA (P<0.001) compared with 45CS.

Discussion

With recent advancements in our understanding that the FA profile of fat supplements alters digestibility, metabolism, and production responses of dairy cows, it is important to examine if the form of the supplement also affects cow performance. Therefore, we designed a study to evaluate the effects of FA profile and form (prilled fat vs. Ca-salt) of a supplement on nutrient digestibility and production responses of mid-lactation dairy cows. We utilized two fat supplements, a C16:0-enriched prill and a Ca-salt of palm FA, blended together to achieve a ratio of 70% C16:0 + 20% *cis*-9 C18:1 (70FB) and compared this versus a Ca-salt formulated to contain 70% C16:0 + 20% *cis*-9 C18:1 (70CS) in order to test if the form of a supplement impacts responses. To test the FA profile of a supplement, we compared 2 Ca-salts, the 70CS versus the Ca-salt of palm FA containing 45% C16:0 + 35% *cis*-9 C18:1 (45CS). We hypothesized that there would be no difference between the form of the fat supplements, whereas

we would observe different production responses with the different FA profiles of the Ca-salts. To our knowledge, there is only one other study that has explored differences between FA profile and form, but they used fat supplements containing 80% C16:0 + 10% *cis*-9 C18:1 that were pre-manufactured and not blended to achieve their specific ratios (Shpirer et al., 2023).

We have focused on improving our understanding of how the FA profile of FA supplemented diets impacts cow performance. We have blended FA prills and Ca-salt supplements to create FA blends with our desired FA compositions (e.g. de Souza et al., 2019; Burch et al., 2021; Bales et al., 2023). Due to the limited inclusion of UFA in FA prills, blending of prills with Ca-salts allows us to manipulate both SFA and UFA content of supplemental FA. In the current study, nutrient intake was not different between 70FB and 70CS, similar to the results observed by Shpirer et al. (2023). The lack of difference in nutrient intake is expected, as both treatments supplied similar amounts of *cis*-9 C18:1, thus potential hypophagic effect of *cis*-9 C18:1 affecting secretion of gut peptides associated with satiety would likely be similar (i.e., cholecystokinin and glucagon-like peptide 1; Bradford et al., 2008). Harvatine and Allen (2005) observed that a Ca-salt of palm FA distillate (**PFAD**) reduced DMI compared with a SFA prill, but the FA profile of these supplements differed. Between the results in our current study and those observed Shpirer et al. (2023), DMI will likely be similar across FA supplements that contain similar FA profiles but in different forms.

Contrary to our expectations, the 70FB treatment increased both DM and NDF digestibility (2.6% and 2.8%, respectively) compared with 70CS, suggesting that the form of the supplement influenced nutrient digestibility. Similarly, Shpirer et al. (2023) compared a Ca-salt versus a free FA prill, both containing 80% C16:0 + 10% *cis*-9 C18:1, and observed differences due to the form of the product, although the Ca-salt increased nutrient digestibility compared

with the prill. One potential reason for differences in nutrient digestibility between their study results and ours could be related to the form of the product containing *cis*-9 C18:1. In our 70FB, the *cis*-9 C18:1 was within a Ca-salt, whereas the free FA prill used by Shpirer et al. (2023) likely provided less protection to the rumen microbes against the effects of *cis*-9 C18:1, as it is known that UFA negatively impact microbial growth rates (Maia et al., 2010) and UFA in the form of Ca-salts reduce the negative effects of UFA on fiber digestion (Jenkins and Palmquist, 1984). In contrast, the difference we observed between 70FB and 70CS may be due to dissociation of the prill in the FA blend releasing C16:0 in the rumen. Palmitic acid can positively influence fibrolytic bacteria, as observed with in vitro work with C16:0 supplementation (Sears et al., 2023) and has been observed extensively in vivo (dos Santos Neto et al., 2021a).

Additionally, FA digestibility was affected by the form of the supplement. Shpirer et al. (2023) also observed a similar decrease in 16-carbon FA digestibility with a free FA prill, but in contrast to our results, they also reported lower 18-carbon and total FA digestibility with a free FA prill compared with a Ca-salt containing the same FA profile (80% C16:0 + 10% *cis*-9 C18:1). While their results could be due the lower amount of *cis*-9 C18:1 in their products (10%), as it is known that *cis*-9 C18:1 increases FA digestibility (de Souza et al., 2019; Prom and Lock, 2021), they may also be due in part to the discrepancies in the FA methodology steps regarding saponification, esterification, hydrolysis, and methylation used for FA analysis of feed and fecal samples, which may have confounded digestibility results. Oleic acid has been shown to improve FA digestibility in feeding trials (de Souza et al., 2018a; Burch et al., 2021; Prom and Lock, 2021) as well as in abomasal infusion studies (Prom et al., 2021, 2022). The improvements in FA digestibility are most likely due to *cis*-9 C18:1 increasing micellar

formation (Freeman, 1969) that would allow for greater absorption of not only *cis*-9 C18:1, but of all FA reaching the small intestine. Our 70FB treatment was a blend of 53% prill and 47% Ca-salt, thus the *cis*-9 C18:1 within the Ca-salt portion may have escaped biohydrogenation to a greater extent and aided total FA digestibility (Boerman et al., 2015a). Although we observed minor differences in FA digestibility, total FA absorption was not different between 70FB and 70CS.

Although we observed differences in nutrient digestibility, we did not observe any differences in production responses due to the form of the supplement. While there was no difference in milk fat yield between forms, 70CS increased mixed milk FA yield by 39 g/d compared with 70FB, likely due to the increase in 16-carbon FA absorption observed with 70CS. Due to the increase in NDF digestibility observed with the 70FB treatment, it might be expected for de novo milk FA yield to also increase with 70FB as a result of acetate being produced from fiber fermentation (Piantoni and VandeHaar, 2023), but we did not observe an increase in de novo milk FA yield. In contrast, Shpirer et al. (2023) reported production differences between a Ca-salt and a free FA prill, both containing 80% C16:0 + 10% *cis*-9 C18:1, with the prill outperforming the Ca-salt. As noted by the authors, these results are difficult to interpret as their free FA prill treatment reduced nutrient digestion but increased production and had no impact on BW; thus there is a discrepancy in energy partitioning to explain their results.

In multiple studies, we have shown that the FA profile of FA blends can impact nutrient intake (de Souza et al., 2018a), nutrient digestibility (Burch et al., 2021; Prom and Lock, 2021), and yields of milk and milk components of cows at differing production levels (de Souza et al., 2019; Western et al., 2020b; Burch et al., 2021). All these studies utilized FA blends to alter the FA profile of supplements, resulting in the proportion of FA prills and Ca-salts differing among

treatments. In our study, we were able to evaluate two Ca-salts that contained different ratios of C16:0 + *cis*-9 C18:1 and observed that FA profile of the Ca-salt impacted DMI. This is an important observation as our current results indicate that the form of the product does not impact DMI whereas the FA profile of the supplement can impact DMI responses. This observation was due to the 45CS treatment reducing intake compared with 70CS, most likely due to the greater content of UFA within the supplements (35% vs. 20% *cis*-9 C18:1, respectively), as diets containing Ca-salts of palm FA have been reported to reduce DMI (dos Santos Neto et al., 2021b) and stimulate gut peptides that reduce satiety (Bradford et al., 2008). In contrast, DMI was not affected by different ratios of C16:0 + *cis*-9 C18:1 DMI when comparing two Ca-salts (Shpirer et al., 2023) or comparing FA blends that differ in proportion of prills and Ca-salts (de Souza et al., 2019; Western et al., 2020b), although all studies mentioned had lower levels of *cis*-9 C18:1 than a traditional Ca-salt of PFAD. The contrary results could be impacted by differences in energy demands between cows across the studies, as well as interactions with other dietary ingredients (NASEM, 2021). Since we did not observe the form of the supplements to affect DMI, we propose that the FA profile has a greater impact on nutrient intake.

Research comparing fat supplements have suggested that DM and NDF digestibility are influenced by FA profile, especially when evaluating fat supplements high in SFA. Supplements higher in C16:0, compared with C18:0, increase DM and NDF digestibility (Western et al., 2020a; dos Santos Neto et al., 2021a) whereas supplementation with varying ratios of C16:0 + *cis*-9 C18:1 did not affect digestibility of DM or NDF (de Souza et al., 2019; Western et al., 2020b). Although Burch et al. (2021) observed differences in nutrient digestion between a ratio of 60% C16:0 + 30% *cis*-9 C18:1 and 60% C16:0 + 30% C18:0, the form of the FA blends differed, as one contained prills and Ca-salts while the other was mostly prilled FA, and could

have influenced results. We utilized two different Ca-salts with different ratios of C16:0 + *cis*-9 C18:1 and detected no differences for DM and NDF digestibility, similar to results observed by de Souza et al. (2019) and Western et al. (2020a). FA digestibility was impacted by FA profile of the Ca-salt treatments, which would be expected due to the increased level of *cis*-9 C18:1 in 45CS aiding FA digestibility (Prom et al., 2021).

Milk production responses were affected by FA profile, consistent with previous research (de Souza et al., 2018a, 2019; Bales et al., 2024). The increase in milk fat yield and content observed for 70CS compared with 45CS was due to the higher level of C16:0 in 70CS, as increased amounts of C16:0, at the expense of *cis*-9 C18:1, increased milk fat production in cows averaging ~45 kg/d of milk yield (de Souza et al., 2019; Western et al., 2020a). The 45CS treatment increased yields of milk and milk lactose but decreased de novo milk FA yield, potentially due to glucose being spared for use for lactose synthesis, resulting in a reduction of glucose available for NADPH for de novo FA synthesis (Cant et al., 1993; Palmquist, 2006).

The objective of our study was to compare fat supplements of different FA profiles and forms, but also included a control treatment in order to compare all FA treatments to a diet without FA supplementation. Although this comparison was not our focus, overall DMI was reduced, driven by the 45CS treatment, which has been observed previously with FA supplements higher in UFA (dos Santos Neto et al., 2021b). Overall, nutrient digestibility was increased by FAS, as observed in previous experiments utilizing both prilled fat and Ca-salts of palm FA (dos Santos Neto et al., 2021a; b). Considering that overall FAS increased dietary content of *cis*-9 C18:1, it would be expected for FAS to increase FA digestibility (Prom and Lock, 2021; Prom et al., 2021) through improving micelle formation (Freeman, 1969) as UFA have a higher digestibility than SFA (Boerman et al., 2015a). The lack of differences in ECM

was unexpected for overall FAS, as FA-supplemented diets have been observed to increase milk production yields compared with non-FA control treatments (dos Santos Neto et al., 2021a; b). The ~8% DM inclusion of whole cottonseed potentially influenced milk yield results by helping meet nutrient requirements for milk synthesis. We recently evaluated inclusion rates of WCS and observed that the 8% and 16% DM inclusion had similar milk yield responses (see Chapter 5). Due to this, there was likely reduced potential for increased milk yields in our current study compared to observations in experiments evaluating FA supplementation in diets that had low (<5% DM) inclusion of whole cottonseed and lower total FA content (Burch et al., 2021; Bales et al., 2024). This stresses the importance of how basal diet FA content can influence milk production responses. Overall, FAS increased yields of milk fat and 3.5% FCM and lower body weight gain compared with CON, demonstrating FAS partitioned more energy to milk fat synthesis.

Even though our periods were 21-d in length, we did detect differences for the effect of form, FA profile, as well as differences between CON and FAS. A longer-term study would be needed to observe if DMI, nutrient digestibility, and milk production responses would change as cows increase in DIM and change in energy requirements and reproduction status. Although the form of the supplement had minor effects on nutrient digestibility, production responses were not different, thus we are confident that results of our previous FA blend studies are reflective of the FA profile and not the form of the blend (de Souza et al., 2019; Burch et al., 2021; Bales et al., 2024). Our study is not the first to evaluate FA profile vs. form of supplements, however, our study differs from Shpirer et al. (2023) as they manufactured a free FA prill to contain 80% C16:0 + 10% *cis-9* C18:1 while we blended commercially available FA supplements for our 70FB treatment. This decision was based on melting points, as *cis-9* C18:1 has a higher melting

point than C16:0 (13° versus 62°C, respectively), thus manufacturing techniques for FA supplements that contain higher levels of UFA need to be considered for ease of handling (NASEM, 2021) and the high melting point of *cis*-9 C18:1 does not allow for levels above 10% using current prill technologies. Additionally, manufacturing *cis*-9 C18:1 into a Ca-salt, instead of a free FA prill, can help reduce potential negative effects of UFA on rumen fermentation (Palmquist and Jenkins, 2017). Furthermore, results from Shpirer et al. (2023) should be interpreted with caution. As stated by the authors, their digestibility results did not reflect energy partitioned to milk production, possibly due to methodology issues, as discussed earlier. Additionally, ether extract % DM for diets are lower than values for FA % DM, and since ether extract contains nonnutritive waxes and pigments that are extracted in ether (Sukhija and Palmquist, 1988), it would be expected for ether extract to be higher than FA content. If ether extract content was used for FA digestibility, digestion results would not be accurately calculated. Additionally, there were unexplained changes in diet ingredient inclusion (i.e. protein sources and whole cottonseed differences) and large differences for the individual FA composition of treatment diets that could influence treatment differences.

Conclusion

Our study was designed to evaluate if nutrient digestibility and milk production responses are affected by the form of a fat supplement, the FA profile of the fat supplement, or both. Even though we observed minor differences for digestibility variables, there were no differences for production responses between a 70% C16:0 + 20% *cis*-9 C18:1 FA blend and a Ca-salt formulated to also contain 70% C16:0 + 20% *cis*-9 C18:1. Both FA digestibility and milk production responses were affected by the FA profile of the two Ca-salt treatments, further

indicating that the FA profile of a fat supplement is more important than the form of the fat supplement. Results from our study can have immediate commercial application, and will allow manufactures, nutritionists, and producers to make more informed decisions on specific fat supplements. Nutritionists and dairy producers can supplement diets with either a single form product, or blend multiple products for a specific ratio, and still achieve desired production responses.

CHAPTER 5: EFFECT OF INCREASING DIETARY INCLUSION OF WHOLE COTTONSEED ON NUTRIENT DIGESTIBILITY AND MILK PRODUCTION OF HIGH-PRODUCING DAIRY COWS

Abstract

We determined the effects of increasing dietary inclusion of whole cottonseed (**WCS**) on nutrient digestibility and milk production responses of high-producing dairy cows. Twenty-four multiparous Holstein cows (mean ± standard deviation; 52.7 ± 2.63 kg/d of milk; 104 ± 23 DIM) were randomly assigned to treatment sequences in a replicated 4 × 4 Latin square design with 21-d periods. Treatments were increasing doses of WCS at 0, 8, 16, and 24% dry matter (**DM**), with WCS replacing soybean meal and hulls to maintain similar diet nutrient composition (%DM) of neutral detergent fiber (**NDF**; 32%), forage NDF (21%), starch (27%), and crude protein (17%). Total FA content of each treatment was 1.70, 2.96, 4.20, and 5.40 %DM, respectively. Three preplanned contrasts were used to test the linear, quadratic, and cubic effects of increasing dietary WCS. Increasing dietary WCS from 0 to 24% DM quadratically influenced intakes of DM and NDF, with the highest value being for the 8% WCS, and intakes of 16- and 18-carbon, and total FA, with maximum values obtained at 24% WCS. Increasing dietary WCS affected digestibility of DM (cubic) and NDF (quadratic), with the lowest values being for the 8% WCS, and increased 16-carbon digestibility (quadratic) but decreased digestibility of 18-carbon and total FA (both quadratic), with highest and lowest values for the 24% WCS, respectively. Increasing dietary WCS quadratically increased absorbed 16- and 18-carbon, and total FA, with maximum values obtained for 24% WCS. Increasing dietary WCS increased (quadratic) yields of milk, milk fat, milk protein, milk lactose, 3.5% fat corrected milk, and energy corrected milk, and linearly increased body weight gain. The source of milk FA was affected by dietary WCS,

with a quadratic decrease in the yield of de novo and mixed milk FA and a quadratic increase in preformed milk FA. Increasing dietary WCS linearly increased *trans*-10 C18:1 content. As dietary WCS increased, plasma insulin linearly decreased, and plasma gossypol levels linearly increased. Despite the decrease in total FA digestibility, increasing dietary WCS from 0 to 24% DM increased FA absorption. Increasing dietary inclusion of WCS up to 16% DM increased milk production responses and DM intake. Under the current dietary conditions, high-producing dairy cows increased milk production responses from a diet containing 8-16% DM inclusion of WCS.

Introduction

The continuing rise in milk production of dairy cows increases nutrient and energy demands, thus exploring ways to increase nutrient supply is important. With rising world oil and biodiesel prices affecting fat supplement availability and cost, there is renewed interest in the use of oilseeds. Oilseeds are a readily available feedstuff that can increase the amount of dietary fatty acids (**FA**), and have often been found to increase milk fat production (Banks et al., 1976; Rabiee et al., 2012). Whole cottonseed (**WCS**) is a common by-product ingredient utilized in dairy cow diets and is unique because of its high content of FA and CP, as well as being a source of fiber (Coppock et al., 1987; Moreira et al., 2004). Dietary inclusion of WCS has increased milk fat content and the yields of milk fat and 3.5% FCM (Smith et al., 1981; Clark and Armentano, 1993; de Souza et al., 2018a). In previous research, WCS has often been included in basal diets at a single inclusion rate and was not the focus of the research project (Harvatine and Allen, 2006; Western et al., 2020a; Shpirer et al., 2023).

To our knowledge, there are very few studies evaluating increasing levels of WCS inclusion in the diet. Coppock et al. (1985) evaluated increasing dietary WCS at levels of 0, 15, and 30% DM, and found a linear decrease in DMI but an increase in ether extract and crude protein digestibility; however milk production responses were not evaluated. Smith et al. (1981) supplemented WCS at 0, 5, 15, and 25% DM to four cows averaging 21 kg/d of milk, and reported no effect on DMI or milk yield, but increased yields of milk fat and 4% FCM. Recently, Pierce et al. (2023) included WCS as 0, 3.4, 6.8, and 9.9% DM and observed a linear decrease in DMI but no impact on production responses in cows averaging ~ 40 kg/d. In a recent meta-regression, dos Santos Neto et al. (2022) evaluated the available literature with WCS inclusion of up to 17% DM and found that for every 1-percentage increase in inclusion level, yields of milk fat, milk protein, and ECM were increased. The authors noted that cows used in the studies in the meta-regression had a low level of milk production (average 29.5 kg/d). Although the addition of WCS to dairy cow diets is not a new practice, determining optimal inclusion levels for high producing dairy cattle is important. Therefore, the objective of our study was to evaluate the effects of feeding WCS at 0, 8, 16, and 24% DM on nutrient digestibility and milk production responses of high-producing dairy cows. We hypothesized that as dietary WCS increases, nutrient digestibility and production responses will increase. In order to keep nutrient composition of the diets similar, WCS replaced soybean meal and hulls in treatment diets, thus treatment diets contained similar contents of NDF, forage NDF, CP, and starch.

Material and Methods

Design and Treatments

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University, East Lansing. Twenty-four mid-lactation, multiparous Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center were randomly assigned to a treatment sequence in a replicated 4 × 4 Latin square design balanced for carryover effects in four 21-d periods. All animals received a common diet with no WCS during a 14-d preliminary period to obtain baseline values. The baseline average for all animals, with mean ± standard deviation, were 52.7 ± 2.63 kg/d of milk yield, 104 ± 22 DIM, and 706 ± 64 kg of BW.

The treatments consisted of increasing WCS inclusion at 0, 8, 16, and 24% of diet DM. Doses were chosen based on inclusion levels of basal diets in the literature and discussions with commercial nutritionists. The WCS replaced soybean meal and soybean hulls to keep NDF and CP levels similar across all diets. Soybean meal and soybean hulls are more readily available than cottonseed by-products in the Midwest, allowing for these treatment diets to have immediate application in the region. All experimental diets were formulated to meet the nutrient requirements of the average cow (Table 15; NASEM, 2021). The DM concentration of forages was determined twice weekly, and diets adjusted when necessary. Cows were fed 115% expected intake at 8000 h daily. Feed access was blocked from 0600 to 8000 h for orts collection and offering of new feed. Cows were milked 3x/d and housed in individual tie-stalls throughout the experiment with water available ab libitum in each stall which were bedded with sawdust and cleaned twice daily.

Data and Sample Collection

Samples and production data were collected during the last 5 d of each treatment period (d 17-21). Samples of all diet ingredients (0.5 kg) and orts (12.5%) were collected daily and composited by cow/period for analysis. Milk yield was recorded, and two milk samples were collected at each milking. One aliquot was collected in a sealed tube with preservative and stored at 4°C for milk component analysis. The second aliquot was stored without preservative at -20°C until analyzed for FA composition. Blood (~15 mL) samples were collected every 15 h, resulting in 8 samples/cow per period and stored on ice until centrifugation at 2,000 × *g* for 15 min at 4°C. Plasma was transferred into microcentrifuge tubes and stored at −20°C until composited by cow per period. One additional plasma aliquot following the afternoon milking on d 4 of sampling periods was immediately stored at −80°C for gossypol analysis. Fecal samples were taken every 15 h, resulting in 8 samples/cow per period and stored in a sealed plastic cup at −20°C. Fecal samples were later dried and composited by cow per period for analysis. BW was measured 3 times per week following the afternoon milking with changes in BW determined according to Boerman et al. (2015b). On the last day of each period, BCS was determined by three trained investigators on a 5-point scale in 0.25 increments (Wildman et al., 1982).

Sample Analysis

Dietary ingredients, orts, and feces were dried at 55°C in a forced-air oven for 72 h for DM determination. Dried samples were ground in a Wiley mill (1 mm screen; Arthur H. Thomas, Philadelphia, PA). Samples of feed ingredients, orts, and feces were analyzed for NDF, indigestible NDF (iNDF), starch, and CP according to Boerman et al. (2017) and FA analysis according to Bales et al. (2023). Milk samples were analyzed for fat, true protein, and lactose concentrations by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan

Dairy Herd Improvement Association (Central Star DHI, Grand Ledge, MI). Yields of milk components, FCM, and ECM were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each period. Milk samples used for analysis of FA composition were composited based on milk fat yield (d 17-21 per period). Milk lipids were extracted, FAME prepared, and analyzed by gas chromatography as described previously (Lock et al., 2013). Yields of individual FA (g/d) in milk fat was calculated using milk fat yield and FA concentration to determine yield on a mass basis using the molecular weight of each FA while correcting for glycerol content and other milk lipid classes (Piantoni et al., 2013). Plasma insulin samples were analyzed in duplicate and quantified using a bovine insulin ELISA using a solid phase 2-site enzyme immunoassay (Mercodia) at the Michigan State University Veterinary Diagnostic Laboratory (coefficient of variation of <5% between duplicates; Lansing, MI). Plasma gossypol were analyzed for (-) and (+) isomers and total gossypol and WCS analyzed for total gossypol using the method described by Bullock et al. (2010) based on AOCS Recommended Practice BA 8a-99 (AAOCS, 1998) at the USDA-ARS, Southern Regional Research Center (New Orleans, LA).

Statistical Analysis

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

$$Y_{ijkl} = \mu + C_i(S_j) + P_k + T_l + S_j + P_k \times T_l + e_{ijkl},$$

Where Y_{ijkl} = the dependent variable, μ = the overall mean, $C_i(S_j)$ = random effect of cow nested within square (i = 1 to 4), P_k = fixed effect of period (k = 1 to 4), T_l = fixed effect of treatment (l

= 1 to 4), S_j = fixed effect of square (j = 1 to 6), and e_{ijkl} = residual error. The interaction of $P_k \times T_l$ was initially included in the model and removed when not significant ($P > 0.20$; de Souza et al., 2020; Prom et al., 2021). Main effects were declared significant at $P \leq 0.05$ and tendencies $P \leq 0.10$. Three orthogonal contrasts were evaluated to test the linear, quadratic, and cubic effects of increasing WCS in the diet.

Results

Nutrient Composition, Intake and Digestibility

Intakes of WCS and gossypol were 2.82, 5.54, and 7.82 kg/d and 18.7, 36.8, and 52.0 g/d for the 8%, 16%, and 24% treatments, respectively.

Increasing dietary WCS from 0 to 24% DM quadratically influenced DMI and NDF intake (both $P<0.001$; Table 16), with the highest value being for the 8% WCS. Intakes of 16- and 18-carbon, and total FA were increased as dietary WCS level increased (quadratic; all $P<0.001$), with the highest value being for the 24% treatment. Total FA intake increased by 481, 896, and 1,208 g/d for 8, 16, and 24% WCS, respectively.

Increasing dietary WCS affected the digestibility of DM (cubic, $P = 0.03$; Table 16) and NDF (quadratic; $P<0.01$), with the lowest value being for 8% WCS. Increasing WCS increased 16-carbon digestibility (cubic; $P<0.001$), with the highest value being for 24% WCS. Increasing WCS decreased digestibility of 18-carbon and total FA (quadratic; both $P<0.001$), with the lowest digestibility of 18-carbon observed at the 24% WCS, and the lowest digestibility of total FA observed at 16 and 24% WCS. However, increasing WCS increased absorbed 16-carbon (86.6, 161, and 202 g/d, respectively; quadratic, $P<0.001$), 18-carbon (267, 449, and 609 g/d,

respectively; quadratic, $P<0.001$), and total FA (361, 624, and 862 g/d, respectively; quadratic, $P<0.001$), with the highest value being for 24% WCS.

Production Responses

Increasing dietary WCS from 0 to 24% DM increased yields of milk, 3.5% FCM, ECM, milk fat, milk protein, and milk lactose (all quadratic, $P < 0.001$; Table 17), with the highest values being for 8% and 16% WCS. Increasing WCS tended to increase milk fat content (quadratic; $P = 0.08$), with the highest value being for 16% WCS. Increasing WCS had a quadratic effect on milk protein yield ($P < 0.001$), with an increase at 8% WCS, followed by a decrease up to 24% WCS. Increasing WCS increased feed efficiency (ECM/DMI; quadratic, $P < 0.001$) and BW change (linear; $P = 0.02$), with the highest values being for 24% WCS. Increasing dietary WCS quadratically increased BCS change ($P < 0.01$) up to 16% WCS, followed by a decrease at 24% WCS. There was no effect of increasing WCS for BW ($P \geq 0.47$) or BCS ($P \geq 0.21$).

Milk FA Content and Yield

Milk FA are derived from two sources: <16 carbon FA (de novo) from de novo synthesis in the mammary gland and >16 carbon FA (preformed) originating from extraction from plasma. Mixed source 16-carbon FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis in the mammary gland and extraction from plasma.

Increasing dietary WCS from 0 to 24% DM decreased the yields of de novo and mixed milk FA (quadratic, both $P <0.001$; Table 18) and increased the yield of preformed milk FA (quadratic; $P <0.001$), with the lowest and highest values, respectively being for 24% WCS. For individual de novo milk FA, increasing WCS increased yields of C4:0 (quadratic; $P <0.001$), with the highest value being for 16% WCS, increased C6:0 and C8:0 (quadratic; both $P <0.001$),

with the highest value being for 8% WCS. and decreased C10:0-C14:0 (quadratic; all P <0.001), with the lowest value being for 24% WCS. The reduction in mixed milk FA was due to reductions in both C16:0 and *cis*-9 C16:1 (quadratic; both P <0.001), with the lowest value being for 24% WCS. For individual preformed milk FA, increasing WCS increased C18:0, *trans*-6 to 8 C18:1, *trans*-9 C18:1, *trans*-10 C18:1, *trans*-11 C18:1, *trans*-12 C18:1, *cis*-9 C18:1, *cis*-9, *cis*-12 C18:2 (quadratic; all P <0.001) and decreased *cis*-9, *cis*-12, *cis*-15 C18:3 (quadratic; P <0.001), with the highest and lowest values, respectively, being for 24% WCS. Milk FA content followed a similar pattern as milk FA yields with increasing dietary WCS (Table 19).

Plasma Metabolites

Increasing dietary WCS from 0 to 24% DM decreased plasma insulin (linear, P <0.01; Table 20), and increased plasma content of (-) gossypol, (+) gossypol, and total gossypol (linear; all P <0.001). Treatment by period interactions were observed for plasma gossypol, with a linear effect observed across periods (P <0.001; Figure 6). We observed that 0% WCS had the lowest gossypol level in period 1 compared with periods 2, 3, and 4 (P=0.03, P=0.07, and P=0.05, respectively) suggesting a carry-over effect of gossypol in plasma across periods, although there were no other period differences within WCS treatments.

Discussion

Due to large variations in dietary inclusion of WCS in the literature and increasing milk yields, the objective of our study was to evaluate the effects of increasing dietary WCS on nutrient digestibility and production responses of high-producing, mid-lactation dairy cows. We hypothesized that as WCS level increased, there would be an increase in nutrient digestibility and milk production responses. Previous studies examining dietary WCS inclusions levels have

done so in lower producing cows (~ 20 kg/d milk yield) and did not balance diets for starch and protein (Coppock et al., 1985) or forage content (Smith et al., 1981), thus results could be confounded due to differences in diet nutrient composition. Similar to Pierce et al. (2023), we balanced diets for NDF, forage NDF, starch, and CP; however, we chose to remove soybean hulls and soybean meal from diets when adding WCS rather than cottonseed hulls and meal. We acknowledge that soybean protein differs from the protein in WCS, but we utilized soybean hulls and soybean meal to adjust NDF and CP because they are more readily available and commonly used feed ingredients in the Midwest compared with cottonseed hulls and meals and represents a more realistic substitution in the dairy industry. In a recent study we replaced cottonseed hulls and meal with WCS (Bales et al., 2023) and observed increased milk production, similar to previous studies that replaced soybean hulls and soybean meal with WCS (Rico et al., 2017; de Souza et al., 2018a). Additionally, Abel-Caines et al. (1997) observed that a combination of soybeans and soybean hulls was an adequate replacement for WCS, thus we are comfortable that our decision to replace soybean hulls and soybean meal with WCS. Importantly, our diets reflect formulations that can have immediate application in the industry.

We observed an increase in DMI with WCS up to 8% DM and a decrease with the 24% level. Compared to diets without WCS, inclusion levels between 4.6 – 9.2% DM either increased (Johnson et al., 2002) or did not effect DMI (de Souza et al., 2018), while inclusion of 16.7-18.5% decreased DMI (Hawkins et al., 1985; Rico et al., 2017). When evaluating increasing dietary WCS, Coppock et al. (1985) and Pierce et al. (2023) observed linear decreases up to WCS 30 and ~10% DM, respectively, whereas Smith et al. (1981) reported no effect on DMI up to 25% DM. Although our dietary WCS levels were similar to those of Smith et al. (1981), there were differences in average intake of WCS as we had intakes of 0, 2.8, 5.5, and 7.8 kg/d of WCS

whereas their intakes were 0, 1.0, 2.9, and 5.1 kg/d of WCS, which could explain the difference in DMI results. In a recent meta-regression, WCS to 17% DM had no effect on DMI (dos Santos Neto et al., 2022), although DMI responses to WCS could also be due to other dietary factors and the concentration of other nutrients in the diet, which could explain the variation in results across studies (Arieli, 1998). Dietary factors impacting DMI with greater dietary WCS could be physically effective fiber, with NDF stimulating ruminal distention (Allen, 2000; NASEM, 2021) or related to dietary unsaturated FA signaling secretion of gut peptides associated with satiety (i.e., cholecystokinin and glucagon-like peptide 1; Bradford et al., 2008). Although gossypol intake increased with higher levels of WCS in our study, we do not believe gossypol intake directly impacted DMI, as Blauwiekel et al. (1997) compared diets with high and low levels of gossypol with WCS supplementation, and found no difference in DMI. Results from our study indicate that feeding WCS up to 16% DM had no effect on DMI of high-producing dairy cows. Digestibility of DM and NDF were opposite to DM and NDF intake, as 8% WCS lowered both digestibility of DM and NDF while 24% WCS increased nutrient digestibility. This result could be expected, as increased DMI negatively impacts nutrient digestibility (de Souza et al., 2018b). Coppock et al. (1985) observed similar results, as 15% WCS decreased fiber digestibility compared with 0 and 30% inclusion. Recently, a meta-regression reported that for every 1-percantage increase in dietary WCS, both DM and NDF digestibility were decreased (dos Santos et al., 2022) and a feeding trial up to ~10% DM WCS decreased nutrient digestion (Pierce et al., 2023). In contrast, Smith et al. (1981) reported no significant effect of increasing dietary WCS on fiber digestibility. The difference in digestibility could be due to multiple factors, including gut peptides associated with satiety affecting passage rates (Bradford et al., 2008; Piantoni et al., 2013) and diet fermentability (NASEM, 2021; Oba and Kammes-Main, 2023) that can effect

rumen retention time. In addition to the effects of DM and NDF intake, increasing intake of C16:0 could also have played a role in the complex digestibility dynamics, influencing the treatment responses we observed. Increasing WCS increased C16:0 content of our treatment diets, and in a recent in vitro experiment, Sears et al. (2023) observed that C16:0 increased bacterial growth, enhanced bacterial groups responsible for fiber digestion, and ultimately increased fiber digestion, similar to observations in vivo with C16:0 supplementation (dos Santos Neto et al., 2021a). Further research investigating the impact of dietary FA on rumen bacteria populations, along with different dietary ingredients, could be useful in determining differences observed in nutrient digestibility across studies.

Increasing dietary WCS increased 16-carbon FA digestibility but decreased 18-carbon and total FA digestibility, similar to results with up to 10% DM WCS (Pierce et al., 2023) and studies evaluating basal diets containing WCS (Rico et al., 2017; de Souza et al., 2018). In contrast with these results, Coppock et al. (1985) and Smith et al. (1981) reported an increase in ether extract digestibility with increasing dietary WCS up to 30% DM, though it is important to note that ether extract also contains nonnutritive waxes and pigments that are extracted in ether (Sukhija and Palmquist, 1988) and thus do not give an accurate estimation of FA digestibility. Some factors affecting FA digestibility are overall FA intake, total flow and profile of FA reaching the duodenum, and carbon chain length and saturation of FA (Boerman et al., 2015a). 16- and 18-carbon, and total FA intakes increased by 279, 905, and 1208 g/d, for the 24% DM treatment, which could explain our decrease in 18-carbon and total FA digestibility. This decrease in FA digestibility with increased FA intake has also been observed in experiments with FA supplements (e.g. Boerman et al., 2017; Prom and Lock, 2021). The major FA in WCS is *cis*-9*, cis*-12 C18:2, which is extensively converted to stearic acid (C18:0) during biohydrogenation

(Jenkins et al., 2008) thus increasing dietary WCS should increase C18:0 flow to the small intestine. As we observed, it is established that greater C18:0 flow to the small intestine decreases FA digestibility (Boerman et al., 2015a; Burch et al., 2021; Prom and Lock, 2021), and increasing supplementation of C18:0 was found to further decrease FA digestion (Boerman et al., 2017). There is some resistance of the seed coat of WCS to degradation in the rumen (Mohamed et al., 1988) thus it is likely that increasing dietary WCS increased the amount of unsaturated FA entering the small intestine that should aid FA digestibility (Boerman et al., 2015a). This concept is highlighted when comparing results from our current study with previous studies. In studies evaluating increasing dietary C18:0 using a 50% C18:0 FA blend and a C18:0-enriched prill, an increase in 18-carbon intake of ~ 250 and 750 g/d decreased 18-carbon FA digestibility by 20 and 30 percentage units, respectively (Boerman et al., 2017; Prom and Lock, 2021). Whereas in our study, when dietary WCS increased 18-carbon intake by ~ 360 and 900 g/d 18-carbon FA digestibility only decreased by 4 and 9 percentage units, respectively. While we observed decreases in FA digestibility with increasing dietary WCS inclusion, 16-, 18-carbon and total FA absorption increased.

Milk production responses were impacted by dietary WCS level, with 8 and 16% WCS increasing yields of milk, milk fat, milk lactose, ECM, and 3.5% FCM, and 8% WCS also increasing milk protein yield. The decrease in milk production yields for 24% WCS could be due to decreased DMI and potentially greater absorption of biohydrogenation intermediates that may partition more energy towards body reserves (Jenkins et al., 2008). Although both 8 and 16% WCS had similar milk production, 16% WCS doubled body weight gain per d, thus indicating differences in energy partitioning at higher inclusion levels. In general, the use of oilseeds to increase dietary FA content results in increased yields of milk and milk fat (Rabiee et al., 2012),

and increasing dietary WCS has been found to linearly increase milk fat production (Smith et al., 1981; dos Santos Neto et al., 2022). There has been some variation in results for milk production responses with WCS inclusion, as Rico et al. (2017) reported no difference in production responses with 16.7% WCS inclusion while de Souza et al. (2018) observed a decrease in milk yield but an increase in milk fat yield with 8.6% WCS, compared to soyhulls. While our 8% WCS increased yields of milk and ECM, Pierce et al. (2023) did not observe an impact on production responses up to ~10% dietary WCS, although the lack of response could be due to the low incremental inclusion rates. Despite our 8% WCS increasing milk protein yield, the 16 and 24% levels resulted in a decrease. Our diets were formulated to be isonitrogenous, so the variation in milk protein when feeding WCS above 8% could also be related to ruminal feed-protein degradation and microbial protein synthesis (Arieli 1998) and dietary lipids depressing milk protein synthesis (DePeters and Cant, 1992) and DMI. Increasing dietary WCS can improve milk production responses, although effects are dependent on feeding rate, and further research is needed to determine how to consistently increase milk protein production with increasing dietary WCS inclusion.

Milk fat yields for 8% and 16% WCS were similar (1.98 and 1.99 kg/d, respectively), although the composition of milk fat differed. When evaluating milk FA sources, as we increased dietary WCS, yields of de novo and mixed milk FA decreased whereas preformed milk FA increased, resulting in different proportions of de novo and preformed milk FA contributing to milk fat across treatments. A meta-analysis evaluating the interdependence between the yields of 18-carbon and de novo milk FA in milk fat, proposed that milk fat synthesis in the mammary gland is dependent upon the simultaneous supply of both sources of milk FA (Glasser et al., 2008b). Additionally, when 18-carbon FA are supplemented in diets, there appears to be a

substitution effect where de novo milk FA are compensated for by an increase in preformed milk FA (Burch et al., 2021; dos Santos Neto et al., 2021b; Bales et al., 2023). The increase in milk fat yield for 8% WCS was due to an increase in preformed milk FA while maintaining de novo and mixed milk FA constant compared to 0%, similar to results observed by de Souza et al. (2018). In contrast, 16% WCS increased milk fat yield due to the substitution of preformed milk FA for de novo milk FA. Although 16% WCS increased milk fat yield, it also increased the yields of many *trans*-18:1 FA, including *trans*-10 18:1, a marker for altered biohydrogenation pathways that can cause milk fat depression (Lock et al., 2007). Thus, the increase in *trans*-10 18:1 and increased BW change are indicative of changes in the biohydrogenation pathways (Jenkins et al., 2008) due to the higher load of unsaturated FA provided by the WCS overcoming normal rumen biohydrogenation capacity. Although we did not detect *trans*-10, *cis*-12 conjugated linoleic acid in any diet, compared to 8 and 16% WCS the 24% WCS diet resulted in the highest *trans*-10 18:1 yield and content in milk fat, a decrease in yields of milk fat and de novo milk FA, and the greatest BW change, indicating that at the highest inclusion level, normal biohydrogenation pathways were altered. This suggests the potential for a mild milk fat depression situation where energy was repartitioned toward body reserves (Bauman et al., 2011; de Souza et al., 2018a), as 16% and 24% WCS increased body weight gain by 0.25 kg/d.

Gossypol is a toxin in the pigment glands of WCS seeds and has been shown to be detrimental to fertility of bulls (Chenoweth et al., 2000) and there is some evidence that gossypol could negatively impact fertility of dairy cows and the development of embryos (Santos et al., 2003). Although our study was not designed to study the impacts of gossypol on health and reproductive performance, we analyzed plasma for (+) and (-) isomers, and total gossypol concentrations to evaluate the effects of our WCS inclusion levels on plasma gossypol levels. A

proposed upper safe limit of total plasma gossypol was proposed to be 5.0 μg/ml (Mena et al., 2001), and increasing dietary WCS in our study increased total plasma gossypol up to 5.87 μg/ml, although only the 24% WCS treatment reached above this suggested limit. In both short and long-term studies, increased plasma gossypol levels did not negatively affect milk production responses (Mena et al., 2001; Santos et al., 2002; Prieto et al., 2003), despite Mena et al. (2001) observing elevated erythrocyte fragility in a short-term study. We did observe a treatment by period interaction, indicating a carry-over effect for plasma gossypol. Reports in white-tail deer suggest 35 days without WCS will lower plasma gossypol to <1 μg/ml (Bullock et al., 2010) and 2 wk of a gossypol-free diet fed to dairy cows lowered plasma gossypol from 0.27 to 0.07 μg/ml (Mena et al., 2001), demonstrating that ruminants have the ability to metabolize and excrete this compound. We also analyzed plasma insulin levels, and observed that increasing dietary WCS linearly decreased plasma insulin, potentially due to the reduction of DMI and the increased intake of unsaturated FA (Relling and Reynolds, 2007). In contrast, Mohamed et al. (1988) reported a decrease in DMI without an effect on plasma insulin with a diet containing WCS. Insulin is an antilipolytic hormone, and higher insulin concentrations can increase lipogenesis in adipose tissue (Vernon, 2005), although that is not reflective in our study as BW change increased with higher dietary inclusion of WCS, suggesting that other factors, such as biohydrogenation intermediates, are contributing to the increased BW gain observed in 16 and 24% WCS.

The objective of our study was to examine the effects of increased levels of WCS in diets of high producing dairy cows. Diets were designed to have similar NDF, forage NDF, CP, and starch content, but an increased FA content that resulted in non-isoenergetic treatments. Therefore, results should be attributed to the nutrients supplied by the WCS. We acknowledge

that the utilization of soybean hulls and soybean meal to balance NDF and CP across the diets resulted in different types of non-forage fiber and protein sources that could have affected rumen fermentation and digestion. As stated previously, our choice to use soybean by-products rather than cotton by-products was because these ingredients are more representative of Midwest dairy cow diets, thus our results will have immediate application in the field. Importantly, we were interested in the effects of WCS and not just cottonseed oil. Abel-Caines et al. (1997) fed diets containing either WCS or whole soybean and soybean hulls, with formulations containing similar NDF, forage NDF, CP and starch sources. There was no difference between treatments for DMI and yields of milk fat and protein and the authors concluded that the soybean products were an effective replacement for WCS, and vice versa. Thus, we do not believe that results from our study were influenced by using soybean meal and soybean hulls and rather that WCS inclusion altered production responses. Although we are not the first to design an experiment to evaluate increasing dietary WCS inclusion to mid-lactation animals, we were able to test the upper limits of WCS inclusion in diets of today's high-producing dairy cows. Our study design was similar to that of Pierce et al. (2023), however, there are some differences between these studies that can explain differences in production responses. First, our lowest dietary WCS inclusion was similar to their highest WCS level (8.11 vs. 9.9%, respectively), and although our dietary NDF% was similar, we included less alfalfa silage in our diets (~ 9.6 vs 16.4% DM, respectively) and consequently included more non-forage fiber sources to replace WCS. Second, we utilized only multiparous cows that averaged ~10 kg/d greater milk yield than the multiparous cows used by Pierce et al. (2023). Therefore, the differences in production responses may be explained by production level of the cows, incremental differences in WCS inclusion levels, and diet formulations.

Conclusion

Our study was designed to determine dietary levels of WCS that improve performance of high-producing, mid-lactation dairy cows. Although our study is not the first to evaluate increasing dietary WCS, it is the first dose response study in high producing dairy cows (≥ 50 kg/d of milk) to test the upper limits of possible WCS inclusion. We observed that nutrient intake and digestibility was affected by dietary WCS, with the greatest intake for 8% WCS while 24% WCS decreased nutrient intake, whereas nutrient digestibility was lowered with 8% WCS but increased up to 24%. Increasing dietary WCS increased FA intakes, lowered total FA digestibility, and increased FA absorption available for utilization. Milk production responses were impacted by WCS inclusion level, with 8 and 16% WCS increasing yields of milk, milk fat, 3.5% FCM, and ECM, although the increases observed for milk fat differed by milk FA sources across treatments. Although milk production responses were similar between 8 and 16% WCS, the 16% inclusion doubled body weight gain. Additionally, higher levels of dietary WCS could potentially further increase milk fat yield if the reduction in de novo milk FA could be prevented. Overall cow performance was greatest for 8 and 16% inclusion and reduced with 24% inclusion of WCS.

CHAPTER 6: INCREASING DIETARY INCLUSION OF HIGH OLEIC ACID SOYBEANS INCREASES MILK PRODUCTION OF HIGH-PRODUCING DAIRY COWS

Abstract

Recent research has highlighted the importance of dietary fatty acid profile of fatty acid supplements on production responses of high-producing dairy cows. Conventional soybeans contain ~15% oleic acid and ~50% linoleic acid whereas high oleic acid soybeans (**HOSB**) contain ~75% oleic acid and ~7% linoleic acid. We determined the effect of increasing dietary inclusion of roasted and ground HOSB on production responses of high-producing dairy cows. Twenty-four multiparous Holstein cows (50.7 ± 4.45 kg/d of milk; 122 ± 57 DIM) were randomly assigned to treatment sequences in a replicated 4 × 4 Latin square design with 21-d periods. Treatments were increasing doses of HOSB at 0, 8, 16, and 24% DM. HOSB replaced conventional soybean meal and hulls to maintain similar diet nutrient composition (% dry matter [**DM**]) of 27.4 – 29.4% (**NDF**), 20.6% forage NDF, 27.5% starch, and 15.9 – 16.5% CP. Total fatty acid content of treatments was 1.65, 3.11, 4.52, and 5.97% DM, respectively. Pre-planned contrasts included the linear, quadratic, and cubic effects of increasing HOSB. Increasing dietary inclusion of HOSB linearly decreased DM intake and milk urea nitrogen and increased yields of milk, 3.5% fat corrected milk, energy corrected milk, and milk fat, and quadratically increased milk protein. The increased response to milk fat was due to an increase in preformed milk fatty acids. Due to the increase in milk component yields and decrease in DMI, there was an increase in feed efficiency. Increasing HOSB had no effect on body weight change or BCS change. In summary, increasing dietary inclusion of HOSB up to 24% DM increased production responses of high-producing dairy cows and did not affect body reserves.

Introduction

The review by Palmquist and Jenkins (1980) outlines the benefits of supplementing fat to diets for higher yielding cows because increased milk yields requiring more nutrients to support lactation. Supplemental fat can be fed in multiple forms, such as oilseeds and fatty acid (**FA**) supplements, many of which have different FA profiles. Research has shown positive effects of different FA supplements on milk production responses (dos Santos Neto et al., 2021a; b), and our lab has emphasized the importance of considering the FA profile of supplements as cow responses differ due to different individual FA and combinations of FA depending on production level (de Souza et al., 2019; Burch et al., 2021; Bales et al., 2024). In particular, recent research in our lab has observed that higher producing cows increase milk production with higher levels of oleic acid (***cis*-9 C18:1)** in FA blends (de Souza et al., 2019; Western et al., 2020; Burch et al., 2021).

Oilseeds also differ in FA profile, but contain mostly unsaturated FA (**UFA**; Glasser et al., 2008a). Soybeans are a rich source of linoleic acid (**C18:2**), a FA known to increase the risk of milk fat depression (Bauman et al., 2011; Dorea and Armentano, 2017) and thus, low inclusion rates of soybeans have typically been utilized in dairy cattle diets. Recently, novel varieties of soybeans have been developed that are enriched in *cis*-9 C18:1, at the expense of C18:2. High oleic soybeans (**HOSB**) contain approximately 70% *cis*-9 C18:1 and <10% C18:2 (Weld and Armentano, 2018) which is vastly different from conventional soybeans that contain 22 – 26% *cis*-9 C18:1 and 47 – 54% C18:2 (Glasser et al., 2008a; Weld and Armentano, 2018). Based on the increased content of *cis*-9 C18:1 and lower content of C18:2, we propose that HOSB have the potential to increase milk production of high yielding cows.

There are few studies that have evaluated HOSB in dairy cow diets. Weld and Armentano (2018) observed that raw, whole HOSB increased milk fat yield compared with raw, whole conventional soybeans. Additionally, they assessed particle size within soybean variety, and observed an increase in milk fat yield with ground HOSB versus ground conventional soybeans. Overall, HOSB also reduced the content of *trans*-10 C18:1 in milk, a FA that is part of the altered rumen biohydrogenation pathways associated with milk fat depression (Lock et al., 2007; Bauman et al., 2011). Lopes et al. (2017) evaluated processing method of soybeans and observed an increase in milk fat content with HOSB (whole and heated HOSB and extruded meal) compared with conventional extruded soybean meal. Although initial research with HOSB suggests an increase in milk energy output, further research is needed to establish suitable feeding rates. To our knowledge, only one other study has previously evaluated feeding rates of soybeans, but the experiment utilized whole, roasted conventional soybeans (Knapp et al., 1991).

The practice of heat-treating soybeans is common to increase RUP content (Grummer et al., 1994), as well as to denature a trypsin inhibitor that could otherwise reduce protein digestibility in the small intestine (NASEM, 2021). When compared with raw soybeans, roasted soybeans has been found to either increase milk production (Tice et al., 1993; Dhiman et al., 1997) or have no impact on responses (Bernard, 1990; Scott et al., 1991). Weld and Armentano (2018) only utilized raw HOSB, thus their results may have been different if the soybeans were roasted. Additionally, the soybeans were all fed at similar feeding rates (~15-19% DM); therefore, milk production may have been improved if fed at higher or lower inclusion rates. Knapp et al. (1991) fed whole, roasted conventional soybeans at 0, 12, 18, and 24% DM and reported increased 3.5% FCM at the highest levels, but the soybeans replaced soybean meal and ground corn, thus also altering dietary starch content. Therefore, we designed a study to evaluate

increasing dietary inclusion of roasted and ground HOSB at 0, 8, 16, and 24% DM. We hypothesized that as soybean inclusion rate increased, DMI and milk production responses would also increase. We replaced conventional soybean meal and soybean hulls when adding HOSB to diets in order to have similar (% DM) NDF, forage NDF, starch and CP across treatment diets.

Material and Methods

Design and Treatments

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University, East Lansing. Twenty-four mid-lactation, multiparous Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center were randomly assigned to a treatment sequence in a replicated 4 × 4 Latin square design balanced for carryover effects in four 21-d periods. All animals received a common diet during a 14-d preliminary period to obtain baseline values. The baseline average for all animals, with mean ± standard deviation, were 122 ± 57 DIM and 50.7 ± 4.45 kg/d of milk.

The treatments consisted of increasing HOSB (Plenish; Pioneer, Johnston, IA) inclusion at 0, 8, 16, and 24% DM of the diet. The HOSB replaced soybean meal and soybean hulls to keep NDF, forage NDF, starch, and CP levels similar across all diets (Table 21). The HOSB used in the trial were roasted at 157°C for 2 hours using a Dilts-Wetzel roaster (Dilts-Wetzel Manufacturing Co, Ithaca, MI) and then ground in a Dalex hammer mill (Dalex Fabrication and Machining Inc, Terre Haute, IN) using a 10 mm screen, with a mean particle size of 771 ± 2.56 microns. All experimental diets were formulated to meet the nutrient requirements of the average cow (Table 21; Nutrient Requirements of Dairy Cattle, 2021). The DM concentration of forages

was determined twice weekly, and diets adjusted accordingly. Cows were milked 3x/d and fed 115% of expected intake daily. Feed access was blocked from 0600 to 8000 h for orts collection and offering of new feed at 8000 h. Cows were housed in individual tie-stalls throughout the experiment with water available ab libitum in each stall and were bedded with sawdust and cleaned twice daily.

Data and Sample Collection

Samples and production data were collected during the last 5 d of each treatment period (d 17-21). Samples of all diet ingredients (0.5 kg) and orts (12.5%) were collected daily and composited by cow/period for analysis. Milk yield was recorded, and two milk samples were collected at each milking. One aliquot was collected in a sealed tube with preservative and stored at 4ºC for milk component analysis. The second aliquot was stored without preservative at -20ºC until analyzed for FA composition. Blood (~15 mL) samples were collected every 15 h, resulting in 8 samples/cow per period and stored on ice until centrifugation at 2,000 × *g* for 15 min at 4°C. Plasma was transferred into microcentrifuge tubes and stored at −20°C until composited by cow per period. Body weight was measured 3 times per week following the afternoon milking with changes in BW determined according to Boerman et al. (2015b). On the last day of each period, BCS was determined by three trained investigators on a 5-point scale in 0.25 increments (Wildman et al., 1982).

Sample Analysis

Dietary ingredients and orts were dried at 55ºC in a forced-air oven for 72 h for DM determination. Dried samples were ground in a Wiley mill (1 mm screen; Arthur H. Thomas, Philadelphia, PA). Samples of feed ingredients were analyzed for NDF, starch and CP according to Boerman et al. (2017) and analyzed for total FA content and FA profile according to Bales et

al. (2023). Milk samples were analyzed for fat, true protein, and lactose concentrations by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan Dairy Herd Improvement Association (Central Star DHI, Grand Ledge, MI). Plasma samples were analyzed at the Michigan State University Veterinary Diagnostic Laboratory (Lansing, MI). Plasma insulin was quantified with a bovine insulin ELISA using a solid phase 2-site enzyme immunoassay (Mercodia) and blood urea nitrogen was measured on a AU680 analyzer using an adapted enzymatic method according to Talke and Schubert (1965). Yields of milk components, 3.5% FCM, and ECM were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each period.

Milk samples used for analysis of FA composition were composited based on milk fat yield of individual samples taken at each milking during the collection period (d 17-21 per period). The composite samples (~40 mL) were then centrifuged at 17,800 × *g* for 30 min at 4°C for separation of the fat cake for collection for further analysis. We used an adopted protocol of the extraction procedure adapted from Hara and Radin (1978) using *n*-hexane/isopropanol (3:2, vol/vol) for the extraction of total lipids from the fat cake (Lock et al., 2013). The FAME were prepared by mixing 2.5 mL of *n*-hexane containing 25 mg of lipids and 0.5 mL of 0.5 M sodium methoxide solution in methanol for 5 min. Next, 1 gram of sodium bisulfate was added, the vial was vortexed, and then centrifuged at 6,000 × g for 5 min. The supernatant containing the FAME was transferred into a 2-mL vial and used directly for GLC analysis. Fatty acid composition covering approximately 70 FA in the range C4:0 to C24:0 was determined by a GC-2010 Plus gas chromatograph (Shimadzu, Kyoto, Japan) with a split injector (1:100 split ratio) and a flame-ionization detector (FID) using a CP-Sil 88 WCOT (wall-coated open tubular) fused-silica column (100 m × 0.25 mm i.d. × 0.2-μm film thickness; Varian Inc., Lake Forest, CA).

Hydrogen was used as the carrier gas at a flow rate of 1 mL/min and for the FID at 40 mL/min. The other FID gases were purified air at 400 mL/min and nitrogen makeup gas at 30 mL/ min. Injector and detector temperature was kept at 250°C. The oven program was: initial temperature of 40°C and held for 4 min, programmed at 13°C/min to 175°C and held for 27 min, then programmed at 4°C/ min to 215°C and held for 35 min. Injection volume was 1 μL. Integration and quantification (GCsolution software version 2.32.00; Shimadzu) were based on FID response. Individual FAME were identified by comparison of retention times with known FAME standards (GLC reference standard 463, GLC reference standard 481-B, and conjugated octadecadienoic mix-ture #UC-59-M from Nu-Chek Prep Inc., Elysian, MN; Supelco 37 component FAME mix, *cis/trans* FAME mix, bacterial acid methyl ester mix, and PUFA No. 3 mix from Supelco Inc., Bellefonte, PA). Short-chain FAME were corrected for mass discrepancy using the response factors published by Ulberth and Schrammel (1995). Even though all quantified FA were used for summation by source and concentration calculations, only select FA are included in the tables.

Yields of individual FA (g/d) were calculated using milk fat yield and FA concentration to determine yield on a mass basis using the molecular weight of each FA while correcting for glycerol and other milk lipid classes (Piantoni et al., 2013). Preformed milk FA yield response to additional 18-carbon FA intake (**FAYR-INT**) and absorbed 18-carbon FA (**FAYR-ABS**) were calculated as: FAYR-INT (%) = [(preformed milk FA yield for HOSB treatment – preformed milk FA yield for 0% HOSB supplementation)/(18-carbon FA intake for HOSB treatment – 18-carbon FA intake for 0% HOSB supplementation)] and FAYR-ABS (%) = [(preformed milk FA yield for HOSB treatment – preformed milk FA yield for 0% HOSB supplementation)/(absorbed 18-carbon for HOSB treatment – absorbed 18-carbon FA for 0% HOSB supplementation)].

Statistical Analysis

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

$$Y_{ijkl} = \mu + C_i(S_j) + P_k + T_l + S_j + P_k \times T_l + e_{ijkl},$$

Where Y_{ijkl} = the dependent variable, μ = the overall mean, $C_i(S_j)$ = random effect of cow nested within square (i = 1 to 4), P_k = fixed effect of period (k = 1 to 4), T_l = fixed effect of treatment (l = 1 to 4), S_j = fixed effect of square (j = 1 to 5), and e_{ijkl} = residual error. The interaction of $P_k \times T_l$ were initially included in the model and removed when not significant ($P > 0.20$; Prom et al., 2021). Main effects were declared significant at $P \leq 0.05$ and tendencies $P \leq 0.10$. Three orthogonal contrasts evaluated the linear, quadratic, and cubic effects of increasing HOSB inclusion in the diet.

Results

Production Responses

Increasing dietary inclusion of HOSB from 0 to 24% DM decreased DMI (linear; P=0.01; Table 22) up to 0.70 kg/d. Intake of HOSB was 2.58, 4.97, and 7.43 kg/d and resulted in additional intakes of *cis*-9 C18:1 of 416, 803, and 1,120 g/d for the 8, 16, and 24% inclusion levels, respectively.

Increasing dietary HOSB from 0 to 24% DM increased yields of 3.5% FCM, ECM, milk fat, and feed efficiency (ECM/DMI, linear, all $P < 0.001$; Table 22) and increased milk yield (quadratic, $P < 0.01$), milk fat content (quadratic, $P < 0.01$), and milk lactose yield and content

(quadratic, both $P < 0.001$), with the highest value being for 24% treatment. Increasing HOSB inclusion increased milk protein yield (quadratic, $P < 0.01$) with the highest value being for the 8 and 16%. Increasing HOSB inclusion decreased milk urea nitrogen (linear, $P < 0.001$) and milk protein content (quadratic, $P = 0.02$), with the highest value being for the control treatment. There was no effect of increasing HOSB inclusion on BW (P =0.25), BW change ($P = 0.20$), BCS ($P = 0.52$), or BCS change ($P = 0.19$).

Milk FA Yield and Content

Milk FA are derived from two sources: <16 carbon FA (de novo) from de novo synthesis in the mammary gland and >16 carbon FA (preformed) originating from extraction from plasma. Mixed source 16-carbon FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis in the mammary gland and extraction from plasma.

Increasing dietary inclusion of HOSB from 0 to 24% DM decreased the yield of de novo and mixed milk FA (both quadratic, P =0.05 and P <0.01; Table 23), with the lowest value being for the 24% treatment, whereas increasing HOSB increased preformed milk FA yield (quadratic; P =0.04), with highest values being for the 24% treatment. For select individual milk FA, increasing HOSB inclusion increased yields of C4:0 (linear; P <0.001), C18:0 (quadratic; P =0.02),), *trans*-9 C18:1 (quadratic; P =0.01), *trans*-10 C18:1 (quadratic; P =0.04),), *trans*-11 C18:1 (linear; P <0.001), and *cis*-9 C18:1 (quadratic; P <0.01), with the highest values being for the 24% treatment. Increasing dietary inclusion of HOSB decreased C16:0 yield (quadratic; P =0.01), with the lowest value being for the 24% treatment. Increasing dietary inclusion of HOSB from 0 to 24% DM decreased the content of de novo (linear, P <0.001; Table 24) and mixed milk FA (quadratic; P <0.001), with the lowest value being for the 24% treatment, but increased preformed milk FA content (quadratic; P <0.001), with highest values being for the 24%

treatment. Increasing dietary inclusion of HOSB increased *trans*-10 C18:1 content (linear; $P < 0.001$) but *trans*-10, *cis*-12 CLA was not detected.

Plasma Metabolites

Increasing dietary inclusion of HOSB decreased blood urea nitrogen (linear, $P < 0.001$; Table 25) and tended to decrease plasma insulin (linear; $P = 0.08$).

Discussion

The use of soybeans in dairy cattle diets has been a common nutrition practice, but typically at low dietary inclusions due to high levels of C18:2 increasing the risk of milk fat depression. New varieties of soybeans containing high levels of *cis*-9 C18:1 and low levels of C18:2 have recently been developed and could be a good alternative to conventional soybeans for feeding to lactating dairy cows. Our group has observed that increasing the *cis*-9 C18:1 content of FA supplements improved milk production responses in fresh cows (de Souza et al., 2021) and post-peak, high producing cows (>55 kg/d milk yield; de Souza et al., 2019; Western et al., 2020; Burch et al., 2021). Although there is research on the effects of *cis*-9 C18:1 in FA supplements (de Souza et al., 2019; Western et al., 2020b; dos Santos Neto et al., 2021b), there is limited research with the feeding HOSB to dairy cattle, resulting in uncertainties regarding processing methods and feeding rates with this new soybean variety. Therefore, the objective of our study was to evaluate roasted and ground HOSB at increasing dietary inclusions of 0, 8, 16, and 24% DM on intake and production responses of high-producing, mid-lactation dairy cows.

To our knowledge our study is the first to assess roasted and ground HOSB, as well as the first to consider potential optimal feeding rates. We designed our treatment diets to have a similar nutrient composition by replacing soybean meal and soybean hulls with HOSB, therefore

NDF, forage NDF, starch, and CP (%DM) were similar across treatment diets. We acknowledge that protein fractions would be different, as roasted soybeans contain more RUP than soybean meal, and that FA content was increased with increased inclusion of HOSB. As a result of our diet formulations, we are confident that responses were due to roasted, ground HOSB inclusion. Currently there are only two published studies evaluating HOSB, one assessed raw HOSB (Weld and Armentano, 2018) and the other compared whole HOSB to an extruded HOSB meal (Lopes et al., 2017). There is one study evaluating inclusion rates of conventional soybeans up to 24% DM, although Knapp et al. (1991) used whole, roasted conventional soybeans and replaced soybean meal and ground corn with the soybeans. Consequently, Knapp et al. (1991) increased CP (% DM) with increased soybean inclusion, and we would expect a difference in starch content, but the authors did not report this nutrient in their diet table. Therefore, the observed increases in milk production responses could be confounded based on the difference in nutrient composition of the treatment diets. Additionally, we utilized high producing dairy cows (~50 kg/d milk yield) whereas the cows in Knapp et al. (1991) produced between 30 and 38 kg/d of milk, thus nutrient demand and utilization for milk production and physiological state between these two group of cows would differ (NASEM, 2021).

The decrease in DMI with greater inclusion of HOSB was expected as HOSB supplied more *cis*-9 C18:1 and increased flow of unsaturated FA has been observed to increase gut peptides associated with satiety and decreased DMI (Choi et al., 2000; Bradford et al., 2008). Variability in DMI has been observed for FA sources rich in *cis*-9 18:1 and could be due to type of FA source; for example, evaluation of high *cis*-9 18:1 sunflower seed and safflower oil did not affect DMI (Casper et al., 1988; He and Armentano, 2011), whereas crushed canola seed, naturally high in *cis*-9 18:1, increased DMI compared with other seeds containing high levels of

18:2 and 18:3 (linolenic acid; Beauchemin et al., 2009). In a meta-analysis evaluating impact of Ca-salts of palm fat (average 38% *cis*-9 18:1), DMI was decreased compared to a diet with no additional FA supplementation (dos Santos Neto et al., 2021b). There was no effect on DMI with utilization of raw HOSB inclusion (~19% DM; Weld and Armentano, 2018) or inclusion of whole, roasted HOSB and extruded HOSB meal (7 and 17% DM, respectively; Lopes et al., 2017), though these studies did not evaluate different inclusion rates. Additionally, Knapp et al. (1991) observed no effect on DMI when increasing whole, roasted conventional soybeans from 0 to 24% inclusion. Although we observed a decrease in DMI with the highest HOSB inclusion level, the grind size of the soybeans did not change, thus we believe other dietary factors had a larger influence on DMI, such as the impact of increased intake of *cis*-9 C18:1.

Milk production responses to soybean supplementation are variable, but our results for increased milk yield with increasing dietary inclusion are similar to results observed by Knapp et al. (1991) when conventional soybeans were included at 0, 12, 18, and 24% DM. In contrast, Lopes et al. (2017) and Weld and Armentano (2018) observed no difference or a decrease in milk yield with HOSB, compared to a high *cis*-9 C18:1 extruded soybean meal or whole, raw conventional soybeans, respectively. Other studies evaluating high *cis*-9 C18:1 sunflower seed (79%; Casper et al., 1988) and high *cis*-9 C18:1 peanut oil (52%; Kelly et al., 1998) observed no effect on milk yield up to 5.8% DM inclusion, whereas rapeseed oil (61% *cis*-9 C18:1) at 2.0% DM increased milk yield compared with other 18-carbon oils (Dai et al., 2011), although the average production of cows in these studies was ~20 kg/d less than the cows in our current study. Supplementation of FA supplements containing up to 30% *cis*-9 C18:1 increased milk yield in high producing cows (>50 kg/d milk yield; de Souza et al., 2019; Western et al., 2020; Burch et al., 2021), and the increase in milk yield observed in our study may be due to the production

level of our cows responding positively to the HOSB. Processing of oilseeds can play a key factor in milk yield responses, as we used roasted HOSB and Knapp et al. (1991) used roasted conventional soybeans, whereas previously high *cis*-9 C18:1 oilseed (sunflower seeds and HOSB) studies used raw seeds (Casper et al., 1988; Weld and Armentano, 2018). Our results indicate that high-producing dairy cows respond positively to roasted, ground HOSB up to 24% DM.

Increasing dietary HOSB increased yields of milk and milk lactose and decreased de novo FA yield. Since mammary glucose supply is imperative for adequate milk production and lactose is the main osmotic regulator of milk volume (Linzell, 1972), the reduction in de novo FA synthesis with increasing inclusion of HOSB would spare glucose for other purposes, such as lactose synthesis (Cant et al., 1993). Soybeans contain an average of 40% CP, with ~6% being lysine (NASEM, 2021), and roasting soybeans increases RUP content (Grummer et al., 1994), thus likely supplying more rumen-bypass AA for milk synthesis (Piantoni and VandeHaar, 2023). Lysine is one of the limiting AA for milk production (NRC, 2001) but is extracted by the mammary gland at a higher rate than used for milk protein synthesis (NASEM, 2021), which could contribute to the increased milk yield in our study with increased intake of HOSB. Also, mitochondrial activity in the mammary gland is important to support lactation and fuel precursors that support milk biosynthesis (Favorit et al., 2021). In cultures of mammary epithelial cells treated with *cis*-9 C18:1, mitochondrial numbers increased and a higher expression of a mitochondrial activity marker was observed (Cohen et al., 2015), further supporting the increase in milk yield with increased inclusion of *cis*-9 C18:1 in FA blends (de Souza et al., 2019; Western et al., 2020). Additionally, abomasal infusion of *cis*-9 C18:1 increased mitochondrion biogenesis in adipose tissue of lactating cows (Abou-Rjeileh et al.,

2023). The increased milk yield observed with increasing dietary inclusion of HOSB could be due to a synergistic effect of glucose-sparing mechanisms, lysine content of the soybeans, and effects of *cis*-9 C18:1 on mammary epithelial cells.

Increasing HOSB inclusion in our study also improved milk component yields, and coupled with the increase in milk yield, resulted in increased yields of 3.5% FCM and ECM. Milk protein yield was increased with higher HOSB inclusion in our study, which is in contrast with both Lopes et al. (2017) and Weld and Armentano (2018), as they observed no effect of HOSB on milk protein yield. Even though we kept CP % similar across our treatment diets, the RDP and RUP content of our diets varied due to the differing amounts of solvent extracted soybean meal and roasted HOSB used (34% versus 66% RUP [%CP], respectively; Lin and Kung, 1999). High-producing dairy cows need protein sources high in RUP, as microbial protein alone is inadequate for milk production requirements (Wang et al., 2010), and we most likely met protein requirements needed for increased milk protein yield with increasing HOSB inclusion. Using the NASEM (2021) diet formulation model and RUP values for solvent extracted soybean meal and roasted soybeans from Lin and Kung (1999), predicted AA supply was increased 6, 8, and 15 percentage units for the 8, 16, and 24% HOSB, respectively, compared to 0% HOSB. This is further supported by the decrease in urea nitrogen concentrations in both blood and milk, suggesting improved AA balance and efficiency (Wang et al., 2010). Future experiments should evaluate balancing RDP and RUP content of diets, and the impacts of roasted versus raw HOSB in diets fed to high-producing dairy cows.

As expected, increasing dietary inclusion of HOSB increased milk fat yield, as observed previously when high-producing dairy cows were supplemented with increased amounts of *cis*-9 18:1 (de Souza et al., 2019; Western et al., 2020b). Knapp et al. (1991) observed that 12, 18, and

24% inclusion of whole, roasted conventional soybeans increased milk fat yield compared with 0%, although 18 and 24% inclusion decreased milk fat yield compared with the 12% treatment, potentially due to the increased level of 18:2 provided by the conventional soybeans, which is different from our observation with HOSB. Raw HOSB increased milk fat yield compared with conventional soybeans, independent of particle size (Weld and Armentano, 2018). Additionally, Lopes et al. (2017) observed a tendency for HOSB (whole, roasted and extruded soybean meal) to increase milk fat yield compared with conventional extruded soybean meal. Due to Weld and Armentano (2018) including different soybean varieties and processing types at similar feeding rates (conventional ~16% DM and HOSB ~19% DM), the observed increases in milk fat yield are a direct result of the *cis*-9 C18:1 content of the HOSB. When evaluating bovine mammary epithelial cells, Liang et al. (2014) observed that *cis*-9 18:1 enhanced fatty acid binding protein 3 by positively impacting milk fat synthesis signaling pathways and increased lipid droplet accumulation. Additionally, lysine stimulated expression and maturation of sterol regulatory element binding protein 1c and fatty acid binding protein 5, which was further enhanced in the presence of FA, one of which was *cis*-9 C18:1 (Li et al., 2019). These are all key proteins involved in milk fat synthesis and could explain our increase in milk fat response as increasing HOSB inclusion level increased *cis*-9 18:1, thus increased substrates (preformed FA) for milk fat synthesis. We recognize that a significant amount of the dietary *cis*-9 18:1 undergoes biohydrogenation in the rumen; but using ruminal biohydrogenation extents for oilseeds of 30% (Jenkins and Bridges, 2007) to 60% (Barletta et al., 2016), we would expect approximately 160 to 300, 320 to 560, and 450 to 780 g/d of *cis*-9 C18:1 from HOSB reaching the small intestine for the 8, 16, and 24% inclusion rates, respectively. Greater supply of *cis*-9 18:1 to the small intestine will increase absorption of FA (Prom et al., 2021), thus increasing FA available for

utilization in the mammary gland and other tissues. The improvement in milk fat with increasing HOSB inclusion in our study could be attributed to the greater amount of dietary *cis*-9 18:1 that may have enhanced mechanisms involved in milk fat synthesis and increasing substrate supply of long-chain FA utilized for preformed FA.

The milk fat response to increasing HOSB was driven by an increase in preformed milk FA yield, with an almost 2-fold increase for the 24% inclusion. Similar responses were observed in a meta-analysis evaluating oilseed supplementation on milk FA composition (Glasser et al., 2008a), as oilseeds predominantly contain 18-carbon FA and supply more long-chain FA which are available for absorption and utilization. In our study, we calculated 18-carbon transfer efficiency for dietary 18-carbon and absorbed 18-carbon FA, and observed that the 8, 16, and 24% HOSB treatments had FAYR-INT of 43, 38, and 37% and FAYR-ABS of 62, 60, and 62%, respectively. We also observed that increasing HOSB inclusion decreased de novo milk FA, which agrees with the meta-analysis by Dorea and Armentano (2017), as they observed a negative relationship between dietary *cis*-9 C18:1 content and de novo milk FA yield. This could be described as a substitution effect, as the decrease in de novo FA are compensated for by an increase in preformed FA when supplementing FA in the diet (Glasser et al., 2008b). Additionally, *cis*-9 C18:1 can affect the esterification of triglycerides because it has a high affinity for all *sn*-positions on the glycerol backbone, thus potentially outcompeting short and medium chain FA for the *sn*-3 and *sn*-2 and positions, respectively (Jensen et al., 1991). Although overall de novo FA yield was reduced, increasing HOSB inclusion increased the yield of C4:0, similar to Weld and Armentano (2018) whom reported increased C4:0 yield with HOSB compared with conventional soybeans. This increase in C4:0 in milk is likely due to the regulation of milk fat fluidity (Barbano and Sherbon, 1980) as there are mechanisms in the

mammary gland to ensure that milk fat is fluid at body temperature, indicated by shifts in yields of individual milk FA. The melting point of FA will influence milk fat fluidity and is impacted by both carbon chain length and degree of unsaturation (Dils, 1986), thus esterification of specific combinations of FA on the triglyceride backbone maintains the melting point at ~39°C to provide fluid fat globules in milk (Timmen and Patton, 1988). Similarly, greater *cis*-9 C18:1 content corresponds with fluidity regulation, and we observed over a 2-fold increase in the yield of *cis*-9 C18:1 in milk fat. Although a portion of C18:0 is converted to *cis*-9 C18:1 by the stearoyl Co-A desaturase enzyme to ensure milk fat fluidity (Mosley and McGuire, 2007), a large portion could be due to greater absorption of *cis*-9 C18:1 with increased HOSB inclusion, as Weld and Armentano (2018) observed increased incorporation of *cis*-9 C18:1 in milk fat, with no effect on C18:0 milk FA yield, when feeding ground, raw HOSB compared to ground, raw conventional soybeans. Future research is needed to evaluate whether nutritional strategies can mitigate reductions in de novo milk FA with increased supply of pre-formed FA to potentially further increase milk fat yields in response to HOSB feeding.

It is important to consider the UFA content of specific feed ingredients when formulating diets, as increased intake of UFA in the rumen can disrupt normal biohydrogenation pathways that produce intermediates that impact milk fat synthesis (Bauman et al., 2011). Through alternative pathways, C18:2 can be biohydrogenated to *trans*-10-, *cis*-12 conjugated linoleic acid (CLA), a potent bioactive FA that has negative effects on milk fat synthesis, which can be further biohydrogenated to *trans*-10 C18:1, a biohydrogenation intermediate that either has no effect on milk fat synthesis (Lock et al., 2007), or is much less potent than *trans*-10-, *cis*-12 CLA (Shingfield et al., 2009). Oleic acid is readily biohydrogenated in the rumen and can produce several *trans*-FA, including *trans*-10 C18:1, but is unlikely to produce *trans*-10-, *cis*-12 CLA

(Dewanckele et al., 2020). Although we did observe an increase in *trans*-10 C18:1 content and yield in milk fat with increased inclusion of HOSB, suggesting evidence of alternate biohydrogenation pathways, we did not detect appreciable amounts of *trans*-10-, *cis*-12 CLA. Additionally, increasing HOSB inclusion increased milk fat yield in our study, thus we did not induce milk fat depression. Weld and Armentano (2018) did detect *trans*-10-, *cis*-12 CLA, although there was no difference between soybean varieties, but they observed an increase in *trans*-10 C18:1 and a decrease in milk fat output with conventional soybeans compared with HOSB, more indicative of a milk fat depression scenario. Additionally, we did not utilize HOSB meal or hulls in our control treatment, but the difference in C18:2 content across diets was only 0.02%, thus we would not expect this to impact production responses. Our study demonstrates that under the dietary conditions tested, incorporation of HOSB in the diet of lactating cows did not induce milk fat depression, even with an increase in *trans*-18:1 isomers.

Although all cows were gaining BW, we did not observe as effect of increasing HOSB inclusion on BW gain, but longer-term studies are needed to examine this further. Changing dietary inclusion of *cis*-9 C18:1 has been shown to decrease BW loss in fresh cows (de Souza et al., 2021) through limiting lipolysis in adipose tissue (Abou-Rjeileh et al., 2023). Additionally, increasing *cis*-9 C18:1 content in FA blends either increased (de Souza et al., 2018a, 2019) or had no effect (Western et al., 2020b; Burch et al., 2021) on BW change in mid-lactation cows. Increases in BW with increased dietary *cis*-9 C18:1 have been associated with an increase in plasma insulin (de Souza et al., 2018a, 2019), as insulin is an antilipolytic hormone that can increase adipose tissue lipogenesis (Vernon, 2005). The tendency for increased HOSB inclusion to decrease plasma insulin in our study did not impact BW and most likely is associated with the

decrease in DMI, similar to results observed with increased inclusion of a Ca-salt reducing both DMI and insulin release (Choi and Palmquist, 1996).

We designed our study to evaluate feeding rates for roasted and ground HOSB, due to the lack of available literature regarding dose responses with HOSB, particularly with this processing method. Although Weld and Armentano (2018) were able to assess differences between soybean variety and particle size, they only utilized raw soybeans. Though our study and Weld and Armentano (2018) have answered important questions for the dairy industry, there is still missing information regarding potential differences between raw and roasted HOSB, as milk production differences have been observed between whole raw and whole roasted conventional soybeans (Tice et al., 1993). Therefore, future research should evaluate milk production responses in high-producing cows to roasted or raw HOSB. A longer-term study (>3 weeks) would be important to evaluate if feeding HOSB has sustained positive impacts on milk production as cows increase DIM. Finally, by design our diets were not formulated to be isoenergetic, as our objective was to investigate the effect of increasing HOSB inclusion on production, thus increasing total FA content of diets. Further research with HOSB could consider formulating diets to have similar FA contents, although it is important to note that FA supplements containing different FA profiles to that of the HOSB, will also influence results (de Souza et al., 2018a, 2019; Bales et al., 2024).

Conclusion

Our study is unique as we are the first to evaluate roasted and ground high *cis*-9 C18:1 soybeans and the first to assess a dose response with this soybean variety. Increasing roasted, ground HOSB increased the yields of milk and milk component but decreased DMI by 0.70 kg/d

up to 24% inclusion. Due to this soybean variety containing a high level of *cis*-9 C18:1, HOSB allows for greater inclusion rates and higher dietary FA levels that can positively impact cow performance. Further research is needed to evaluate potential differences in milk production between raw and roasted HOSB, the effect of manipulating protein fractions with inclusion of HOSB, and dietary strategies to mitigate the reduction in de novo milk FA yield to further increase milk fat responses.

CHAPTER 7: EFFECTS OF RAW AND ROASTED HIGH OLEIC SOYBEANS ON MILK PRODUCTION RESPONSES OF HIGH-PRODUCING DAIRY COWS

Abstract

Processing method of soybeans has the potential to influence dairy cow production performance, therefore we determined the effect feeding roasted or raw, ground high oleic acid soybeans (HOSB) on production responses of high-producing dairy cows. Thirty-six multiparous Holstein cows (45.6 ± 6.22 kg/d of milk; 110 ± 61 DIM) were randomly assigned to treatment sequences in a 4 × 2 Truncated Latin square design with 35-d periods. Treatments were: 1) control (CON) with no soybean inclusion, 2) 16% roasted and ground HOSB (RST), 3) 16% raw and ground HOSB (RAW-D), and 4) 16% raw and ground HOSB + additional rumen undegradeable protein (RAW-U). HOSB replaced conventional soybean meal and hulls in the control diet and rumen by-pass protein replaced soybean meal in RAW-U to maintain diet nutrient composition (% dry matter [DM]) of 28.0% neutral detergent fiber (NDF), 21.3% forage NDF, 27.3% starch, and 17.8% CP. Ether extract of each treatment was formulated to contain 3.25, 5.80, 5.80, and 5.80 %DM, respectively. Pre-planned contrasts included the overall effect of HOSB inclusion {CON vs. SOY [1/3 (RST + RAW-D + RAW-U)]}, the effect of soybean processing {RST vs. RAW [1/2 (RAW-D + RAW-U)]}, and the effect of increasing RUP content within the raw HOSB treatments (RAW-D vs RAW-U). Results are presented in the following order: CON, RST, RAW-D, and RAW-U. For most variables tested, there were significant interactions between treatment and week, as SOY increased production variables compared with CON and RST increased production responses compared with RAW, although the magnitude of difference varied between weeks. Overall, SOY increased DMI and yields of milk, 3.5% FCM, ECM, and milk fat, but did not affect milk protein yield. RST did not impact

DMI but increased yields of milk, 3.5% FCM, ECM, milk fat, and milk protein. RAW-U did not impact DMI and increased yields of milk and milk protein and tended to increase ECM. Overall, HOSB inclusion of 16% DM increased production responses in high-producing dairy cows, but heat treatment had a greater impact than raw HOSB, and the addition of rumen-bypass protein positively affected milk protein response.

Introduction

Oilseeds are a good source of fat and protein and have been found to increase milk fat production (Banks et al., 1976; Rabiee et al., 2012). Soybeans are a commonly used oilseed that can increase FCM (Tice et al., 1993) and increase milk component yields at higher inclusion levels (Knapp et al., 1991). Although soybeans can increase milk fat yield, they contain high levels of linoleic acid (**C18:2**), a fatty acid (**FA**) that can increase the risk of milk fat depression (Bauman et al., 2011). It is known that dietary oils high in oleic acid (***cis*-9 C18:1**) have less of a negative impact on milk fat secretion compared to oils high in C18:2 (He et al., 2012; Dorea and Armentano, 2017). While conventional soybeans contain ~50% C18:2 (Glasser et al., 2008a), development of a new soybean enriched in *cis*-9 C18:1 (**HOSB**; ~70%) and low in C18:2 (<10%; Weld and Armentano, 2018) has shown the potential to increase milk fat production compared with conventional soybeans (Weld and Armentano, 2018; Bomberger et al., 2019). In Chapter 6, we observed increases in milk production responses when increasing dietary inclusion level of roasted, ground HOSB up to 24% diet DM in place of solvent extracted soybean meal and soyhulls. While increased production responses have been observed with HOSB, both roasted (Bomberger et al., 2019; Chapter 6) and raw (Weld and Armentano, 2018), to our

knowledge there has not been a direct comparison of raw and roasted HOSB to evaluate potential milk production differences in high-producing dairy cows.

Many studies have evaluated the effects of roasting and processing methods of conventional soybeans in dairy cow diets, with differences in digestion and milk production dependent on processing (Bernard, 1990; Tice et al., 1993; Grummer et al., 1994). Soybeans are often heat-treated to increase RUP content (Grummer et al., 1994), as well as to denature a trypsin inhibitor that could otherwise reduce protein digestibility in the small intestine (NASEM, 2021). Compared with raw soybeans, roasting conventional soybeans either had no effect (Bernard, 1990; Scott et al., 1991) or increased milk production (Tice et al., 1993; Dhiman et al., 1997). Grummer et al. (1994) observed no difference in milk component yield between diets containing either roasted soybeans or raw soybeans and a source of by-pass protein. Therefore, we designed a study to evaluate the effects of raw and roasted HOSB on milk production responses of high-producing dairy cows. We had two hypotheses: 1.) roasted, ground HOSB would improve milk production compared with raw, ground HOSB and 2.) milk production responses will be increased with a raw, ground HOSB diets containg additional by-pass protein compared to a raw, ground HOSB. We utilized conventional soybean meal and soybean hulls to replace HOSB in our control diet in order to maintain similar NDF, forage NDF, CP and starch (%DM) across treatment diets.

Material and Methods

Design and Treatments

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University, East Lansing. Thirty-six mid-lactation, multiparous

Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center (Lansing, MI) were randomly assigned to a treatment sequence in a balanced 4 × 2 truncated Latin square design in 2 consecutive 35-d periods. Truncated Latin square designs have been used previously in dairy science research (Clark and Armentano, 1999; de Souza et al., 2019; Western et al., 2020a). Our truncated Latin square design allowed for 4 treatments across 2 periods, with multiples of 12 cows required to balance treatment sequences; therefore, each cow was assigned to 2 treatments in 2 different periods. We chose this truncated design to utilize longer experimental periods to evaluate BW across time, compared with more common 2- to 3-wk periods often used in complete Latin square designs. All animals received a common diet during a 7-d preliminary period to obtain baseline values. The baseline average for all animals, with mean ± standard deviation, were 45.6 ± 6.22 kg/d of milk; 110 ± 61 DIM.

The treatments consisted of 1) control containing soybean meal and soybean hulls (**CON**); 2) inclusion of 16% DM of roasted and ground HOSB (**RST**); 3) inclusion of 16% DM of raw and ground HOSB (**RAW-D**); and 4) inclusion of 16% DM of raw and ground HOSB + additional rumen undegradable protein (**RAW-U**). In order to keep diet nutrient composition similar across treatments, HOSB replaced soybean meal and soybean hulls in CON and the rumen by-pass protein supplement replaced soybean meal in RAW-U (Table 26). The 16% DM inclusion of HOSB was based on results from our previous study (Chapter 6). Whole HOSB were delivered in bulk at the beginning of the experiment and then were divided into two groups for additional processing. The first portion of the HOSB were sent to a local feed mill (Mathews Elevator, Fowler, MI) for roasting and grinding for the RST treatment. These soybeans were roasted at 157°C for 2 hours using a Dilts-Wetzel roaster (Dilts-Wetzel Manufacturing Co, Ithaca, MI) and then ground in a Dalex hammer mill (Dalex Fabrication and Machining Inc,

Terre Haute, IN) using a 10 mm screen. The roasted and ground HOSB were subsequently delivered to the Michigan State University Dairy Cattle Teaching and Research Center and stored in a steel silo for the duration of the experiment. For the RAW-D and RAW-U treatments, raw HOSB from the remaining HOSB were ground once per week at the Michigan State University Dairy Cattle Teaching and Research Center using a Lancaster hammer mill (Lancaster Parts & Equipment, Lancaster, PA) with a 3.18 screen. Although we utilized two different hammer mills and screen sizes, we were able to meet our goal of similar particle size across roasted and raw HOSB, with mean ± standard deviation of 725 ± 2.59 microns and 794 ± 2.52 microns for roasted and raw HOSB, respectively. The decision to grind raw HOSB weekly was based on the processing methods of Weld and Armentano (2018) in order to minimize oxidation. After grinding, raw HOSB were stored on concrete in a cool, dry feed bay within the feed center, and any unused raw HOSB after one week were discarded.

All experimental diets were formulated to meet the nutrient requirements of the average cow (Table 26; NASEM 2021). The DM concentration of forages was determined twice weekly, and diets adjusted accordingly. Cows were fed 115% expected intake at 8000 h daily. Feed access was blocked from 0600 to 8000 h for orts collection and offering of new feed. Cows were milked 3x/d and housed in individual tie-stalls throughout the experiment. Water was available ab libitum in each stall and stalls were bedded with sawdust and cleaned twice daily.

Data and Sample Collection

Milk yield and samples were collected twice weekly during the first 4 wk of each period to monitor milk components and production data. The milk samples were collected in a sealed tube with preservative and stored at 4^{o}C for milk component analysis. During wk 5 of each period, milk samples and yield were collected 3 times to represent every other day of the

sampling period (d 31, 33, and 35). On d 31, 33, and 35, two milk samples were collected at each milking, with one aliquot collected in a sealed tube with preservative and stored at 4ºC for milk component analysis and the second aliquot stored without preservative at -20ºC until analyzed for FA composition. Feed ingredients and orts were collected d 30-35 and blood samples twice on d 35. Samples of all diet ingredients (0.5 kg) and orts (12.5%) were composited by cow/period for analysis. Blood (~15 mL) samples were collected at 0400 and 1300 h based on times of feed access, resulting in 2 samples/cow per period and stored on ice until centrifugation at 2,000 × *g* for 15 min at 4°C. Plasma was transferred into microcentrifuge tubes and stored at −20°C until composited by cow per period. BW was measured 3 times per wk following the afternoon milking with changes in BW determined according to Boerman et al. (2015b). On the last day of each period, BCS was determined by 3 trained investigators on a 5-point scale in 0.25 increments (Wildman et al., 1982).

Sample Analysis

Dietary ingredients and orts were dried at 55ºC in a forced-air oven for 72 h for DM determination. Dried samples were ground in a Wiley mill (1 mm screen; Arthur H. Thomas, Philadelphia, PA). Samples of feed ingredients were analyzed for NDF, starch, and CP according to Boerman et al. (2017) and total FA content and FA profile according to Bales et al. (2023). Milk samples were analyzed for fat, true protein, and lactose concentrations by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan Dairy Herd Improvement Association (Central Star DHI, Grand Ledge, MI). Yields of milk components, 3.5% FCM, and ECM were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each period. Milk samples used for analysis of FA composition were composited based on milk fat yield (d 31, 33, and 35 per period). Milk lipids were

extracted, FAME prepared, and analyzed by gas chromatography as described previously (Lock et al., 2013). Yields of individual FA (g/d) in milk fat was calculated using milk fat yield and FA concentration to determine yield on a mass basis using the molecular weight of each FA while correcting for glycerol content and other milk lipid classes (Piantoni et al., 2013). Plasma samples were analyzed at the Michigan State University Veterinary Diagnostic Laboratory (Lansing, MI) for plasma insulin which was quantified with a bovine insulin ELISA using a solid phase 2-site enzyme immunoassay (Mercodia) and for blood urea nitrogen which was determined on a AU680 analyzer using an adapted enzymatic method based on Talke and Schubert (1965).

Statistical Analysis

Data were analyzed utilizing two models. The first model analyzed yield of milk and milk components that were collected weekly. The second model analyzed data that was only collected at the end of each period (d 30-35) and BW parameters.

For model 1, weekly production data were analyzed using the GLIMMIX model procedure of SAS (version 9.4, SAS Institute, Cary, NC) using the following model:

$$Y_{ijklm} = \mu + C_i(S_j) + P_k + W_m + T_l + S_j + W_m \times T_l + e_{ijklm},$$

Where Y_{ijklm} = the dependent variable, μ = the overall mean, $C_i(S_j)$ = random effect of cow nested within square (i = 1 to 4), P_k = fixed effect of period (k = 1 to 2), W_m = fixed effect of week within period, T_l = fixed effect of treatment (l = 1 to 4), S_j = fixed effect of square (j = 1 to 9), $W_m \times T_l$ = the fixed effect of the interaction between week and treatment, and e_{ijklm} = residual error.

For model 2, milk FA, DMI, BW, and BCS were analyzed using the GLIMMIX model procedure of SAS (version 9.4, SAS Institute, Cary, NC) using the following model:

$$Y_{ijklm} = \mu + C_i(S_j) + P_k + T_l + S_j + P_k \times T_l + e_{ijklm},$$

Where Y_{ijklm} = the dependent variable, μ = the overall mean, $C_i(S_j)$ = random effect of cow nested within square (i = 1 to 4), P_k = fixed effect of period (k = 1 to 2), T_l = fixed effect of treatment (l = 1 to 4), S_j = fixed effect of square (j = 1 to 9), $P_k \times T_l$ = the fixed effect of the interaction between period and treatment, and e_{ijklm} = residual error.

Unless otherwise specified, compound symmetry was the covariate structure used for analysis because it resulted in the lowest Bayesian information criterion for most of the variables measured. Significance was determined at $P \leq 0.05$ for main effects and $P \leq 0.10$ for interactions. Tendencies were determined at $P \leq 0.10$ for main effects and $P \leq 0.15$ for interactions. When interactions were at $P \leq 0.15$, the slice option was used to evaluate treatment effects within week ($W_m \times T_l$) or period ($P_k \times T_l$), and interactions were removed when not significant ($P > 0.20$; de Souza et al., 2020; Prom et al., 2021). Three contrasts evaluated the overall effect of HOSB inclusion {CON vs. the average of the HOSB treatments (**SOY**) [1/3 (RST + RAW-D + RAW-U)]}, the effect of heat-treatment of HOSB {RST vs. the average of the raw HOSB treatments (**RAW**) [1/2 (RAW-D + RAW-U)]}, and the effect of additional undegradable within the raw HOSB treatments (**PRTN**; RAW-D vs. RAW-U).

Results

Production Responses

Overall effect of HOSB inclusion

Overall, compared with CON, SOY increased DMI by 0.67 kg/d (P=0.04; Table 27) and increased the yields of milk (P<0.01), 3.5% FCM, ECM, milk fat, and milk lactose (all P<0.001), content of milk fat and lactose (all P<0.001), and feed efficiency (ECM/DMI; P < 0.01), but decreased milk protein and MUN content (both P<0.001) and BW (P<0.01). There was no effect of treatment for milk protein yield (P=0.21), BW change (P=0.60), BCS (P=0.76), or BCS change (P=0.73).

For most of the experiment, treatment × week interactions were observed, although the magnitude of change varied across weeks. Overall, compared with CON, SOY did not impact week 1 milk yield (P=0.85; Figure 7) but increased milk yield during weeks 2 – 5 (P<0.01, P<0.01, P=0.10, and P<0.001, respectively). For weeks 1 – 5, compared to CON, SOY increased yields of ECM, (P=0.01, P<0.001, P<0.001, P=0.03, and P<0.001, respectively) 3.5% FCM (P<0.01, P<0.001, P<0.001, P<0.01, and P<0.001, respectively), and milk fat (P<0.01, P<0.001, P<0.001, P<0.001, and P<0.001, respectively). Compared with CON, SOY increased milk protein yield for week 2 (P=0.03) and week 5 (P=0.01) but did not impact week 1, 3, or 4 (P=0.13, P=0.26, and P=0.24). Overall, compared with CON, SOY decreased milk urea nitrogen concentration for weeks 1 – 5 (all P<0.001).

Effect of heat-treatment

For the effect of roasted vs. raw HOSB, compared with RAW, RST increased the yields of milk, 3.5% FCM, ECM, milk fat, milk protein, and milk lactose (all (P<0.001; Table 27), BW (P<0.001), and decreased milk protein and MUN content (both P<0.01), and tended to decrease

BW change (P=0.08). There was no effect of RST vs. RAW for DMI (P=0.91), milk fat content (P=0.23), milk lactose content (P=0.48), ECM/DMI (P=0.25), BCS (P=0.78), or BCS change (P=0.32).

For most of the experiment, treatment × week interactions were observed, although the magnitude of change varied across weeks (Figure 7). For weeks 1 – 5, compared with RAW, RST increased yields of milk (P<0.01, P<0.001, P<0.001, P<0.01, and P<0.01, respectively), ECM (P<0.001, P<0.001, P<0.001, P<0.01, and P=0.03, respectively), FCM (P<0.001, P<0.001, P<0.001, P<0.001, and P=0.01, respectively), milk fat (P<0.01, P<0.001, P<0.001, P=0.01, and P=0.03, respectively), and milk lactose (P<0.01, P<0.001, P<0.001, P<0.001, and P<0.01, respectively). Compared with RAW, RST increased milk protein yield during weeks 1 – 4 (P<0.001, P<0.001, P<0.001, and P=0.01, respectively) but not in week 5 (P=0.31). Compared with RAW, RST decreased MUN content during weeks 1, 2, 4, and 5 (P<0.001, P<0.001, P<0.001, and P=0.01, respectively) and tended to decrease it in week 3 (P=0.07).

Effect additional by-pass protein for raw HOSB treatments

For the effect of protein, compared with RAW-D, RAW-U increased yields of milk and lactose (both P<0.01; Table 27), and protein (P=0.01), and tended to increase ECM (P=0.07). Compared with RAW-D, RAW-U decreased content of milk fat (P<0.01), milk protein (P=0.03), and milk urea nitrogen (P<0.01). RAW-U increased BW (P<0.01) but did not affect BW change (P=0.40), and decreased BCS (P=0.03) and BCS change (P=0.04). Protein source did not impact DMI (P=0.90), milk fat yield (P=0.85), or ECM/DMI (P=0.24).

For specific weeks, treatment × week interactions were observed for the effect of protein (Figure 7). Compared with RAW-D, RAW-U increased yields of milk (P<0.01), milk protein (P=0.02) and milk lactose (P=0.01), and tended to increase ECM (P=0.09) for week 2,

increased yields of milk (P<0.01), ECM (P=0.03), 3.5% FCM (P=0.04), milk protein (P<0.01), and milk lactose (P<0.01) and tended to increase milk fat yield (P=0.08) in week 4, and tended to increase yields of milk (P=0.08) and milk lactose (P=0.09) in week 5. Protein source did not impact yields of milk, ECM, 3.5% FCM, milk fat, milk protein, or milk lactose for weeks 1 and 3 (all P >0.23) and did not impact yields of ECM, 3.5% FCM, milk fat, or milk protein for week 5 (all P >0.32). Compared with RAW-D, RAW-U increased MUN content during weeks 1, 2, 4, and 5 (P<0.01, P=0.04, P=0.02, and P=0.05, respectively), but did not impact it during week 3 (P=0.18).

Milk FA Content and Yield

Milk FA are derived from two sources: <16 carbon FA (de novo) from de novo synthesis in the mammary gland and >16 carbon FA (preformed) originating from extraction from plasma. Mixed source 16-carbon FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis in the mammary gland and extraction from plasma.

Overall effect of HOSB inclusion

Overall, compared with CON, SOY did not affect the yields de novo milk FA (P =0.94; Table 28), decreased mixed milk FA (P <0.001), and increased preformed milk FA (P <0.001). For select individual milk FA, compared with CON, SOY increased the yields of C4:0, C18:0, and *cis*-9 C18:1 (all P <0.001) but decreased the yield of C16:0 (P <0.001).

Effect of heat-treatment

For the effect of roasted vs. raw, compared with RAW, RST did not affect the yields of de novo (P =0.42; Table 28) or mixed milk FA (P =0.75), but increased preformed milk FA (P =0.01). For select individual milk FA, compared with RAW, RST increased yields of C4:0 (P

=0.02), *trans*-10 C18:1 (*P*<0.001), and *cis*-9 C18:1 (*P* <0.01) but did not affect yields of C16:0 (*P* =0.72) or C18:0 (*P* =0.21).

Effect additional by-pass protein for raw HOSB treatments

For the effect of protein, there was no effect of treatment between RAW-D and RAW-U for the yields of de novo (*P* =0.47; Table 28), mixed (*P* =0.38), or preformed milk FA (*P* =0.79). Additionally, there was no difference between RAW-D and RAW-U for select individual milk FA.

Plasma Metabolites

Compared with CON, SOY decreased plasma insulin (*P* = 0.03; Table 30) and plasma BUN (*P* < 0.001). Compared with RAW, RST decreased plasma BUN (*P* < 0.001) but did not affect plasma insulin (*P* = 0.94) and compared with RAW-D, RAW-U decreased plasma BUN (*P* = 0.03) but did not affect plasma insulin (*P* = 0.70).

Discussion

The use of conventional soybeans in dairy cattle diets has been a common nutrition practice, but often at low dietary inclusions due to high levels of C18:2 increasing the risk for milk fat depression (Bauman et al., 2011). Since dietary oils high in C18:2 can reduce milk fat secretion compared to oils high in *cis*-9 C18:1 (He et al., 2012; Dorea and Armentano, 2017), the high *cis*-9 C18:1 content of HOSB, compared with conventional soybeans, has the potential to reduce the risk of depressing milk fat synthesis (Weld and Armentano, 2018). Additionally, high-producing cows increase milk production in response to a higher content of *cis*-9 C18:1 in supplemental FA blends (de Souza et al., 2019; Western et al., 2020b; Burch et al., 2021).

There is limited literature evaluating milk production responses to HOSB feeding and there is no direct comparison determining if cow performance is impacted by roasted or raw HOSB. Additionally, supplementing by-pass protein to manipulate protein content (RDP vs. RUP) with inclusion of HOSB in dairy cow diets has yet to be evaluated. This information would be important to dairy producers as there is variability and costs associated with heat-treating soybeans. Bales and Lock (2023) was the first to evaluate increasing feeding rates of roasted and ground HOSB. The decision to grind the HOSB was based on results from Weld and Armentano (2018), as the authors observed ground HOSB increased milk fat yield compared with whole, HOSB. We evaluated 0, 8, 16, and 24% DM of HOSB inclusion to diets of high-producing dairy cows and observed a linear increase in milk production responses up to inclusion of 24%, but DMI was reduced with the highest level. Since our first study only utilized roasted HOSB and Weld and Armentano (2018) only used raw HOSB, it was necessary to determine the impact of roasted and raw HOSB on milk production responses. To our knowledge, only one study has directly compared roasted and raw conventional soybeans with or without additional by-pass protein (Grummer et al., 1994). Although the authors observed no difference between the roasted soybeans and raw soybeans + by-pass protein, they did observe that both of these treatments increased milk production compared to the diet containing only raw soybeans. Therefore, the objective of our study was to evaluate the effects of raw vs. roasted HOSB on milk production responses of high-producing, mid-lactation dairy cows. To our knowledge, this study is the first to assess differing processing methods of HOSB within a single study. We designed our HOSB treatments to contain 16% DM of HOSB, based on results from our initial study Bales and Lock (2023), and the HOSB replaced soybean meal and soybean hulls in the control treatment to keep NDF, forage NDF, CP, and starch (%DM) similar across diets. Additionally, to test protein

sources, we replaced soybean meal with a source of rumen by-pass soybean protein in one of the raw HOSB treatments to evaluate the impact of manipulating dietary RDP and RUP levels.

In our study, overall SOY increased DMI by ~0.50 kg/d compared to CON, but we did not observe an impact of heat treatment or protein source on DMI. This is similar to previous research evaluating differences between roasted and raw conventional soybeans, both whole and ground (Mielke and Schingoethe, 1981; Bernard, 1990; Tice et al., 1993) and when evaluating particle size of raw conventional soybeans and HOSB (Weld and Armentano, 2018). Grummer et al. (1994) also reported no difference in DMI when comparing RDP and RUP levels of raw or roasted, whole conventional soybeans. Although Tice et al. (1993) observed no difference between roasted vs. raw, whole soybeans, the authors did observe an increase in DMI with a smaller particle size of roasted soybeans. Additionally, increasing inclusion levels of both conventional soybeans and HOSB have been found to either decrease DMI at higher levels (Venturelli et al., 2015; Bales and Lock, 2023, respectively) or did not impact DMI (Knapp et al., 1991). The HOSB in our study were included at 16% DM and were ground to contain similar particle sizes (725 and 794 microns for roasted and raw HOSB, respectively), thus we could have potentially observed differences in DMI if we had different inclusion levels and grind sizes. Additionally, though DMI can be impacted when replacing plant protein with animal protein (Polan et al., 1997), we utilized a soy by-pass protein to replace soybean meal to adjust dietary RUP content; thus, the AA profile would be similar to that of soybean meal, with the largest difference being the degradability in the rumen. The increase in DMI was most likely related to the greater demand for nutrients required for increased milk production (NASEM, 2021).

High-producing dairy cows need protein sources high in RUP, as microbial protein alone is inadequate for milk production (VandeHaar and St-Pierre, 2006; Wang et al., 2010), thus the

increase in milk production when comparing roasted and raw HOSB is likely due to the increased RUP content (Chalupa, 1975; Grummer et al., 1994) supplying more rumen-bypass AA required for milk synthesis (Piantoni and VandeHaar, 2023). Milk production responses were impacted by roasting, as RST increased yields of milk and milk components compared with RAW. Roasted, whole conventional soybeans have been found to increase milk production compared with raw, whole conventional soybeans (Tice et al., 1993; Dhiman et al., 1997) whereas smaller particle size of roasted or raw conventional soybeans did not impact milk production yields (Chouinard et al., 1997; Amanlou et al., 2012). Faldet and Satter (1991) observed increased milk production across lactation when feeding roasted and cracked soybeans compared to soybeans that were raw and cracked. The authors further summarized 10 literature comparisons of roasted and raw soybeans and reported roasted soybeans to increase milk and 4% FCM yields by 1.4 and 1.0 kg/d, respectively, compared with raw soybeans. Although they attributed a large increase in milk production to alfalfa silage being the only forage in the diet, the authors also concluded that roasted soybeans improved milk production due to more lysine available to the small intestine and increased protein digestion (Faldet and Satter, 1991). Therefore, our RST treatment likely improved protein supply compared to RAW, due to improvements in AA balance and efficiency also indicated by the decrease in urea nitrogen concentrations in both plasma and milk (Wang et al., 2010). Also, raw soybeans contain a trypsin inhibitor (Mielke and Schingoethe, 1981), a compound that decreases protein digestion by degradation of dietary protein in the rumen that results in a reduced flow of AA available for absorption and utilization (Stern et al., 1985). Lysine is an AA that has been shown to have a greater loss during protein degradation in the rumen than other AA (Clark et al., 1987) but is also an AA in proteins involved in milk fat synthesis (Li et al., 2019). Thus, if the RAW treatments

had greater lysine degradation causing a smaller quantity absorbed, it may have negatively impacted milk fat synthesis. Overall, our results indicate that heat-treatment of HOSB will increase production responses compared to raw soybeans, and this should be considered when utilizing HOSB in diets for high-producing dairy cows.

Although there was a lack of difference for DMI, milk production responses were impacted by the addition of by-pass protein. Replacing soybean meal with a soy by-pass protein in the RAW-U treatment may have supplied more RUP compared with RAW-D, potentially providing more by-pass AA for mammary milk and milk protein synthesis (Piantoni and VandeHaar, 2023). Our results are similar to Grummer et al. (1994), who reported increased milk production with higher RUP diets (roasted, conventional and raw, conventional soybeans + by-pass protein) compared with raw, whole conventional soybeans. Although RAW-U increase milk protein yield compared with RAW-D, it did not alter milk fat yield, consistent with results of Nichols et al. (2018) where dietary protein adjustments impacted milk protein yield but did not impact milk fat response. Adjusting dietary RUP content to diets containing raw, ground HOSB improved milk production responses, suggesting that other dietary protein sources are an important consideration if utilizing raw HOSB in diets for high-producing dairy cows.

Overall inclusion of HOSB at 16% DM increased milk production responses, similar to observations with inclusion of conventional soybeans to dairy cow diets compared to control treatments (Tice et al., 1993; Amanlou et al., 2012).The increase in milk production responses due to SOY was expected, as higher-producing cows often benefit from rations with supplemental FA (Palmquist and Jenkins, 1980) and oilseeds have been observed to increase milk fat production (Banks et al., 1976; Rabiee et al., 2012). Additionally, SOY increased the intake of *cis*-9 C18:1, and research has shown that high-producing cows increase ECM and 3.5%

FCM with more *cis*-9 C18:1 in supplemental FA blends (de Souza et al., 2019; Western et al., 2020b; Burch et al., 2021). The increase in milk production responses to increased supply of *cis*-9 C18:1 could be attributed to the positive impacts of *cis*-9 C18:1 on bovine mammary epithelial cells, specifically mitochondrial activity and key proteins for milk fat synthesis (Liang et al., 2014; Cohen et al., 2015; Li et al., 2019). Milk protein yield was not impacted with SOY, similar to when comparing different particle sizes of HOSB (Weld and Armentano, 2018) or heat treatments of conventional soybeans (Amanlou et al., 2012). The lack of milk protein response between CON and SOY in our study was due to the raw HOSB treatments reducing the average milk protein yield for SOY. This could be due to protein degradation by the trypsin enzyme mentioned earlier, and if lysine absorption was impacted it would further explain the lack of protein response as lysine is a limiting essential AA (NASEM, 2021). This further emphasizes the importance of protein sources when feeding high-producing dairy cows. Overall, inclusion of HOSB in the diet of high-producing dairy cows was beneficial for improving milk production responses.

Overall HOSB inclusion and heat treatment impacted milk FA sources. SOY increased milk fat due to an increase in preformed milk FA, with *cis*-9 C18:1 making up 67% of the preformed milk FA yield. Weld and Armentano (2018) also observed raw HOSB to increase total preformed FA yield compared to a low-fat control diet, though the authors additionally reported a decrease in de novo milk FA yield. The observed increase in milk fat for RST was due to an increase in preformed milk FA yield, with *cis*-9 C18:1 making up ~75% of the 46 g/d increase in performed FA yield. Although Dorea and Armentano (2017) reported a negative relationship between dietary *cis*-9 C18:1 content and de novo milk FA yield in a meta-analysis, de Souza et al. (2019) reported an interdependence between de novo and preformed milk FA, as

high-producing cows receiving more *cis*-9 C18:1 in a FA blend increased yields of both de novo and preformed milk FA. This could be due to the positive effects of *cis*-9 C18:1 on mitochondrial activity that support milk biosynthesis and proteins involved in milk fat synthesis (Liang et al., 2014; Cohen et al., 2015; Favorit et al., 2021). We acknowledge that a portion of the increased *cis*-9 C18:1 in milk fat is due to the stearoyl Co-A desaturase enzyme converting C18:0 to *cis*-9 C18:1 for milk fluidity (Mosley and McGuire, 2007). It is important to also acknowledge that SOY decreased mixed milk FA yield, likely from de novo synthesis, but increased short chain milk FA, thus there was a shift in the FA profile from C4:0 to C16:0. The increase in milk fat yield was due predominantly to an increase in preformed milk FA, as de novo milk FA was not impacted by heat treatment or overall HOSB inclusion.

The use of the truncated Latin square design allowed us to evaluate BW change across longer-term periods. Though all cows were in a positive energy balance and gaining BW, there was a tendency for RST to have less BW gain compared to RAW (0.25 vs 0.45 kg/d), and overall SOY and protein source did not impact BW parameters. Thus, inclusion of HOSB to dairy cow diets increases milk production responses and maintains body condition. Due to HOSB containing mostly *cis*-9 C18:1, there will be extensive biohydrogenation resulting in other 18-carbon FA leaving the rumen, predominantly C18:0 (Maia et al., 2010), and although RST increased the yields of many *trans*-C18:1 FA, there is no indication of milk fat depression. Additionally, there was no *trans*-10, *cis*-12 CLA detected in milk fat samples of cows receiving HOSB, further indicating no negative impact on milk fat synthesis. Whole soybeans have greater protection than when the seed is processed (Jenkins and Bridges, 2007) but ruminal loss of *cis*-9 C18:1 in oilseeds has been estimated to be ~30-60% (Jenkins and Bridges, 2007; Barletta et al., 2016). Using these BH values and intakes of HOSB, we would expect a range of ~300 – 500 g/d

of *cis*-9 C18:1 for the HOSB treatments to reach the small intestine for absorption and utilization. Although the increase in *trans*-C18:1 FA could imply that roasting and grinding soybeans in milk fat have less protection for UFA in the rumen, the increase in *trans*-FA could also indicate greater FA digestibility and absorption, as we observed an increase in milk fat with roasted HOSB. Oleic acid is known to increase FA digestion and absorption compared with other FA (Boerman et al., 2015a; Prom and Lock, 2021; Prom et al., 2021), thus there is potential that the RST treatment increased FA absorption compared with the RAW treatments, indicated by the increase in *trans*-FA found in the milk fat. Additionally, there could be differences in rumen fermentation between the roasted and raw HOSB, as well as the potential for trypsin to disrupt the shield protecting the oil within the soybean seed (Huang, 1992) that could further impact fermentation and digestion.

Our results have practical implications and can be readily applied to nutrition programs. We designed the study to evaluate the effects of roasting HOSB, as the equipment needed to roast soybeans may not be cost effective for every dairy producer. Our results indicate that inclusion of HOSB, regardless of heat-treatment, can increase milk production responses, suggesting that raw HOSB can be utilized. However, our results would also suggest supplementing a rumen by-pass protein to raw HOSB diets to increase milk protein synthesis and support milk production. Due to RST increasing milk and milk components compared to RAW, roasting HOSB appears to maximize milk production responses to HOSB. Although our main objectives were to evaluate the roasted and raw HOSB, utilizing soybean meal and soybean hulls to replace HOSB, the control treatment was not isoenergetic compared with the HOSB treatments. Continued research examining HOSB should consider increasing the total FA content of a control diet to examine isoenergetic treatments, but it is more important to consider that the

addition of a FA supplement will not have the same FA profile as the HOSB. This will potentially influence results as cows will respond differently to supplements with different FA profiles (de Souza et al., 2018a, 2019; Burch et al., 2021). Also, future research should evaluate different nutrition strategies to minimize the reduction in de novo milk FA to continue to increase milk fat yield. Additionally, there should be consideration if there is potential that utilization of HOSB could replace some fat supplements or may have an additive effect to existing diets with supplemental FA, such as diets already containing a C16:0-enriched supplement.

Conclusion

The inclusion of ground HOSB at 16% diet DM to dairy cow diets increased DMI and increased yields of milk and milk components. Additionally, when evaluating heat treatment of ground HOSB, roasted improved milk production responses compared to raw, ground HOSB. When comparing protein sources, the inclusion of additional rumen by-pass protein to a raw HOSB diet increased milk protein yield and tended to increase ECM yield. This is the first study to evaluate the effects of heat treatment and protein source manipulation utilizing raw HOSB in diets formulated for high-producing dairy cows.

CHAPTER 8: OVERALL CONCLUSIONS

Inclusion of FA supplements and oilseeds can increase yields of milk and milk components and thus milk income. Oleic acid is a fatty acid we previously found to increase FA digestibility and milk components and reduce body weight loss early lactation and high-producing cows. It is critical to further understand how *cis*-9 C18:1 impacts milk production responses of dairy cattle and how this information can be best utilized in the feed of high-producing dairy cows. This will advance our understanding of the utilization and functionality of FA supplements and oilseeds in order to improve dairy cattle nutrition and thus increasing farm profitability. The main objective of this dissertation was to examine the effects of different levels of *cis-9* C18:1 in both FA supplements and oilseeds on nutrient digestibility and production responses of high-producing, mid-lactation dairy cows.

In Chapter 3, we evaluated the effectiveness of a *cis*-9 C18:1-enriched Ca-salt. In order to assess the efficacy of this novel Ca-salt, we tested the supplement alongside abomasal and ruminal infusions of *cis*-9 C18:1. The *cis*-9 C18:1-enriched Ca-salt increased nutrient digestibility compared with ruminal infusions of *cis*-9 C18:1, indicating the Ca-salt supplied additional *cis*-9 C18:1 past the rumen. Although milk production responses were not impacted by treatment, the study was not designed to determine milk production responses. Overall, a Ca-salt enriched in *cis*-9 C18:1 can effectively supply *cis-9* C18:1 past the rumen and therefore offers potential as a supplement to deliver *cis*-9 C18:1 in order to improve nutrient absorption and utilization.

Chapter 4 further examined the effect of *cis*-9 C18:1 in FA supplements, as we investigated if cow performance was due to the FA profile or the form of a FA supplement. We observed that nutrient digestibility was affected by the form of a FA supplement whereas milk

production responses were impacted only by the FA profile of the supplement. The 70% C16:0 + 20% *cis*-9 C18:1 FA blend increased digestibility of DM and NDF compared with a 70% C16:0 + 20% *cis*-9 C18:1 Ca-salt, although total FA digestibility and milk production responses were not different. We observed no difference between a 70% C16:0 + 20% *cis*-9 C18:1 Ca-salt and a 45% C16:0 + 35% *cis*-9 C18:1 Ca-salt for digestibility of DM and NDF, but the 45% C16:0 + 35% *cis-9* C18:1 Ca-salt increased total FA digestibility compared with the 70% C16:0 + 20% *cis*-9 C18:1 Ca-salt, likely due to the increase in *cis*-9 C18:1 content. Additionally, milk production responses differed between the two Ca-salts, further demonstrating that FA profile is more important than the form of a FA supplement. Results from this chapter will allow manufactures, nutritionists, and producers to make more informed decisions on specific fat supplements. Increased milk production responses can be achieved by using either a single product or a blend of multiple products to achieve a desired ratio of supplemental FA.

Chapters 5 and 6 were designed to examine the upper limits of oilseed feeding, and evaluated increasing inclusion of WCS and HOSB up to 24% DM. In Chapter 5, increasing inclusion of WCS impacted nutrient digestibility and milk production responses in high-producing dairy cows. DMI was greatest at 8% WCS while 24% WCS decreased nutrient intake. Although increasing WCS decreased FA digestibility, FA absorption increased. Yields of milk, milk fat, and milk protein were increased with 8 and 16% WCS, with the 16% WCS also doubling body weight gain. In Chapter 6, increasing HOSB up to 24% DM decreased DMI but increased yields of milk, milk fat, and milk protein, without an effect on BW. The increase in milk fat was due to an almost 2-fold increase in preformed milk FA; however improvements in fat yield were limited since increasing HOSB also decreased the yield of de novo milk FA.

In Chapter 7, we evaluated heat treatment of HOSB, as well as the impact of protein source when feeding raw HOSB. Overall inclusion of ground HOSB at 16% DM increased DMI and milk production responses. Roasted, ground HOSB increased yields of milk and milk components compared with raw HOSB. Additionally, supplementation of additional rumen by-pass protein increased milk protein in a diet containing raw HOSB. Results indicate that roasting HOSB will improve milk production responses, although consideration for the cost of equipment needed to roast soybeans may not be cost effective for every dairy producer.

Across these studies, milk fat yield was positively impacted by the use of FA supplements and oilseeds, primarily due to increases in preformed milk FA. However, decreases in the yield of de novo milk FA were also observed which somewhat limited potential further improvements in fat yields. The results in these experimental chapters lay the foundation for future research to continue to investigate dietary strategies to mitigate reductions in de novo milk FA yields and further increase milk fat responses.

Results discussed in this thesis support recent research in our lab and continue to improve nutritional strategies for high producing cows in the dairy industry. The results from Chapters 4 – 7 have direct impact on the dairy industry and provide important information that has immediate application.

REFERENCES

Abel-Caines, S.F., R.J. Grant, and S.G. Haddad. 1997. Whole Cottonseeds or a Combination of Soybeans and Soybean Hulls in the Diets of Lactating Dairy Cows. J. Dairy Sci. 80:1353–1357. doi:10.3168/jds.S0022-0302(97)76063-6.

Abou-Rjeileh, U., J.M. dos Santos Neto, M. Chirivi, N. O'Boyle, D. Salcedo, C.M. Prom, J. Laguna, J. Parales-Giron, A.L. Lock, and G.A. Contreras. 2023. Oleic acid abomasal infusion limits lipolysis and improves insulin sensitivity in adipose tissue from periparturient dairy cows. J. Dairy Sci.. doi:10.3168/jds.2022-22402.

Adams, A.L., B. Harris, H.H. Van Horn, and C.J. Wilcox. 1995. Effects of Varying Forage Types on Milk Production Responses to Whole Cottonseed, Tallow, and Yeast. J. Dairy Sci. 78:573–581. doi:10.3168/jds.S0022-0302(95)76668-1.

Allen, M.S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624. doi:10.3168/jds.S0022-0302(00)75030-2.

Amanlou, H., N. Sis, S. Bassiri, N. author No author, A. Aghazadeh, R. Salamatdust, and A. Karimi. 2012. Nutritional value of raw soybeans, extruded soybeans, roasted soybeans and tallow as fat sources in early lactating dairy cows. Open Vet. J. 5:88. doi:10.5455/ovj.2012.v2.i0.p88.

Arieli, A. 1998. Whole cottonseed in dairy cattle feeding: A review. Anim. Feed Sci. Technol. 72:97–110. doi:10.1016/S0377-8401(97)00169-7.

Bales, A.M., M.E. Cinzori, and A.L.L. Lock. 2024. Increasing palmitic acid and reducing stearic acid content of supplemental fatty acid blends improves production performance of mid-lactation dairy cows. J. Dairy Sci. 107:278–287. doi:10.3168/jds.2023-23874.

Bales, A.M., J. de Souza, and A.L. Lock. 2023. Milk production responses of dairy cows to fatty acid supplements with different ratios of palmitic and oleic acids in low- and high-fat basal diets. JDS Commun.. doi:10.3168/jdsc.2023-0415.

Banks, W., J.L. Clapperton, M.E. Ferrie, and A.G. Wilson. 1976. Effect of feeding fat to dairy cows receiving a fat-deficient basal diet I. Milk Yield and Composition. J. Dairy Res. 43:219–227. doi:10.1017/S0022029900015776.

Barbano, D.M., and J.W. Sherbon. 1980. Polyunsaturated Protected Lipid: Effect on Triglyceride Molecular Weight Distribution. J. Dairy Sci. 63:731–740. doi:10.3168/jds.S0022-0302(80)83000-1.

Barber, M.C., R.A. Clegg, M.T. Travers, and R.G. Vernon. 1997. Lipid metabolism in the

lactating mammary gland. Biochim. Biophys. Acta - Lipids Lipid Metab. 1347:101–126. doi:10.1016/S0005-2760(97)00079-9.

Barletta, R. V., J.R. Gandra, V.P. Bettero, C.E. Araújo, T.A. Del Valle, G.F. d. Almeida, E. Ferreira de Jesus, R.D. Mingoti, B.C. Benevento, J.E. d. Freitas Júnior, and F.P. Rennó. 2016. Ruminal biohydrogenation and abomasal flow of fatty acids in lactating cows: Oilseed provides ruminal protection for fatty acids. Anim. Feed Sci. Technol. 219:111–121. doi:10.1016/j.anifeedsci.2016.06.011.

Bauchart, D. 1993. Lipid Absorption and Transport in Ruminants. J. Dairy Sci. 76:3864–3881. doi:10.3168/jds.S0022-0302(93)77728-0.

Bauman, D. E., and C. L. Davis. 1974. Biosynthesis of milk fat. In: B. L. Larson, and V. R. Smith (Eds.) Lactation: A Comprehensive Treatise. Vol. 2. Academic Press, New York, pp. 31- 75.

Bauman, D.E., and J.M. Griinari. 2003. Nutritional Regulation of Milk Fat Synthesis. Annu. Rev. Nutr. 23:203–227. doi:10.1146/annurev.nutr.23.011702.073408.

Bauman, D.E., K.J. Harvatine, and A.L. Lock. 2011. Nutrigenomics, Rumen-Derived Bioactive Fatty Acids, and the Regulation of Milk Fat Synthesis. Annu. Rev. Nutr. 31:299–319. doi:10.1146/annurev.nutr.012809.104648.

Beauchemin, K.A., S.M. McGinn, C. Benchaar, and L. Holtshausen. 2009. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: Effects on methane production, rumen fermentation, and milk production. J. Dairy Sci. 92:2118–2127. doi:10.3168/jds.2008-1903.

Bernard, J.K. 1990. Effect of Raw or Roasted Whole Soybeans on Digestibility of Dietary Nutrients and Milk Production of Lactating Dairy Cows. J. Dairy Sci. 73:3231–3236. doi:10.3168/jds.S0022-0302(90)79015-7.

Blauwiekel, R., S. Xu, J.H. Harrison, K.A. Loney, R.E. Riley, and M.C. Calhoun. 1997. Effect of Whole Cottonseed, Gossypol, and Ruminally Protected Lysine Supplementation on Milk Yield and Composition. J. Dairy Sci. 80:1358–1365. doi:10.3168/jds.S0022-0302(97)76064-8.

Boerman, J.P., J.L. Firkins, N.R. St-Pierre, and A.L. Lock. 2015a. Intestinal digestibility of long-chain fatty acids in lactating dairy cows: A meta-analysis and meta-regression. J. Dairy Sci. 98:8889–8903. doi:10.3168/jds.2015-9592.

Boerman, J.P., S.B. Potts, M.J. VandeHaar, and A.L. Lock. 2015b. Effects of partly replacing dietary starch with fiber and fat on milk production and energy partitioning. J. Dairy Sci. 98:7264–7276. doi:10.3168/jds.2015-9467.

Boerman, J.P., J. de Souza, and A.L. Lock. 2017. Milk production and nutrient digestibility responses to increasing levels of stearic acid supplementation of dairy cows. J. Dairy Sci. 100:2729–2738. doi:10.3168/jds.2016-12101.

Bomberger, R., E. Barnoff, and K. J. Harvatine. 2019. High oleic soybeans increase milk fat yield at high and low inclusion levels. J. Dairy Sci. 102(Suppl. 1):317.

Bradford, B.J., K.J. Harvatine, and M.S. Allen. 2008. Dietary unsaturated fatty acids increase plasma glucagon-like peptide-1 and cholecystokinin and may decrease premeal ghrelin in lactating dairy cows. J. Dairy Sci. 91:1443–1450. doi:10.3168/jds.2007-0670.

Buccioni, A., M. Decandia, S. Minieri, G. Molle, and A. Cabiddu. 2012. Lipid metabolism in the rumen: New insights on lipolysis and biohydrogenation with an emphasis on the role of endogenous plant factors. Anim. Feed Sci. Technol. 174:1–25. doi:10.1016/j.anifeedsci.2012.02.009.

Bullock, S.L., D.G. Hewitt, R.L. Stanko, M.K. Dowd, J. Rutledge, and D.A. Draeger. 2010. Plasma gossypol dynamics in white-tailed deer: Implications for whole cottonseed as a supplemental feed. Small Rumin. Res. 93:165–170. doi:10.1016/j.smallrumres.2010.05.018.

Burch, A.M., A. Pineda, and A.L. Lock. 2021. Effect of palmitic acid-enriched supplements containing stearic or oleic acid on nutrient digestibility and milk production of low- and high-producing dairy cows. J. Dairy Sci. 104:8673–8684. doi:10.3168/jds.2020-19913.

Cant, J.P., E.J. DePeters, and R.L. Baldwin. 1993. Mammary Amino Acid Utilization in Dairy Cows Fed Fat and Its Relationship to Milk Protein Depression. J. Dairy Sci. 76:762–774. doi:10.3168/jds.S0022-0302(93)77400-7.

Casper, D.P., D.J. Schingoethe, R.P. Middaugh, and R.J. Baer. 1988. Lactational Responses of Dairy Cows to Diets Containing Regular and High Oleic Acid Sunflower Seeds. J. Dairy Sci. 71:1267–1274. doi:10.3168/jds.S0022-0302(88)79682-4.

Chalupa, W. 1975. Rumen Bypass and Protection of Proteins and Amino Acids. J. Dairy Sci. 58:1198–1218. doi:10.3168/jds.S0022-0302(75)84697-2.

Chalupa, W., J.L. Evans, and M.C. Stillions. 1964. Influence of ethanol on rumen fermentation and nitrogen metabolism. J. Anim. Sci. 23:802–807. doi:https://doi.org/10.2527/jas1964.233802x.

Chalupa, W., B. Vecchiarelli, A.E. Elser, D.S. Kronfeld, D. Sklan, and D.L. Palmquist. 1986. Ruminal Fermentation In Vivo as Influenced by Long-Chain Fatty Acids. J. Dairy Sci. 69:1293–1301. doi:10.3168/jds.S0022-0302(86)80535-5.

Chelikani, P.K., J.A. Bell, and J.J. Kennelly. 2004. Effect of feeding or abosomal infusion of canola oil in Holstein cows 1. Nutrient digestion and milk composition. J. Dairy Res. 71:279–287. doi:10.1017/S0022029904000287.

Chenoweth, P.J., C.C. Chase, C.A. Risco, and R.E. Larsen. 2000. Characterization of gossypol-induced sperm abnormalities in bulls. Theriogenology 53:1193–1203.

Choi, B.R., and D.L. Palmquist. 1996. High fat diets increase plasma cholecystokinin and pancreatic polypeptide, and decrease plasma insulin and feed intake in lactating cows.. J. Nutr. 126:2913–9. doi:10.1093/jn/126.11.2913.

Choi, B.R., D.L. Palmquist, and M.S. Allen. 2000. Cholecystokinin mediates depression of feed intake in dairy cattle fed high fat diets. Domest. Anim. Endocrinol. 19:159–175. doi:10.1016/S0739-7240(00)00075-8.

Chouinard, P.Y., V. Girard, and G.J. Brisson. 1997. Performance and Profiles of Milk Fatty Acids of Cows Fed Full Fat, Heat-Treated Soybeans Using Various Processing Methods. J. Dairy Sci. 80:334–342. doi:10.3168/jds.S0022-0302(97)75943-5.

Cifarelli, V., and N.A. Abumrad. 2018. Intestinal CD36 and other key proteins of lipid utilization: Role in absorption and gut homeostasis. Compr. Physiol. 8:493–507. doi:10.1002/cphy.c170026.

Clark, J.H., M.R. Murphy, and B.A. Crooker. 1987. Supplying the Protein Needs of Dairy Cattle from By-Product Feeds. J. Dairy Sci. 70:1092–1109. doi:10.3168/jds.S0022-0302(87)80116-9.

Clark, P.W., and L.E. Armentano. 1993. Effectiveness of Neutral Detergent Fiber in Whole Cottonseed and Dried Distillers Grains Compared with Alfalfa Haylage. J. Dairy Sci. 76:2644–2650. doi:10.3168/jds.S0022-0302(93)77600-6.

Clark, P.W., and L.E. Armentano. 1999. Influence of particle size on the effectiveness of the fiber in corn silage. J. Dairy Sci. 82:581–588. doi:10.3168/jds.S0022-0302(99)75270-7.

Cochran, R.C., D.C. Adams, J.D. Wallace, and M.L. Galyean. 1986. Predicting Digestbility of Different Diets with Internal Markers: Evaluation of Four Potential Markers 1476–1483.

Cohen, B.C., A. Shamay, and N. Argov-Argaman. 2015. Regulation of lipid droplet size in mammary epithelial cells by remodeling of membrane lipid composition-a potential mechanism. PLoS One 10:1–19. doi:10.1371/journal.pone.0121645.

Coppock, C.E., J.K. Lanham, and J.I. Horner. 1987. A Review of the Nutritive Value and Utilization of Whole Cottonseed, Cottonseed Meal and Associated By-Products by Dairy

Cattle. Anim. Feed Sci. Technol. 18:89–129.

Coppock, C.E., J.W. West, J.R. Moya, D.H. Nave, J.M. LaBore, K.G. Thompson, L.D. Rowe, and C.E. Gates. 1985. Effects of Amount of Whole Cottonseed on Intake, Digestibility, and Physiological Responses of Dairy Cows. J. Dairy Sci. 68:2248–2258. doi:10.3168/jds.S0022-0302(85)81097-3.

Dai, X.J., C. Wang, and Q. Zhu. 2011. Milk performance of dairy cows supplemented with rapeseed oil, peanut oil and sunflower seed oil. Czech J. Anim. Sci. 56:181–191. doi:10.17221/1434-cjas.

de Souza, J., C.L. Preseault, and A.L. Lock. 2018a. Altering the ratio of dietary palmitic, stearic, and oleic acids in diets with or without whole cottonseed affects nutrient digestibility, energy partitioning, and production responses of dairy cows. J. Dairy Sci. 101:172–185. doi:10.3168/jds.2017-13460.

de Souza, J., C.M. Prom, and A.L. Lock. 2021. Altering the ratio of dietary palmitic and oleic acids affects production responses during the immediate postpartum and carryover periods in dairy cows. J. Dairy Sci. 104:2896–2909. doi:10.3168/jds.2020-19311.

de Souza, J., N.R. St-Pierre, and A.L. Lock. 2019. Altering the ratio of dietary C16:0 and cis-9 C18:1 interacts with production level in dairy cows: Effects on production responses and energy partitioning. J. Dairy Sci. 102:9842–9856. doi:10.3168/jds.2019-16374.

de Souza, J., M.M. Western, and A.L. Lock. 2020. Abomasal infusion of an exogenous emulsifier improves fatty acid digestibility and milk fat yield of lactating dairy cows. J. Dairy Sci. 103:6167–6177. doi:10.3168/jds.2020-18239.

de Souza, R.A., R.J. Tempelman, M.S. Allen, W.P. Weiss, J.K. Bernard, and M.J. VandeHaar. 2018b. Predicting nutrient digestibility in high-producing dairy cows. J. Dairy Sci. 101:1123–1135. doi:10.3168/jds.2017-13344.

DePeters, E.J., and J.P. Cant. 1992. Nutritional Factors Influencing the Nitrogen Composition of Bovine Milk: A Review. J. Dairy Sci. 75:2043–2070. doi:10.3168/jds.S0022-0302(92)77964-8.

Dewanckele, L., P.G. Toral, B. Vlaeminck, and V. Fievez. 2020. Invited review: Role of rumen biohydrogenation intermediates and rumen microbes in diet-induced milk fat depression: An update. J. Dairy Sci. 103:7655–7681. doi:10.3168/jds.2019-17662.

Dhiman, T.R., A.C. Korevaar, and L.D. Satter. 1997. Particle Size of Roasted Soybeans and the Effect on Milk Production of Dairy Cows. J. Dairy Sci. 80:1722–1727. doi:10.3168/jds.S0022-0302(97)76104-6.

Dils, R.R. 1986. Comparative Aspects of Milk Fat Synthesis. J. Dairy Sci. 69:904–910. doi:10.3168/jds.S0022-0302(86)80480-5.

dos Santos Neto, J. M., A. M. Burch, and A. L. Lock. 2022. A meta-regression evaluating the effect of increasing dietary whole cottonseed on nutrient digestibility and production responses of lactating dairy cows. J. Dairy Sci. 105(Suppl. 1):363

dos Santos Neto, J.M., C.M. Prom, and A.L. Lock. 2023a. Comparison of rumen and abomasal infusions of an exogenous emulsifier on fatty acid digestibility of lactating dairy cows. J. Dairy Sci. 106:6789–6797. doi:10.3168/jds.2022-23143.

dos Santos Neto, J.M., C.M. Prom, and A.L. Lock. 2023b. Abomasal infusion of oleic acid and exogenous emulsifier alter fatty acid digestibility and production responses of lactating dairy cows. J. Dairy Sci. 106:7591–7601. doi:10.3168/jds.2022-23038.

dos Santos Neto, J.M., J. de Souza, and A.L. Lock. 2021a. Nutrient digetibility and production responses of lactating dairy cows when saturated free fatty acid supplements are included in diets: A meta-analysis. J. Dairy Sci. 104:12628–12646. doi:10.3168/jds.2021-20699.

dos Santos Neto, J.M., J. de Souza, and A.L. Lock. 2021b. Effects of calcium salts of palm fatty acids on nutrient digestibility and production responses of lactating dairy cows: A meta-analysis and meta-regression. J. Dairy Sci.. doi:10.3168/jds.2020-19936.

Dorea, J.R.R., and L.E. Armentano. 2017. Effects of common dietary fatty acids on milk yield and concentrations of fat and fatty acids in dairy cattle. Anim. Prod. Sci. 57:2224–2236. doi:10.1071/AN17335.

Douglas, G.N., J. Rehage, A.D. Beaulieu, A.O. Bahaa, and J.K. Drackley. 2007. Prepartum nutrition alters fatty acid composition in plasma, adipose tissue, and liver lipids of periparturient dairy cows. J. Dairy Sci. 90:2941–2959. doi:10.3168/jds.2006-225.

Drackley, J.K. 2004. Overview of fat digestion and metabolism in dairy cows.

Drackley, J.K., T.R. Overton, G. Ortiz-Gonzalez, A.D. Beaulieu, D.M. Barbano, J.M. Lynch, and E.G. Perkinst. 2007. Responses to increasing amounts of high-oleic sunflower fatty acids infused into the abomasum of lactating dairy cows. J. Dairy Sci. 90:5165–5175. doi:10.3168/jds.2007-0122.

Durix, A., C. Jean-Blain, H.P. Sallmann, and J.P. Jouany. 1991. Use of a semicontinuous culture system (RUSITEC) to study the metabolism of ethanol in the rumen and its effects on ruminal digestion. Can. J. Anim. Sci. 71:115–123. doi:10.4141/cjas91-013.

Emery, R.S. 1973. Biosynthesis of Milk Fat. J. Dairy Sci. 56:1187–1195. doi:10.3168/jds.S0022-

0302(73)85334-2.

Enjalbert, F., M.C. Nicot, C. Bayourthe, and R. Moncoulon. 1998. Duodenal infusions of palmitic, stearic or oleic acids differently affect mammary gland metabolism of fatty acids in lactating dairy cows. J. Nutr. 128:1525–1532. doi:10.1093/jn/128.9.1525.

Faldet, M.A., and L.D. Satter. 1991. Feeding Heat-Treated Full Fat Soybeans to Cows in Early Lactation. J. Dairy Sci. 74:3047–3054. doi:10.3168/jds.S0022-0302(91)78490-7.

Favorit, V., W.R. Hood, A.N. Kavazis, P. Villamediana, K.N. Yap, H.A. Parry, and A.L. Skibiel. 2021. Mitochondrial bioenergetics of extramammary tissues in lactating dairy cattle. Animals 11. doi:10.3390/ani11092647.

Freeman, C.P. 1969. Properties of fatty acids in dispersions of emulsified lipid and bile salt and the significance of these properties in fat absorption in the pig and the sheep. Br. J. Nutr. 23:249–263. doi:10.1079/bjn19690032.

Glasser, F., A. Ferlay, and Y. Chilliard. 2008a. Oilseed lipid supplements and fatty acid composition of cow milk: A meta-analysis. J. Dairy Sci. 91:4687–4703. doi:10.3168/jds.2008-0987.

Glasser, F., A. Ferlay, M. Doreau, P. Schmidely, D. Sauvant, and Y. Chilliard. 2008b. Long-chain fatty acid metabolism in dairy cows: A meta-analysis of milk fatty acid yield in relation to duodenal flows and De Novo synthesis. J. Dairy Sci. 91:2771–2785. doi:10.3168/jds.2007-0383.

Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications). Agri- cultural Handbook no. 379. USDA Agricultural Research Service.

Grummer, R.R., M.L. Luck, and J.A. Barmore. 1994. Lactational Performance of Dairy Cows Fed Raw Soybeans, with or Without Animal By-product Proteins, or Roasted Soybeans. J. Dairy Sci. 77:1354–1359. doi:10.3168/jds.S0022-0302(94)77074-0.

Hackmann, T.J., and J.L. Firkins. 2015. Maximizing efficiency of rumen microbial protein production 6:1–16. doi:10.3389/fmicb.2015.00465.

Hara, A., and N.S. Radin. 1978. Lipid Extraction of Tissues. Anal. Biochem. 90:420–426.

Harvatine, K.J., and M.S. Allen. 2005. The effect of production level on feed intake, milk yield, and endocrine responses to two fatty acid supplements in lactating cows. J. Dairy Sci. 88:4018–4027. doi:10.3168/jds.S0022-0302(05)73088-5.

Harvatine, K.J., and M.S. Allen. 2006. Effects of fatty acid supplements on milk yield and energy balance of lactating dairy cows. J. Dairy Sci. 89:1081–1091. doi:10.3168/jds.S0022-0302(06)72176-2.

Hauser, H., G.P. Hazlewood, and R.M.C. Dawson. 1979. Membrane fluidity of a fatty acid auxotroph grown with palmitic acid [18]. Nature 279:536–538. doi:10.1038/279536a0.

Hawkins, G.E., K.A. Cummins, M. Silverio, and J.J. Jilek. 1985. Physiological Effects of Whole Cottonseed in the Diet of Lactating Dairy Cows. J. Dairy Sci. 68:2608–2614. doi:10.3168/jds.S0022-0302(85)81144-9.

He, M., and L.E. Armentano. 2011. Effect of fatty acid profile in vegetable oils and antioxidant supplementation on dairy cattle performance and milk fat depression. J. Dairy Sci. 94:2481–2491. doi:10.3168/jds.2010-3755.

He, M., K.L. Perfield, H.B. Green, and L.E. Armentano. 2012. Effect of dietary fat blend enriched in oleic or linoleic acid and monensin supplementation on dairy cattle performance, milk fatty acid profiles, and milk fat depression. J. Dairy Sci. 95:1447–1461. doi:10.3168/jds.2011-4635.

Huang, A.H.C. 1992. Oil bodies and oleosins in seeds. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43:177–200. doi:10.1146/annurev.pp.43.060192.001141.

Jenkins, T.C. 1993. Lipid Metabolism in the Rumen. J. Dairy Sci. 76:3851–3863. doi:10.3168/jds.S0022-0302(93)77727-9.

Jenkins, T.C., and W.C. Bridges. 2007. Protection of fatty acids against ruminal biohydrogenation in cattle. Eur. J. Lipid Sci. Technol. 109:778–789. doi:10.1002/ejlt.200700022.

Jenkins, T.C., and K.J. Harvatine. 2014. Lipid Feeding and Milk Fat Depression. Vet. Clin. North Am. - Food Anim. Pract. 30:623–642. doi:10.1016/j.cvfa.2014.07.006.

Jenkins, T.C., and D.L. Palmquist. 1984. Effect of Fatty Acids or Calcium Soaps on Rumen and Total Nutrient Digestibility of Dairy Rations. J. Dairy Sci. 67:978–986. doi:10.3168/jds.S0022-0302(84)81396-X.

Jenkins, T.C., R.J. Wallace, P.J. Moate, and E.E. Mosley. 2008. Board-Invited Review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. J. Anim. Sci. 86:397–412. doi:10.2527/jas.2007-0588.

Jensen, R.G. 2002. The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 85:295–350. doi:10.3168/jds.S0022-0302(02)74079-4.

Jensen, R.G., A.M. Ferris, and C.J. Lammi-Keefe. 1991. The Composition of Milk Fat. J. Dairy Sci. 74:3228–3243. doi:10.3168/jds.S0022-0302(91)78509-3.

Johnson, K.A., R.L. Kincaid, H.H. Westberg, C.T. Gaskins, B.K. Lamb, and J.D. Cronrath. 2002. The effect of oilseeds in diets of lactating cows on milk production and methane emissions. J. Dairy Sci. 85:1509–1515. doi:10.3168/jds.S0022-0302(02)74220-3.

Kadegowda, A.K.G., M. Bionaz, L.S. Piperova, R.A. Erdman, and J.J. Loor. 2009. Peroxisome proliferator-activated receptor-γ activation and long-chain fatty acids alter lipogenic gene networks in bovine mammary epithelial cells to various extents. J. Dairy Sci. 92:4276–4289. doi:10.3168/jds.2008-1932.

Kazama, R., C. Côrtes, D. da Silva-Kazama, N. Gagnon, C. Benchaar, L.M. Zeoula, G.T.D. Santos, and H. V. Petit. 2010. Abomasal or ruminal administration of flax oil and hulls on milk production, digestibility, and milk fatty acid profile of dairy cows. J. Dairy Sci. 93:4781–4790. doi:10.3168/jds.2010-3219.

Kelly, M.L., J.R. Berry, D.A. Dwyer, J.M. Griinari, P.Y. Chouinard, M.E. Van Amburgh, and D.E. Bauman. 1998. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr. 128:881–885. doi:10.1093/jn/128.5.881.

Kinsella, J.E., D. Bruns, and J.P. Infante. 1975. Fatty acid synthetase of bovine mammary: Properties and products. Lipids 10:227–237. doi:10.1007/BF02532485.

Knapp, D.M., R.R. Grummer, and M.R. Dentine. 1991. The Response of Lactating Dairy Cows to Increasing Levels of Whole Roasted Soybeans. J. Dairy Sci. 74:2563–2572. doi:10.3168/jds.S0022-0302(91)78434-8.

Knudsen, J., and I. Grunnet. 1980. Primer Specificity of Mammalian Mammary Gland Fatty Acid Synthetases. Biochem. Biophys. Res. Commun. 95:1808–1814.

LaCount, D.W., J.K. Drackley, S.O. Laesch, and J.H. Clark. 1994. Secretion of Oleic Acid in Milk Fat in Response to Abomasal Infusions of Canola or High Oleic Sunflower Fatty Acids. J. Dairy Sci. 77:1372–1385. doi:10.3168/jds.S0022-0302(94)77076-4.

Li, X., P. Li, L. Wang, M. Zhang, and X. Gao. 2019. Lysine Enhances the Stimulation of Fatty Acids on Milk Fat Synthesis via the GPRC6A-PI3K-FABP5 Signaling in Bovine Mammary Epithelial Cells. J. Agric. Food Chem. 67:7005–7015. doi:10.1021/acs.jafc.9b02160.

Liang, M.Y., X.M. Hou, B. Qu, N. Zhang, N. Li, Y.J. Cui, Q.Z. Li, and X.J. Gao. 2014. Functional analysis of FABP3 in the milk fat synthesis signaling pathway of dairy cow mammary epithelial cells. Vitr. Cell. Dev. Biol. - Anim. 50:865–873. doi:10.1007/s11626-

014-9780-z.

Lin, C., and L. Kung. 1999. Heat Treated Soybeans and Soybean Meal in Ruminant Nutrition. Proc. Tech. Bull. Am. Soy Assoc. 1–18.

Lin, C.Y., and S. Kumar. 1972. Pathway for the synthesis of fatty acids in mamalian tissues.. J. Biol. Chem. 247:604–606. doi:10.1016/s0021-9258(19)45745-1.

Lindmark Månsson, H. 2008. Fatty acids in bovine milk fat. Food Nutr. Res. 52:4–7. doi:10.3402/fnr.v52i0.1821.

Linzell, J.L. 1972. Mechanism of Secretion of the Aqueous Phase of Milk. J. Dairy Sci. 55:1316–1322. doi:10.3168/jds.S0022-0302(72)85670-4.

Litherland, N.B., A.D. Beaulieu, C.K. Reynolds, and J.K. Drackley. 2023. Effects of esterification, saturation and amount of fatty acids infused into the rumen or abomasum in lactating dairy cows. J. Anim. Physiol. Anim. Nutr. (Berl). 107:12–27. doi:10.1111/jpn.13690.

Lock, A.L., K.J. Harvatine, J.K. Drackley, and D.E. Bauman. 2006. Concepts in fat and fatty acid digestion in ruminants. Proc Intermt. Nutr Conf 85–100.

Lock, A.L., K.J. Harvatine, I. Ipharraguerre, M. Van Amburgh, J.K. Drackley, and D.E. Bauman. 2005. The Dynamics of Fat Digestion in Lactating Dairy Cows: What Does the Literature Tell Us? 19:55.

Lock, A.L., C.L. Preseault, J.E. Rico, K.E. DeLand, and M.S. Allen. 2013. Feeding a C16:0-enriched fat supplement increased the yield of milk fat and improved conversion of feed to milk. J. Dairy Sci. 96:6650–6659. doi:10.3168/jds.2013-6892.

Lock, A.L., C. Tyburczy, D.A. Dwyer, K.J. Harvatine, F. Destaillats, Z. Mouloungui, L. Candy, and D.E. Bauman. 2007. Trans-10 octadecenoic acid does not reduce milk fat synthesis in dairy cows. J. Nutr. 137:71–76. doi:10.1093/jn/137.1.71.

Lopes, J.C., M.T. Harper, F. Giallongo, J. Oh, L. Smith, A.M. Ortega-Perez, S.A. Harper, A. Melgar, D.M. Kniffen, R.A. Fabin, and A.N. Hristov. 2017. Effect of high-oleic-acid soybeans on production performance, milk fatty acid composition, and enteric methane emission in dairy cows. J. Dairy Sci. 100:1122–1135. doi:10.3168/jds.2016-11911.

Lourenço, M., E. Ramos-Morales, and R.J. Wallace. 2010. The role of microbes in rumen lipolysis and biohydrogenation and their manipulation. Animal 4:1008–1023. doi:10.1017/S175173111000042X.

Mackie, R.I., B.A. White, and M.P. Bryant. 1991. Lipid metabolism in anaerobic ecosystems. Crit. Rev. Microbiol. 17:449–479. doi:10.3109/10408419109115208.

Maia, M.R., L.C. Chaudhary, C.S. Bestwick, A.J. Richardson, N. McKain, T.R. Larson, I.A. Graham, and R.J. Wallace. 2010. Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, Butyrivibrio fibrisolvens. BMC Microbiol. 10:8–10. doi:10.1186/1471-2180-10-52.

Mena, H., J.E.P. Santos, J.T. Huber, J.M. Simas, M. Tarazon, and M.C. Calhoun. 2001. The effects of feeding varying amounts of gossypol from whole cottonseed and cottonseed meal in lactating dairy cows. J. Dairy Sci. 84:2231–2239. doi:10.3168/jds.S0022-0302(01)74670-X.

Mielke, C.D., and D.J. Schingoethe. 1981. Heat-Treated Soybeans for Lactating Cows. J. Dairy Sci. 64:1579–1585. doi:10.3168/jds.S0022-0302(81)82728-2.

Mohamed, O.E., L.D. Satter, R.R. Grummer, and F.R. Ehle. 1988. Influence of Dietary Cottonseed and Soybean on Milk Production and Composition. J. Dairy Sci. 71:2677–2688. doi:10.3168/jds.S0022-0302(88)79861-6.

Moore, J. H. and W. W. Christie. 1979. Lipid metabolism in the mammary gland of ruminant animals. Prog. Lipid. Res. 17:347-395.

Moreira, V.R., L.D. Satter, and B. Harding. 2004. Comparison of conventional linted cottonseed and mechanically delinted cottonseed in diets for dairy cows. J. Dairy Sci. 87:131–138. doi:10.3168/jds.S0022-0302(04)73150-1.

Mosley, E.E., and M.A. McGuire. 2007. Methodology for the in vivo measurement of the Δ9-desaturation of myristic, palmitic, and stearic acids in lactating dairy cattle. Lipids 42:939–945. doi:10.1007/s11745-007-3085-x.

Mosley, E.E., G.L. Powell, M.B. Riley, and T.C. Jenkins. 2002. Microbial biohydrogenation of oleic acid to trans isomers in vitro. J. Lipid Res. 43:290–296.

National Academies of Sciences, Engineering, and M. 2021. Nutrient Requirements of Dairy Cattle. Eigth Revi. The National Academies Press, Washington, DC.

National Research, C. 2001. Nutrient Requirements of Dairy Cattle. Seventh Re. The National Academies Press, Washington., DC.

Nichols, K., A. Bannink, S. Pacheco, H.J. van Valenberg, J. Dijkstra, and H. van Laar. 2018. Feed and nitrogen efficiency are affected differently but milk lactose production is stimulated equally when isoenergetic protein and fat is supplemented in lactating dairy cow

diets. J. Dairy Sci. 101:7857–7870. doi:10.3168/jds.2017-14276.

Noble, R.C. 1978. Digestion, absorption and transport of lipids in ruminant animals. Prog. Lipid Res. 17:55–91. doi:10.1016/0079-6832(78)90005-8.

Oba, M., and K. Kammes-Main. 2023. Symposium review: Effects of carbohydrate digestion on feed intake and fuel supply. J. Dairy Sci. 106:2153–2160. doi:10.3168/jds.2022-22420.

Palmquist, D.L. 1991. Influence of Source and Amount of Dietary Fat on Digestibility in Lactating Cows. J. Dairy Sci. 74:1354–1360. doi:10.3168/jds.S0022-0302(91)78290-8.

Palmquist, D.L. 2006. Milk fat: Origin of fatty acids and influence of nutritional factors thereon. Adv. Dairy Chem. 2:43–92. doi:10.1007/0-387-28813-9_2.

Palmquist, D.L., C.L. Davis, R.E. Brown, and D.S. Sachan. 1969. Availability and Metabolism of Various Substrates in Ruminants. V. Entry Rate into the Body and Incorporation into Milk Fat of d(−)β-Hydroxybutyrate. J. Dairy Sci. 52:633–638. doi:10.3168/jds.S0022-0302(69)86620-8.

Palmquist, D.L., and T.C. Jenkins. 1980. Fat in Lactation Rations: Review. J. Dairy Sci. 63:1–14. doi:10.3168/jds.S0022-0302(80)82881-5.

Palmquist, D.L., and T.C. Jenkins. 2017. A 100-Year Review: Fat feeding of dairy cows. J. Dairy Sci. 100:10061–10077. doi:10.3168/jds.2017-12924.

Palmquist, D.L., A.L. Lock, K.J. Shingfield, and D.E. Bauman. 2005. Biosynthesis of Conjugated Linoleic Acid in Ruminants and Humans. Adv. Food Nutr. Res. 50:179–217. doi:10.1016/S1043-4526(05)50006-8.

Piantoni, P., A.L. Lock, and M.S. Allen. 2013. Palmitic acid increased yields of milk and milk fat and nutrient digestibility across production level of lactating cows. J. Dairy Sci. 96:7143–7154. doi:10.3168/jds.2013-6680.

Piantoni, P., A.L. Lock, and M.S. Allen. 2014. Milk production responses to dietary stearic acid vary by production level in dairy cattle. J. Dairy Sci. 98:1938–1949. doi:10.3168/jds.2014-8634.

Piantoni, P., A.L. Lock, and M.S. Allen. 2015. Saturated fat supplementation interacts with dietary forage neutral detergent fiber content during the immediate postpartum and carryover periods in Holstein cows: Production responses and digestibility of nutrients. J. Dairy Sci. 98:3309–3322. doi:10.3168/jds.2014-8798.

Piantoni, P., and M.J. VandeHaar. 2023. Symposium review: The impact of absorbed nutrients

on energy partitioning throughout lactation. J. Dairy Sci. 106:2167–2180. doi:10.3168/jds.2022-22500.

Pierce, R.B., R. Bomberger, S.R. Goodall, and J. Kevin. 2023. Effect of feeding increasing levels of whole cottonseed on milk and milk components , milk fatty acid profile , and total tract digestibility in lactating dairy cows. J. Dairy Sci.. doi:10.3168/jds.2023-23944.

Polan, C.E., G. Cozzi, P. Berzaghi, and I. Andrighetto. 1997. A Blend of Animal and Cereal Protein or Fish Meal as Partial Replacement for Soybean Meal in the Diets of Lactating Holstein Cows. J. Dairy Sci. 80:160–166. doi:10.3168/jds.S0022-0302(97)75923-X.

Prieto, J.G., E.J. DePeters, P.H. Robinson, J.E.P. Santos, J.W. Pareas, and S.J. Taylor. 2003. Increasing dietary levels of cracked Pima cottonseed increase plasma gossypol but do not influence productive performance of lactating Holstein cows. J. Dairy Sci. 86:254–267. doi:10.3168/jds.S0022-0302(03)73604-2.

Prom, C.M., and A.L. Lock. 2021. Replacing stearic acid with oleic acid in supplemental fat blends improves fatty acid digestibility of lactating dairy cows. J. Dairy Sci. 104:9956–9966. doi:10.3168/jds.2020-19985.

Prom, C.M., J.M. dos Santos Neto, and A.L. Lock. 2022. Abomasal infusion of different exogenous emulsifiers alters fatty acid digestibility and milk fat yield of lactating dairy cows. J. Dairy Sci. 105:3102–3112. doi:10.3168/jds.2021-21315.

Prom, C.M., J.M. dos Santos Neto, J.R. Newbold, and A.L. Lock. 2021. Abomasal infusion of oleic acid increases fatty acid digestibility and plasma insulin of lactating dairy cows. J. Dairy Sci.. doi:10.3168/jds.2021-20954.

Rabiee, A.R., K. Breinhild, W. Scott, H.M. Golder, E. Block, and I.J. Lean. 2012. Effect of fat additions to diets of dairy cattle on milk production and components: A meta-analysis and meta-regression. J. Dairy Sci. 95:3225–3247. doi:10.3168/jds.2011-4895.

Relling, A.E., and C.K. Reynolds. 2007. Feeding rumen-inert fats differing in their degree of saturation decreases intake and increases plasma concentrations of gut peptides in lactating dairy cows. J. Dairy Sci. 90:1506–1515. doi:10.3168/jds.S0022-0302(07)71636-3.

Rico, D.E., Y. Ying, and K.J. Harvatine. 2014a. Effect of a high-palmitic acid fat supplement on milk production and apparent total-tract digestibility in high- and low-milk yield dairy cows. J. Dairy Sci. 97:3739–3751. doi:10.3168/jds.2013-7341.

Rico, J.E., M.S. Allen, and A.L. Lock. 2014b. Compared with stearic acid, palmitic acid increased the yield of milk fat and improved feed efficiency across production level of cows. J. Dairy Sci. 97:1057–1066. doi:10.3168/jds.2013-7432.

Rico, J.E., J. de Souza, M.S. Allen, and A.L. Lock. 2017. Nutrient digestibility and milk production responses to increasing levels of palmitic acid supplementation vary in cows receiving diets with or without whole cottonseed. J. Anim. Sci. 95:436–446. doi:10.2527/JAS.2016.1089.

Romo, G.A., D.P. Casper, R.A. Erdman, and B.B. Teter. 1996. Abomasal Infusion of Cis or Trans Fatty Acid Isomers and Energy Metabolism of Lactating Dairy Cows. J. Dairy Sci. 79:2005–2015. doi:10.3168/jds.S0022-0302(96)76573-6.

Santos, J.E.P., M. Villaseňor, E.J. DePeters, P.H. Robinson, and B.C. Baldwin. 2002. Type of cottonseed and level of gossypol in diets of lactating dairy cows: Effects on lactation performance and plasma gossypol. J. Dairy Sci. 85:1491–1501. doi:10.3168/jds.S0022-0302(02)74218-5.

Santos, J.E.P., M. Villasenor, P.H. Robinson, E.J. DePeters, and C.A. Holmberg. 2003. Type of cottonseed and level of gossypol in diets of lactating dairy cows: Plasma gossypol, health, and reproductive performance. J. Dairy Sci. 86:892–905. doi:10.3168/jds.S0022-0302(03)73672-8.

Scarlet, D., and J.K. Drackley. 2013. Farm Animal Metabolism and Nutrition.

Scott, T.A., D.K. Combs, and R.R. Grummer. 1991. Effects of Roasting, Extrusion, and Particle Size on the Feeding Value of Soybeans for Dairy Cows. J. Dairy Sci. 74:2555–2562. doi:10.3168/jds.S0022-0302(91)78433-6.

Sears, A., F. Hentz, J. de Souza, B. Wenner, R.E. Ward, and F. Batistel. 2023. Supply of palmitic, stearic, and oleic acid changes rumen fiber digestibility and microbial composition. J. Dairy Sci. 902–916. doi:10.3168/jds.2023-23568.

Shingfield, K.J., A. Sæbø, P.C. Sæbø, V. Toivonen, and J.M. Griinari. 2009. Effect of abomasal infusions of a mixture of octadecenoic acids on milk fat synthesis in lactating cows. J. Dairy Sci. 92:4317–4329. doi:10.3168/jds.2008-2002.

Shorten, P.R., T.B. Pleasants, and G.C. Upreti. 2004. A mathematical model for mammary fatty acid synthesis and triglyceride assembly: The role of stearoyl CoA desaturase (SCD). J. Dairy Res. 71:385–397. doi:10.1017/S0022029904000354.

Shpirer, J., L. Livshits, H. Kamer, T. Alon, Y. Portnik, and U. Moallem. 2023. The form more than the fatty acid profile of fat supplements influences digestibility but not necessarily the production performance of dairy cows. J. Dairy Sci.. doi:10.3168/jds.2022-22190.

Shreve, B., N. Thiex, and M. Wolf. 2006. NFTA Method 2.1.4—Dry matter by oven drying for 3 hr at 105C. National Forage Testing Association.

Sklan, D., R. Ashkenazi, A. Braun, A. Devorin, and K. Tabori. 1992. Fatty Acids, Calcium Soaps of Fatty Acids, and Cottonseeds Fed to High Yielding Cows. J. Dairy Sci. 75:2463–2472. doi:10.3168/jds.S0022-0302(92)78008-4.

Smith, G.H., S. Mccarthy, and J.A.F. Rook. 1974. Synthesis of milk fat from β-hydroxybutyrate and acetate in lactating goats. J. Dairy Res. 41:175–191. doi:10.1017/S0022029900019609.

Smith, N.E., L.S. Collar, D.L. Bath, W.L. Dunkley, and A.A. Franke. 1981. Digestibility and Effects of Whole Cottonseed Fed to Lactating Cows. J. Dairy Sci. 64:2209–2215. doi:10.3168/jds.S0022-0302(81)82831-7.

Smith, S., A. Witkowski, and A.K. Joshi. 2003. Structural and functional organization of the animal fatty acid synthase. Prog. Lipid Res. 42:289–317. doi:10.1016/S0163-7827(02)00067-X.

Smith, W.A., B. Harris, H.H. Van Horn, and C.J. Wilcox. 1993. Effects of Forage Type on Production of Dairy Cows Supplemented with Whole Cottonseed, Tallow, and Yeast. J. Dairy Sci. 76:205–215. doi:10.3168/jds.S0022-0302(93)77339-7.

Spitsberg, V.L., E. Matitashvili, and R.C. Gorewit. 1995. Association and Coexpression of Fatty-Acid-Binding Protein and Glycoprotein CD36 in the Bovine Mammary Gland. Eur. J. Biochem. 230:872–878. doi:10.1111/j.1432-1033.1995.tb20630.x.

St-Pierre, N.R. 2017. The economic value of milk components in the Northeast. Proceeding from Penn State Dairy Nutrition Workshop. Page November 15-16, 2017, Grantville, PA.

Stern, M.O., K.A. Santos, and L.D. Satter. 1985. Protein Degradation in Rumen and Amino Acid Absorption in Small Intestine of Lactating Dairy Cattle Fed Heat-Treated Whole Soybeans. J. Dairy Sci. 68:45–56. doi:10.3168/jds.S0022-0302(85)80796-7.

Sukhija, P.S., and D.L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36:1202–1206. doi:10.1021/jf00084a019.

Sukhija, P.S., and D.L. Palmquist. 1990. Dissociation of Calcium Soaps of Long-Chain Fatty Acids in Rumen Fluid. J. Dairy Sci. 73:1784–1787. doi:10.3168/jds.S0022-0302(90)78858-3.

Talke, H., and G.E. Schubert. 1965. Enzymatische harnstoffbestimmung in blut und serum im optischen test nach warburg. Klin. Wochenschr. 43:174–175.

Tice, E.M., M.L. Eastridge, and J.L. Firkins. 1993. Raw Soybeans and Roasted Soybeans of Different Particle Sizes. 1. Digestibility and Utilization by Lactating Cows. J. Dairy Sci.

76:224–235. doi:10.3168/jds.S0022-0302(93)77341-5.

Tice, E.M., M.L. Eastridge, and J.L. Firkins. 1994. Raw Soybeans and Roasted Soybeans of Different Particle Sizes. 2. Fatty Acid Utilization by Lactating Cows. J. Dairy Sci. 77:166–180. doi:10.3168/jds.S0022-0302(94)76939-3.

Timmen, H., and S. Patton. 1988. Milk fat globules: Fatty acid composition, size and in vivo regulation of fat liquidity. Lipids 23:685–689. doi:10.1007/BF02535669.

Toral, P.G., R. Gervais, G. Hervás, M.P. Létourneau-Montminy, and P. Frutos. 2020. Relationships between trans-10 shift indicators and milk fat traits in dairy ewes: Insights into milk fat depression. Anim. Feed Sci. Technol. 261:114389. doi:10.1016/j.anifeedsci.2020.114389.

Tyrrell, H.F., and J.T. Reid. 1965. Prediction of the Energy Value of Cow's Milk. J. Dairy Sci. 48:1215–1223. doi:10.3168/jds.S0022-0302(65)88430-2.

Ulberth, F., and F. Schrammel. 1995. Accurate quantitation of short-, medium-, and long-chain fatty acid methyl esters by split-injection capillary gas-liquid chromatography. J. Chromatogr. A 704:455–463. doi:10.1016/0021-9673(95)00224-B.

VandeHaar, M.J., and N.R. St-Pierre. 2006. Major advances in nutrition: Relevance to the sustainability of the dairy industry. J. Dairy Sci. 89:1280–1291. doi:10.3168/jds.S0022-0302(06)72196-8.

Venturelli, B.C., J.E. de Freitas Júnior, C.S. Takiya, A.P.C. de Araújo, M.C.B. Santos, G.D. Calomeni, R. Gardinal, T.H.A. Vendramini, and F.P. Rennó. 2015. Total tract nutrient digestion and milk fatty acid profile of dairy cows fed diets containing different levels of whole raw soya beans. J. Anim. Physiol. Anim. Nutr. (Berl). 99:1149–1160. doi:10.1111/jpn.12297.

Vernon, R.G. 2005. Lipid metabolism during lactation: A review of adipose tissue-liver interactions and the development of fatty liver. J. Dairy Res. 72:460–469. doi:10.1017/S0022029905001299.

Wang, C., H.Y. Liu, Y.M. Wang, Z.Q. Yang, J.X. Liu, Y.M. Wu, T. Yan, and H.W. Ye. 2010. Effects of dietary supplementation of methionine and lysine on milk production and nitrogen utilization in dairy cows. J. Dairy Sci. 93:3661–3670. doi:10.3168/jds.2009-2750.

Weimer, P.J., D.M. Stevenson, and D.R. Mertens. 2010. Shifts in bacterial community composition in the rumen of lactating dairy cows under milk fat-depressing conditions. J. Dairy Sci. 93:265–278. doi:10.3168/jds.2009-2206.

Weld, K.A., and L.E. Armentano. 2017. The effects of adding fat to diets of lactating dairy cows on total-tract neutral detergent fiber digestibility: A meta-analysis. J. Dairy Sci. 100:1766–1779. doi:10.3168/jds.2016-11500.

Weld, K.A., and L.E. Armentano. 2018. Feeding high oleic acid soybeans in place of conventional soybeans increases milk fat concentration. J. Dairy Sci. 101:9768–9776. doi:10.3168/jds.2018-14498.

Western, M.M., J. de Souza, and A.L. Lock. 2020a. Effects of commercially available palmitic and stearic acid supplements on nutrient digestibility and production responses of lactating dairy cows. J. Dairy Sci. 103:5131–5142. doi:10.3168/jds.2019-17242.

Western, M.M., J. de Souza, and A.L. Lock. 2020b. Milk production responses to altering the dietary ratio of palmitic and oleic acids varies with production level in dairy cattle. J Dairy Sci 103:11472–11482. doi:10.3168/jds.2020-18936.

Westreicher-Kristen, E., and A. Susenbeth. 2017. Technical note: An improved tool to insert lines for abomasal infusion in rumen cannulated cattle. J. Dairy Sci. 100:1951–1954. doi:10.3168/jds.2016-12150.

Wildman, E.E., G.M. Jones, P.E. Wagner, R.L. Boman, H.F. Troutt, and T.N. Lesch. 1982. A Dairy Cow Body Condition Scoring System and Its Relationship to Selected Production Characteristics. J. Dairy Sci. 65:495–501. doi:10.3168/jds.S0022-0302(82)82223-6.

Wu, Z., O.A. Ohajuruka, and D.L. Palmquist. 1991. Ruminal Synthesis, Biohydrogenation, and Digestibility of Fatty Acids by Dairy Cows. J. Dairy Sci. 74:3025–3034. doi:10.3168/jds.S0022-0302(91)78488-9.

Yao, W., J. Luo, H. Tian, H. Niu, X. An, X. Wang, and S. Zang. 2022. Malonyl/Acetyltransferase (MAT) Knockout Decreases Triacylglycerol and Medium-Chain Fatty Acid Contents in Goat Mammary Epithelial Cells. Foods 11. doi:10.3390/foods11091291.

APPENDIX A: LIST OF TABLES

Table 1. FA profile and FA % DM of common feed ingredients.

Feedstuff[1]	C16:0, % FA	C18:0, % FA	C18:1, % FA	C18:2, % FA	C18:3, % FA	FA, %DM
Alfalfa Hay	29.9	4.98	2.99	19.9	30.6	1.71
Alfalfa Silage	21.3	3.54	3.11	20.4	42.3	3.51
Corn Silage	17.3	2.51	22.7	43.9	4.87	3.01
Cottonseed[2]	24.6	2.00	14.8	56.5	0.21	15.9
Distillers	14.0	2.40	24.6	56.1	1.70	7.76
Ground Corn	12.3	1.72	26.5	56.3	1.35	2.43
High Moisture Corn	14.7	1.86	23.1	58.4	1.37	4.90
Pasture Grass, cool	16.0	2.50	3.40	13.2	61.3	1.70
Soybeans, conventional[2]	11.4	4.10	22.3	53.5	7.0	18.8
Soybean, high *cis*-9 C18:1[2,3]	5.58	3.36	82.5	3.72	1.46	19.5
Soyhulls	14.0	5.47	17.4	47.7	10.9	1.55
Tallow	28.7	10.3	46.2	9.50	0.20	53.7
C16:0-enriched prill	90.4	1.59	5.25	1.17	-	98.0
C16:0 + C18:0 prill	30.5	53.4	6.44	0.86	0.57	83.4
Ca-salt of palm fat	47.5	3.85	38.1	7.97	0.25	77.1

[1]Compilation of data from the Lock Laboratory and Caledonia Farmers Elevator.
[2]Whole seeds.
[3]Plenish (Pioneer; Johnston, IA)

Table 2. Stereospecific location of fatty acids (FA) in triglycerides (TG) from bovine milk fat. Fatty acids can be esterified at one of three positions on the TG denoted as *sn*-1, *sn*-2, and *sn*-3. The table represents the molar percentage (mol/100mol FA) of major FA at each of the positions on the TG relative to the individual FA. Table adopted from Jensen (2002).

	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1
sn-1	1.6	3.1	10.3	15.2	23.7	27.3	44.1	54.0	37.3
sn-2	0.3	3.9	55.2	56.6	62.9	65.6	45.4	16.2	21.2
sn-3	98.1	93.0	34.5	28.2	13.4	7.1	10.5	29.8	41.5

Table 3. Composition of FA supplements.

	Treatment	
Item	Oleic acid[1]	Ca-Salt[2]
Total FA content, %	99.1	68.6
FA profile, g/100 g of FA[1]		
C14:0	-	0.34
C16:0	0.67	22.4
C18:0	2.02	3.56
cis-9 C18:1	92.2	64.3
cis-9, *cis*-12 C18:2	3.34	7.87
cis-9, *cis*-12, *cis*-15 C18:3	0.02	0.09

[1]Oleic acid (O1008, Sigma-Aldrich) used for abomasal (ABO) and ruminal (RUM) infusion treatments.
[2]Perdue Agribusiness (Salisbury, MD).

Table 4. Ingredient and nutrient composition of diet.

Item	% of DM
Ingredient	
Corn Silage	39.8
Alfalfa Silage	16.3
Ground Corn	18.5
Soybean Meal	8.09
Soyhulls	4.36
Lactating Cow Supplement[1]	6.53
Cottonseed, Whole	2.96
Vitamin and Mineral Mix[2]	1.81
Saturated Fat Supplement[3]	1.69
Nutrient Composition, % DM[4]	
NDF	30.0
Forage NDF	21.7
CP	16.1
Starch	30.3
FA	3.76
16:0	0.92
18:0	0.94
cis-9 C18:1	0.50
cis-9, *cis*-12 C18:2	1.08
cis-9, *cis*-12, *cis*-15 C18:3	0.15

[1]Lactation supplement contained 39% Amino Plus (Ag Processing Inc), 18% CFE pass (Papillon), 16% sodium sesquinate, 13% calcium carbonate, 10% ground corn, 3% urea, and 1% Smartamine (Adisseo).
[2]Vitamin and mineral mix contained 22% ground corn, 21% MIN AD (Min Ad Inc), 20% calcium carbonate, 19% calcium phosphate, 10% white salt, 5% sodium sesquinate, 2% selenium, <1% tallow, Micro 5 (Alltech), vitamin A, vitamin E, vitamin D.
[3]Energy Booster 100 (Milk Specialties Global). Contained (g/100g of FA) 32.6% C16:0, 52.6% C18:0, 5.86% *cis*-9 C18:1, and 0.90% *cis*-9, *cis*-12 C18:2; 83.6% DM total FA.
[4]Expressed as a percent of as fed.

Table 5. Nutrient intake and digestibility responses of cows receiving treatment (n=8).

	Treatment[1]				SEM[2]	Contrast[3]		
Variable	CON	ABO	RUM	SALT		CON vs OA	ABO vs RUM	RUM vs SALT
Intake, kg/d								
DMI	29.1	28.9	28.6	28.6	0.92	0.39	0.52	0.95
NDF	8.87	8.85	8.72	8.69	0.29	0.53	0.39	0.8
Intake,[4] g/d								
16-carbon	200	197	196	206	5.45	0.28	0.64	0.01
18-carbon	647	688	687	671	16.60	<0.01	0.86	0.09
Total FA	884	926	923	917	28.50	<0.01	0.79	0.60
Digestibility, %								
DM	67.9	68.9	67.0	69.1	0.91	0.97	0.06	0.04
NDF	47.6	48.4	46.3	49.0	1.05	0.77	0.04	0.01
16-carbon	62.2	62.8	55.5	61.3	1.94	0.09	<0.01	<0.01
18-carbon	61.9	62.0	56.2	60.2	2.88	0.13	0.01	0.04
Total FA	62.2	62.5	56.4	60.8	2.52	0.12	<0.01	0.03
Absorbed, g/d								
16-carbon	123	124	107	126	3.97	0.11	<0.01	<0.01
18-carbon	397	427	381	402	18.3	0.60	0.01	0.15
Total FA	546	578	513	554	21.7	0.98	<0.01	0.05

[1]CON = water only; ABO = abomasal infusion of 50 g/d of *cis*-9 C18:1; RUM = ruminal infusion of 50 g/d of *cis*-9 C18:1; SALT = 78 g/d of a *cis*-9 C18:1-enriched Ca-salt.
[2]Greatest SEM.
[3]*P*-values associated with contrasts: (1) CON compared with the overall effect of OA treatment (CON vs. OA), (2) abomasal infusion of *cis*-9 C18:1 vs ruminal infusion of *cis*-9 C18:1 (ABO vs RUM), and (3) the ruminal infusion of *cis*-9 C18:1 vs a *cis*-9 C18:1-enriched Ca-salt added to the rumen (RUM vs SALT).
[4]The amount of FA in the infusate and Ca-salt was included for the intake, digestibility, and absorption of FA.

Table 6. Production responses of cows receiving treatment (n=8).

Variable	Treatment[1]				SEM[2]	Contrast[3]		
	CON	ABO	RUM	SALT		CON vs OA	ABO vs RUM	RUM vs SALT
Yield, kg/d								
Milk	44.1	45.4	44.4	43.6	2.00	0.29	0.24	0.33
3.5% FCM[4]	43.8	45.1	43.9	43.3	1.58	0.36	0.17	0.47
ECM[5]	43.4	44.6	43.7	43.2	1.59	0.63	0.32	0.53
Fat	1.52	1.57	1.52	1.51	0.05	0.70	0.19	0.62
Protein	1.33	1.38	1.34	1.33	0.05	0.19	0.16	0.62
Lactose	2.13	2.20	2.15	2.12	0.10	0.20	0.22	0.41
Milk composition, %								
Fat	3.49	3.47	3.45	3.47	0.12	0.58	0.77	0.70
Protein	3.01	3.05	3.03	3.06	0.08	0.05	0.25	0.04
Lactose	4.81	4.84	4.84	4.85	0.03	0.15	0.84	0.59
ECM/DMI	1.39	1.46	1.44	1.41	0.03	0.05	0.44	0.35
BW, kg	699	701	698	701	3.72	0.89	0.55	0.62
BCS	3.12	3.16	3.12	3.15	0.05	0.37	0.15	0.33
Insulin, ug/mL	0.94	0.85	0.91	0.83	0.04	0.12	0.20	0.06

[1]CON = water only; ABO = abomasal infusion of 50 g/d of *cis*-9 C18:1; RUM = ruminal infusion of 50 g/d of *cis*-9 C18:1; SALT = 78 g/d of a *cis*-9 C18:1-enriched Ca-salt.

[2]Greatest SEM.

[3]*P*-values associated with contrasts: (1) CON compared with the overall effect of OA treatment (CON vs. OA), (2) abomasal infusion of *cis*-9 C18:1 vs ruminal infusion of *cis*-9 C18:1 (ABO vs RUM), and (3) the ruminal infusion of *cis*-9 C18:1 vs a *cis*-9 C18:1-enriched Ca-salt added to the rumen (RUM vs SALT).

[4]3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)] (NRC, 2001).

[5] ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)] (Tyrrell and Reid, 1965).

Table 7. Milk fatty acid yields of cows receiving treatment (n=8).

Variable	Treatment[2]				SEM[3]	Contrast		
	CON	ABO	RUM	SALT		CON vs OA	ABO vs RUM	RUM vs SALT
Summation by source, g/d								
De novo	384	389	382	372	16.9	0.47	0.49	0.33
Mixed	532	519	506	505	15.4	0.04	0.26	0.94
Preformed	539	558	538	535	16.3	0.68	0.08	0.77
Selected individual fatty acids, g/d								
C4:0	44.5	45.0	44.3	43.1	1.72	0.75	0.59	0.32
C6:0	30.7	30.7	30.4	29.3	0.99	0.46	0.75	0.21
C8:0	18.4	18.4	18.3	17.5	0.82	0.51	0.83	0.20
C10:0	46.4	46.6	45.9	44.4	3.24	0.61	0.67	0.37
C12:0	54.9	54.7	53.9	52.3	4.29	0.54	0.73	0.47
C14:0	179	181	175	172	7.53	0.45	0.24	0.37
C16:0	512	498	484	484	14.9	0.04	0.22	0.95
cis-9 C16:1	23.1	20.7	21.0	21.0	0.84	0.03	0.75	0.95
C18:0	136	130	132	128	6.00	0.05	0.61	0.25
trans -6 to -8 C18:1	4.37	4.15	5.07	4.66	0.24	0.06	<0.001	0.01
trans-9 C18:1	3.26	3.96	3.70	3.48	0.15	<0.01	0.03	0.05
trans-10 C18:1	6.68	7.29	7.74	7.98	0.78	0.02	0.27	0.54
trans-11 C18:1	11.0	10.0	10.7	11.1	0.46	0.09	0.03	0.13
cis-9 C18:1	264	287	264	264	8.93	0.11	<0.01	0.94
cis-9, *cis*-12 C18:2	35.6	36.0	34.7	36.3	1.79	0.72	0.21	0.64
cis-9, *cis*-12, *cis*-15 C18:3	5.11	5.09	4.94	5.08	0.28	0.51	0.24	0.29

[1]CON = water only; ABO = abomasal infusion of 50 g/d of *cis*-9 C18:1; RUM = ruminal infusion of 50 g/d of *cis*-9 C18:1; SALT = 78 g/d of a *cis*-9 C18:1-enriched Ca-salt.
[2]Greatest SEM.

Table 7 (cont'd)
[3]*P*-values associated with contrasts: (1) CON compared with the overall effect of OA treatment (CON vs. OA), (2) abomasal infusion of *cis*-9 C18:1 vs ruminal infusion of *cis*-9 C18:1 (ABO vs RUM), and (3) the ruminal infusion of *cis*-9 C18:1 vs a *cis*-9 C18:1-enriched Ca-salt added to the rumen (RUM vs SALT).

Table 8. Composition of fatty acid (FA) supplements used for FA treatments.

	FA Supplements[2]		
Item	ProPalm 85	Perdue Calcium Salts	Ruminer 70
Total FA content, % DM	96.9	74.8	79.5
FA profile, g/100 g of FA[1]			
C14:0	1.72	0.93	1.45
C16:0	90.4	46.9	70.6
C18:0	2.09	4.12	6.47
cis-9 C18:1	4.44	38.4	16.9
cis-9, *cis*-12 C18:2	0.87	7.92	3.67
cis-9, *cis*-12, *cis*-15 C18:3	0.00	0.25	0.11

[1]Average (n=4) composition of FA supplements based on samples taken during the collection period.
[2]FA supplements manufactured by Perdue Agribusiness, Salisbury MD.

Table 9. Proportion of fatty acid (FA) supplements and FA profile for 70FB treatment.

	FA Treatment[1]
Item	70FB
Proportion of FA supplement in treatment blend[2], %	
ProPalm85	52.8
Perdue Calcium Salts	47.2
FA profile of FA blend, g/100 g of FA[3]	
C14:0	1.35
C16:0	69.9
C18:0	3.05
cis-9 C18:1	20.4
cis-9, *cis*-12 C18:2	4.20
cis-9, *cis*-12, *cis*-15 C18:3	0.12

[1]70FB = 2.0% of DM to provide approximately 70% C16:0 + 20% *cis*-9 C18:1.
[2]FA supplements manufactured by Perdue Agribusiness, Salisbury MD.
[3]Average (n=4) composition of FA supplements based on samples taken during the collection period.

Table 10. Ingredient and nutrient composition of treatment diets.

	Treatment[1]			
	CON	70FB	70CS	45CS
Ingredient, % DM				
Corn Silage	36.3	36.3	36.4	36.3
Alfalfa Silage	12.5	12.5	12.5	12.5
Whole Cottonseed	8.12	8.11	8.13	8.11
Ground Corn	19.1	19.1	19.1	19.1
Soybean Meal	8.69	8.68	8.69	8.68
Vitamin and Mineral Mix[2]	1.92	1.92	1.92	1.92
Lactating Cow Mix[3]	5.36	5.36	5.37	5.36
Soyhulls	7.98	5.92	5.53	5.55
70:20 FA Blend	-	2.16	-	-
Ruminer70	-	-	2.37	-
Perdue Calcium Salts	-	-	-	2.52
Nutrient Composition, % DM[4]				
NDF	30.3	28.9	28.7	28.6
Forage NDF	17.5	17.5	17.5	17.5
Starch	29.2	29.2	29.2	29.2
CP	16.6	16.4	16.3	16.3
Fatty Acid (FA)	3.03	4.83	4.92	4.88
16:0	0.54	1.81	1.89	1.42
18:0	0.08	0.14	0.20	0.16
cis-9 18:1	0.52	0.89	0.84	1.24
cis-9, *cis*-12 18:2	1.67	1.74	1.74	1.81
cis-9, *cis*-12, *cis*-15 18:3	0.15	0.15	0.15	0.15

[1]CON = no FA supplementation, 70FB = 2.0% of DM to provide approximately 70% C16:0 + 20% *cis*-9 C18:1, 70CS = 2.0% of DM to provide approximately 70% C16:0 + 20% *cis*-9 C18:1, and 45CS = 2.0% of DM to provide approximately 45% C16:0 + 35% *cis*-9 C18:1.
[2]Vitamin and mineral mix contained 22% ground corn, 21% MIN AD (Min Ad Inc), 20% calcium carbonate, 19% calcium phosphate, 10% white salt, 5% sodium sesquinate, 2% selenium, <1% tallow, Micro 5 (Alltech), vitamin A, vitamin E, vitamin D.
[3]Lactation supplement contained 39% Amino Plus (Ag Processing Inc), 18% CFE pass (Papillon), 16% sodium sesquinate, 13% calcium carbonate, 10% ground corn, 3% urea, and 1% Smartamine (Adisseo).
[4]Expressed as a percent of as fed.

Table 11. Nutrient intake and digestibility of cows fed treatment diets (n=20)[1].

Variable	Treatment[2]				SEM[3]	Contrast[4]		
	CON	70FB	70CS	45CS		CON vs FAS	Form	Profile
Intake, kg/d								
DMI	31.0	30.9	30.4	29.4	0.56	0.02	0.26	0.02
NDF	9.51	9.01	8.87	8.56	0.17	<0.01	0.21	0.01
Intake, g/d								
16-carbon	167	548	568	411	8.00	<0.01	<0.01	<0.01
18-carbon	758	918	904	1007	16.8	<0.01	0.15	<0.01
Total FA	948	1495	1488	1443	23.2	<0.01	0.52	<0.01
Digestibility, %								
DM	66.5	71.8	69.2	70.0	0.39	<0.01	<0.01	0.13
NDF	43.9	51.4	48.6	49.4	0.66	<0.01	<0.01	0.31
16-carbon	72.6	65.5	72.5	76.5	1.22	0.07	<0.01	<0.01
18-carbon	71.7	76.1	73.1	74.3	1.43	<0.001	<0.01	0.12
Total FA	71.2	71.9	72.6	74.7	1.34	<0.01	0.36	0.01
Absorbed, g/d								
16-carbon	123	360	413	316	7.57	<0.001	<0.001	<0.001
18-carbon	546	697	660	757	15.5	<0.001	<0.01	<0.001
Total FA	676	1076	1084	1082	22.0	<0.001	0.55	0.90

[1]Experimental diets fed to 20 cows in replicated 4 × 4 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).
[2]CON = no fatty acid (FA) supplementation, 70FB = 2.0% of DM to provide approximately 70% C16:0 + 20% *cis*-9 C18:1, 70CS = 2.0% of DM to provide approximately 70% C16:0 + 20% *cis*-9 C18:1, and 45CS = 2.0% of DM to provide approximately 45% C16:0 + 35% *cis*-9 C18:1.
[3]Greatest SEM.
[4]CON vs. FAS tested the overall effect of FA supplementation, Form (70FB vs. 70CS) tested the effect of form of the supplement, and Profile (70CS vs. 45CS) tested the effects of FA profile of a Ca-salt.

Table 12. Production responses of cows fed treatment diets (n=20)[1].

Variable	Treatment[2]				SEM[3]	Contrast[4]		
	CON	70FB	70CS	45CS		CON vs FAS	Form	Profile
Yield, kg/d								
Milk	47.1	47.1	46.8	48.7	0.99	0.24	0.48	<0.01
3.5% FCM[5]	48.0	49.4	49.2	49.0	1.08	0.01	0.68	0.74
ECM[6]	48.6	48.7	48.9	49.3	1.10	0.34	0.56	0.48
Fat	1.71	1.77	1.79	1.75	0.05	<0.01	0.28	0.04
Protein	1.54	1.49	1.48	1.50	0.04	<0.001	0.41	0.08
Lactose	2.33	2.30	2.30	2.38	0.05	0.85	0.91	<0.01
Milk composition, %								
Fat	3.66	3.76	3.77	3.61	0.09	0.02	0.90	<0.001
Protein	3.26	3.17	3.15	3.08	0.04	<0.001	0.38	<0.001
Lactose	4.94	4.90	4.89	4.90	0.02	<0.001	0.36	0.18
ECM/DMI	1.54	1.59	1.61	1.64	0.03	<0.01	0.42	0.18
BW, kg	694	695	692	692	14.8	0.72	0.13	0.67
BW change, kg	0.87	0.67	0.51	0.54	0.13	0.04	0.33	0.85
BCS	3.15	3.11	3.12	3.12	0.07	0.03	0.64	0.96
BCS change	0.09	0.00	0.02	0.03	0.02	<0.01	0.55	0.72

[1]Experimental diets fed to 20 cows in replicated 4 × 4 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).
[2]CON = no fatty acid (FA) supplementation, 70FB = 2.0% DM of FA to provide approximately 70% C16:0 + 20% *cis*-9 C18:1, 70CS = 2.0% DM of FA to provide approximately 70% C16:0 + 20% *cis*-9 C18:1, and 45CS = 2.0% DM of FA to provide approximately 45% C16:0 + 35% *cis*-9 C18:1.
[3]Greatest SEM.
[4]CON vs. FAS tested the overall effect of FA supplementation, Form (70FB vs. 70CS) tested the effect of form of the supplement, and Profile (70CS vs. 45CS) tested the effects of FA profile of a Ca-salt.
[5]Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)] (NRC, 2001).
[6]Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein) (Tyrrell and Reid, 1965).

Table 13. Milk fatty acid yield for cows fed treatment diets (n=20)[1].

Variable	Treatment[2]				SEM[3]	Contrast[4]		
	CON	70FB	70CS	45CS		CON vs FAS	Form	Profile
Summation by source, g/d								
De novo	432	391	388	380	12.9	<0.001	0.54	0.20
Mixed	560	622	661	577	20.3	<0.001	<0.001	<0.001
Preformed	599	625	619	675	15.3	<0.001	0.56	<0.001
Select individual fatty acids, g/d								
C4:0	43.2	48.0	48.5	49.3	1.68	<0.001	0.63	0.47
C6:0	31.3	31.4	31.0	31.4	1.19	0.98	0.50	0.53
C8:0	20.7	18.7	18.0	18.6	0.75	<0.001	0.04	0.10
C10:0	55.7	47.3	46.1	44.7	2.01	<0.001	0.25	0.16
C12:0	67.6	54.6	53.5	50.6	2.21	<0.001	0.30	0.01
C14:0	199	179	177	170	5.37	<0.001	0.51	0.01
C16:0	541	602	637	555	20.1	<0.001	<0.001	<0.001
cis-9 C16:1	27.6	27.4	28.5	25.6	1.45	0.25	0.01	<0.001
C18:0	148	154	149	165	6.09	<0.01	0.09	<0.001
trans -6 to -8 C18:1	4.79	5.76	5.63	7.85	0.25	<0.001	0.40	<0.001
trans-9 C18:1	3.51	4.23	4.14	5.34	0.15	<0.001	0.40	<0.001
trans-10 C18:1	9.09	9.70	10.2	12.2	0.76	<0.001	0.14	<0.001
trans-11 C18:1	15.2	15.9	15.7	19.4	1.14	<0.01	0.60	<0.001
cis-9 C18:1	266	291	291	320	7.98	<0.001	0.88	<0.001
cis-9, *cis*-12 C18:2	46.8	44.5	45.5	48.1	1.11	0.16	0.16	<0.01
cis-9, *cis*-12, *cis*-15 C18:3	6.62	6.05	5.96	6.03	0.15	<0.001	0.47	0.61

[1]Experimental diets fed to 20 cows in replicated 4 × 4 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).
[2]CON = no fatty acid (FA) supplementation, 70FB = 2.0% DM of FA to provide approximately 70% C16:0 + 20% *cis*-9 C18:1, 70CS = 2.0% DM of FA to provide approximately 70% C16:0 + 20% *cis*-9 C18:1, and 45CS = 2.0% DM of FA to provide approximately 45% C16:0 + 35% *cis*-9 C18:1.

Table 13 (cont'd)
[3]Greatest SEM.
[4]CON vs. FAS tested the overall effect of FA supplementation, Form (70FB vs. 70CS) tested the effect of form of the supplement, and Profile (70CS vs. 45CS) tested the effects of FA profile of a Ca-salt.

Table 14. Milk fatty acid concentration for cows fed treatment diets (n=20)[1].

Variable	Treatment[2]				SEM[3]	Contrast[4]		
	CON	70FB	70CS	45CS		CON vs FAS	Form	Profile
Summation by source, g/100 g								
De novo	27.1	23.8	23.1	22.8	0.25	<0.001	<0.01	0.09
Mixed	34.8	38.2	39.5	35.4	0.38	<0.001	<0.001	<0.001
Preformed	37.9	38.1	37.2	41.7	0.42	<0.001	<0.01	<0.001
Selected individual fatty acids, g/100 g								
C4:0	2.75	2.93	2.86	2.98	0.07	<0.001	0.01	<0.001
C6:0	1.97	1.92	1.86	1.91	0.04	<0.001	0.01	0.01
C8:0	1.29	1.13	1.08	1.11	0.02	<0.001	<0.001	<0.01
C10:0	3.52	2.86	2.78	2.67	0.07	<0.001	0.03	0.01
C12:0	4.22	3.31	3.20	3.05	0.07	<0.001	0.02	<0.01
C14:0	12.4	10.8	10.6	10.4	0.1	<0.001	<0.01	<0.01
C16:0	33.1	36.5	37.8	33.9	0.34	<0.001	<0.001	<0.001
cis-9 C16:1	1.71	1.66	1.69	1.53	0.06	<0.001	0.07	<0.001
C18:0	9.39	9.42	8.96	9.94	0.33	0.73	<0.01	<0.001
trans -6 to -8 C18:1	0.30	0.36	0.34	0.48	0.01	<0.001	0.16	<0.001
trans-9 C18:1	0.22	0.26	0.25	0.32	0.01	<0.001	0.03	<0.001
trans-10 C18:1	0.61	0.61	0.63	0.77	0.06	<0.001	0.21	<0.001
trans-11 C18:1	0.97	1.00	0.97	1.21	0.07	<0.01	0.34	<0.001
cis-9 C18:1	16.6	17.7	17.5	19.6	0.29	<0.001	0.07	<0.001
cis-9, *cis*-12 C18:2	2.97	2.72	2.74	2.95	0.04	<0.001	0.27	<0.001
cis-9, *cis*-12, *cis*-15 C18:3	0.42	0.37	0.36	0.37	0.01	<0.001	<0.01	<0.001

[1]Experimental diets fed to 20 cows in replicated 4 × 4 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).
[2]CON = no fatty acid (FA) supplementation, 70FB = 2.0% DM of FA to provide approximately 70% C16:0 + 20% *cis*-9 C18:1, 70CS = 2.0% DM of FA to provide approximately 70% C16:0 + 20% *cis*-9 C18:1, and 45CS = 2.0% DM of FA to provide approximately 45% C16:0 + 35% *cis*-9 C18:1.
[3]Greatest SEM.

Table 14 (cont'd)
[4]CON vs. FAS tested the overall effect of FA supplementation, Form (70FB vs. 70CS) tested the effect of form of the supplement, and Profile (70CS vs. 45CS) tested the effects of FA profile of a Ca-salt.

Table 15. Ingredient and nutrient composition of treatment diets.

	Treatment[1]			
	0%	8%	16%	24%
Ingredient, % DM				
Corn Silage	35.9	35.7	35.7	35.5
Alfalfa Silage	9.59	9.52	9.52	9.48
Ground Corn	17.7	17.6	17.6	17.5
Mineral Mix[2]	4.62	4.59	4.59	4.58
Lactation Mix[3]	4.82	4.79	4.79	4.77
Soybean Meal	9.29	6.94	4.78	2.40
Soybean Hulls	18.1	12.8	7.14	1.66
Whole Cottonseed[4]	0.00	8.11	16.0	24.1
Nutrient Composition, % DM				
NDF	32.2	32.1	31.8	31.6
Forage NDF	20.6	20.6	20.6	20.6
Starch	27.6	27.5	27.4	27.4
CP	17.3	17.2	17.1	16.9
Fatty Acid (FA)	1.70	2.96	4.20	5.40
16:0	0.25	0.54	0.82	1.10
18:0	0.06	0.08	0.11	0.13
cis-9 18:1	0.33	0.51	0.69	0.87
cis-9, *cis*-12 18:2	0.90	1.64	2.37	3.07
cis-9, *cis*-12, *cis*-15 18:3	0.13	0.13	0.12	0.12
Gossypol[4]	0.00	0.05	0.11	0.16

[1]Inclusion levels of WCS, %DM

[2]Vitamin and mineral mix contained 26% Amino Plus (Ag Processing Inc), 14% calcium carbonate, 13% Caledonia Pass (Papillon) and sodium sesquicarbonate, 9% calcium phosphate, 6% urea, 5% white salt, 4% magnesium oxide, 3% MIN AD (Min Ad Inc) and potassium chloride red, 2% D&D Ion Pak, 1% selenium, and <1% ferrous sulfate, Micro 5, Smartamine (Adisseo), vitamin D, vitamin E, and vitamin A.

[3]Lactation supplement contained 40% Amino Plus (Ag Processing Inc), 18% CFE pass (Papillon), 16% sodium sesquinate, 13% calcium carbonate, 10% ground corn, 3% urea, and 1% Smartamine (Adisseo).

[4]Whole cottonseed contained 6.65 g/kg of gossypol and a FA profile composed of: (g/100g) 22.5 of C16:0, 2.17 of C18:0, 14.7 of *cis*-9 18:1, 58.3 of *cis*-9, *cis*-12 18:2, and 0.17 of *cis*-9, *cis*-12, *cis*-15 18:3; 16.5 g/100g total FA.

Table 16. Nutrient intake and digestibility of cows fed treatment diets (n=24)[1].

Variable	Treatment[2]				SEM[3]	Contrast[4]		
	0%	8%	16%	24%		Linear	Quadratic	Cubic
Intake, kg/d								
DMI	34.4	35.2	34.6	32.6	0.48	<0.001	<0.001	0.96
NDF	11.2	11.5	11.2	10.4	0.15	<0.001	<0.001	0.57
Intake, g/d								
16-carbon	84.9	195	291	364	2.89	<0.001	<0.001	0.42
18-carbon	495	857	1167	1400	13.1	<0.001	<0.001	0.49
Total FA	594	1075	1490	1802	16.2	<0.001	<0.001	0.45
Digestibility, %								
DM	65.7	63.7	65.4	66.7	0.43	0.01	<0.001	0.03
NDF	45.2	41.9	43.5	45.8	1.33	0.33	<0.01	0.20
16-carbon	71.9	75.7	75.9	77.8	0.62	<0.001	0.10	0.03
18-carbon	80.1	76.2	71.6	71.1	0.80	<0.001	0.01	0.06
Total FA	77.8	75.6	72.3	72.5	0.75	<0.001	0.04	0.07
Absorbed, g/d								
16-carbon	60.4	147	222	283	2.17	<0.001	<0.001	0.97
18-carbon	386	653	835	995	11.7	<0.001	<0.001	0.11
Total FA	451	812	1075	1313	13.7	<0.001	<0.001	0.12

[1]Experimental diets fed to 24 cows in replicated 4 × 4 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).
[2]Inclusion level of WCS, %DM
[3]Greatest SEM.
[4]Contrasts correspond to the linear, quadratic, and cubic effects of increasing WCS inclusion.

Table 17. Production responses of cows fed treatment diets (n=24)[1].

Variable	Treatment[2]				SEM[3]	Contrast[4]		
	0%	8%	16%	24%		Linear	Quadratic	Cubic
Yield, kg/d								
Milk	50.9	52.9	52.8	50.3	0.63	0.32	<0.001	0.93
3.5% FCM[5]	51.9	54.7	54.7	51.6	0.52	0.41	<0.001	0.80
ECM[6]	52.8	54.6	54.5	51.1	0.44	<0.01	<0.001	0.23
Fat	1.87	1.98	1.99	1.87	0.03	0.89	<0.001	0.76
Protein	1.65	1.70	1.65	1.55	0.02	<0.001	<0.001	0.68
Lactose	2.50	2.61	2.60	2.47	0.03	0.28	<0.001	0.95
Milk composition, %								
Fat	3.67	3.73	3.75	3.72	0.06	0.14	0.08	1.00
Protein	3.23	3.17	3.14	3.08	0.03	<0.001	0.92	0.32
Lactose	4.92	4.94	4.93	4.91	0.02	0.04	<0.001	0.89
ECM/DMI	1.45	1.42	1.43	1.57	0.02	<0.001	<0.001	0.09
BW, kg	719	720	719	720	10.5	0.72	0.74	0.47
BW change, kg	0.25	0.25	0.50	0.54	0.12	0.02	0.86	0.33
BCS	3.00	3.02	3.04	3.02	0.04	0.24	0.21	0.23
BCS change	-0.03	0.01	0.05	-0.01	0.02	0.26	<0.01	0.27

[1]Experimental diets fed to 24 cows in replicated 4 × 4 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).
[2]Inclusion level of WCS, %DM
[3]Greatest SEM.
[4]Contrasts correspond to the linear, quadratic, and cubic effects of increasing WCS inclusion.
[6]Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)] (NRC, 2001).
[7]Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein) (Tyrrell and Reid, 1965).

Table 18. Milk fatty acid yields for cows fed treatment diets (n=24)[1].

Variable	Treatment[2]				SEM[3]	Contrast[4]		
	0%	8%	16%	24%		Linear	Quadratic	Cubic
Summation by source, g/d								
De novo	504	500	457	386	9.46	<0.001	<0.001	0.60
Mixed	674	664	632	576	13.6	<0.001	<0.001	0.93
Preformed	565	689	761	777	9.00	<0.001	<0.001	0.88
Select individual fatty acids, g/d								
C4:0	48.3	53.7	53.9	50.8	1.17	0.01	<0.001	0.52
C6:0	35.9	38.5	36.8	32.0	0.85	<0.001	<0.001	0.48
C8:0	23.5	24.3	22.3	18.4	0.56	<0.001	<0.001	0.45
C10:0	66.4	64.3	56.5	44.7	1.68	<0.001	<0.001	0.66
C12:0	81.5	75.5	64.3	50.2	2.01	<0.001	<0.001	0.59
C14:0	227	227	210	179	3.89	<0.001	<0.001	0.67
C16:0	638	634	606	552	12.7	<0.001	<0.001	0.96
cis-9 C16:1	35.7	29.9	25.8	23.1	1.08	<0.001	<0.001	0.75
C18:0	116	180	220	238	5.55	<0.001	<0.001	0.90
trans -6 to -8 C18:1	3.93	5.14	6.26	6.82	0.11	<0.001	<0.001	0.13
trans-9 C18:1	3.15	4.09	5.03	5.28	0.09	<0.001	<0.001	0.01
trans-10 C18:1	8.07	8.92	10.1	10.0	0.36	<0.001	<0.001	0.07
trans-11 C18:1	11.8	17.4	23.8	37.0	1.02	<0.001	<0.001	0.03
trans-12 C18:1	7.03	9.82	11.7	12.3	0.26	<0.001	<0.001	0.72
cis-9 C18:1	258	299	322	328	5.99	<0.001	<0.001	0.94
cis-9, *cis*-12 C18:2	47.8	52.2	51.8	46.3	1.06	0.02	<0.001	0.90
cis-9, *cis*-12, *cis*-15 C18:3	7.25	6.40	4.99	3.50	0.14	<0.001	<0.001	0.13

[1]Experimental diets fed to 24 cows in replicated 4 × 4 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).
[2]Inclusion level of WCS, %DM
[3]Greatest SEM.

Table 18 (cont'd)
[4]Contrasts correspond to the linear, quadratic, and cubic effects of increasing WCS inclusion.

Table 19. Milk fatty acid contents for cows fed treatment diets (n=24)[1].

Variable	Treatment[2]				SEM[3]	Contrast[4]		
	0%	8%	16%	24%		Linear	Quadratic	Cubic
Summation by source, g/100 g								
De novo	28.9	27.0	24.7	22.0	0.30	<0.001	0.01	0.94
Mixed	38.4	35.7	34.0	32.8	0.29	<0.001	<0.001	0.33
Preformed	32.7	37.3	41.3	45.2	0.40	<0.001	0.07	0.37
Selected individual fatty acids, g/100 g								
C4:0	2.73	2.90	2.91	2.88	0.04	<0.001	<0.001	0.22
C6:0	2.02	2.07	1.98	1.82	0.03	<0.001	<0.001	0.20
C8:0	1.34	1.31	1.20	1.05	0.02	<0.001	<0.001	0.35
C10:0	3.76	3.46	3.05	2.55	0.07	<0.001	<0.01	0.85
C12:0	4.72	4.07	3.47	2.87	0.09	<0.001	0.42	0.68
C14:0	13.0	12.3	11.3	10.2	0.13	<0.001	0.01	0.94
C16:0	36.3	34.1	32.7	31.5	0.26	<0.001	<0.001	0.41
cis-9 C16:1	2.08	1.61	1.39	1.32	0.05	<0.001	<0.001	0.10
C18:0	6.67	9.68	11.9	13.6	0.25	<0.001	<0.001	0.57
trans -6 to -8 C18:1	0.23	0.28	0.34	0.39	0.01	<0.001	0.97	0.63
trans-9 C18:1	0.18	0.22	0.27	0.30	0.01	<0.001	0.08	0.02
trans-10 C18:1	0.47	0.49	0.55	0.57	0.02	<0.001	0.80	0.15
trans-11 C18:1	0.70	0.95	1.29	2.11	0.07	<0.001	<0.001	0.03
trans-12 C18:1	0.40	0.53	0.63	0.70	0.02	<0.001	<0.01	0.94
cis-9 C18:1	14.8	16.2	17.5	18.7	0.24	<0.001	0.38	0.84
cis-9, *cis*-12 C18:2	2.77	2.84	2.81	2.65	0.07	<0.001	<0.001	0.56
cis-9, *cis*-12, *cis*-15 C18:3	0.42	0.34	0.27	0.20	0.01	<0.001	0.37	0.45

[1]Experimental diets fed to 24 cows in replicated 4 × 4 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).
[2]Inclusion level of WCS, %DM
[3]Greatest SEM.
[4]Contrasts correspond to the linear, quadratic, and cubic effects of increasing WCS inclusion.

Table 20. Plasma metabolites for cows fed treatment diets (n=24)[1].

	Treatment[2]				SEM[3]	*P*-value	Contrast[5]		
Variable	0%	8%	16%	24%		Trt[4] × Period	Linear	Quadratic	Cubic
Insulin, µg/L	0.96	0.90	0.92	0.87	0.03	0.13	<0.01	0.88	0.09
(-) Gossypol, µg/ml[6]	0.60	1.46	2.31	3.30	0.09	0.05	<0.001	0.41	0.65
(+) Gossypol, µg/ml[6]	0.30	1.00	1.78	2.61	0.05	0.03	<0.001	0.15	0.82
Total Gossypol, µg/ml	0.91	2.46	4.09	5.87	0.14	0.03	<0.001	0.35	0.90

[1]Experimental diets fed to 24 cows in replicated 4 × 4 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).
[2]Inclusion level of WCS, %DM
[3]Greatest SEM.
[4]Trt = treatment.
[5]Contrasts correspond to the linear, quadratic, and cubic effects of increasing WCS inclusion.
[6](-) and (+) refer to the negative and positive isomers of gossypol, respectively.

Table 21. Ingredient and nutrient composition of treatment diets.

	Treatment[1]			
	0%	8%	16%	24%
Ingredient, % DM				
Corn Silage	45.6	45.4	45.4	45.5
Alfalfa Silage	8.11	8.08	8.08	8.09
Ground Corn	15.2	15.1	15.1	15.1
Mineral Mix[2]	1.98	1.97	1.97	1.97
Lactation Mix[3]	2.72	2.71	2.71	2.71
DCAD supplement[4]	0.46	0.45	0.45	0.46
Soybean Meal	18.5	12.6	6.49	0.46
Soybean Hulls	7.47	5.46	3.54	1.31
High Oleic Soybeans[1,5]	0.00	8.23	16.2	24.4
Nutrient Composition, % DM[6]				
NDF	29.4	28.7	28.1	27.4
Forage NDF	20.6	20.5	20.6	20.6
Starch	27.5	27.5	27.5	27.6
CP	16.5	16.4	16.1	15.9
Fatty Acid (FA)	1.65	3.11	4.52	5.97
16:0	0.25	0.31	0.36	0.42
18:0	0.05	0.10	0.14	0.19
cis-9 18:1	0.30	1.61	2.89	4.19
cis-9, *cis*-12 18:2	0.92	0.91	0.91	0.90
cis-9, *cis*-12, *cis*-15 18:3	0.11	0.12	0.14	0.15

[1]Inclusion levels of HOSB, %DM

[2]Vitamin and mineral mix contained 26% Amino Plus (Ag Processing Inc), 14% calcium carbonate, 13% Caledonia Pass (Papillon) and sodium sesquicarbonate, 9% calcium phosphate, 6% urea, 5% white salt, 4% magnesium oxide, 3% MIN AD (Min Ad Inc) and potassium chloride red, 2% D&D Ion Pak, 1% selenium, and <1% ferrous sulfate, Micro 5, Smartamine (Adisseo), vitamin D, vitamin E, and vitamin A.

[3]Lactation supplement contained 40% sodium sesquinate, 20% calcium carbonate, 19% ground corn, 15% Spectrum AgriBlue (Perdue Agribusiness), 3% Smartamine (Adisseo), and 2% urea.

[4]Ion Plus (D&D Ingredients LLC; Delphos, OH).

[5]Plenish Soybean (Dupont Pioneer; Johnston, IA, USA). FA profile composed of: (g/100g) 5.44 of C16:0, 3.40 of C18:0, 81.0 of *cis*-9 18:1, 3.51 of *cis*-9, *cis*-12 18:2, and 1.47 of *cis*-9, *cis*-12, *cis*-15 18:3; 20.3% DM of total FA. Mean particle size: 771 ± 2.56 microns

[6]Expressed as a percent of as fed.

Table 22. DMI and production responses of cows fed treatment diets (n=24)[1].

Variable	Treatment[2]				SEM[3]	Contrast[4]		
	0%	8%	16%	24%		Linear	Quadratic	Cubic
DMI, kg/d	31.2	31.3	30.7	30.5	0.56	0.01	0.66	0.26
Yield, kg/d								
Milk	48.2	51.4	52.0	52.2	1.81	<0.001	<0.01	0.30
3.5% FCM[5]	48.5	50.9	51.5	52.4	1.02	<0.001	0.11	0.25
ECM[6]	49.4	51.3	51.8	52.4	0.91	<0.001	0.12	0.37
Fat	1.66	1.75	1.78	1.83	0.06	<0.001	0.27	0.23
Protein	1.56	1.63	1.63	1.61	0.02	0.01	<0.01	0.46
Lactose	2.33	2.52	2.57	2.58	0.04	<0.001	<0.001	0.25
Milk composition, %								
Fat	3.52	3.47	3.48	3.64	0.12	0.02	<0.01	0.62
Protein	3.29	3.19	3.16	3.14	0.04	<0.001	0.02	0.43
Lactose	4.86	4.91	4.93	4.94	0.02	<0.001	<0.001	0.19
Milk urea nitrogen, mg/dL	11.3	10.4	9.57	8.46	0.26	<0.001	0.39	0.72
ECM/DMI	1.57	1.65	1.69	1.72	0.03	<0.001	0.17	0.89
BW, kg	742	742	743	744	14.7	0.25	0.64	0.44
BW change, kg/d	0.64	0.76	0.58	0.49	0.11	0.20	0.34	0.44
BCS	3.10	3.09	3.10	3.09	0.05	0.81	0.78	0.52
BCS change	0.06	0.01	0.04	0.04	0.02	0.81	0.19	0.24

[1]Experimental diets fed to 24 cows in replicated 4 × 4 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).
[2]Inclusion level of HOSB, %DM
[3]Greatest SEM.
[4]Contrasts correspond to the linear, quadratic, and cubic effects of increasing HOSB inclusion.
[6]Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)] (NRC, 2001)
[7]Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein) (Tyrrell and Reid, 1965).

Table 23. Milk fatty acid yields for cows fed treatment diets (n=24)[1].

Variable	Treatment[2]				SEM[3]	Contrast[4]		
	0%	8%	16%	24%		Linear	Quadratic	Cubic
Summation by source, g/d								
De novo	464	446	412	368	19.1	<0.001	0.05	0.85
Mixed	605	510	443	386	18.2	<0.001	<0.01	0.45
Preformed	519	702	835	978	19.6	<0.001	0.04	0.15
Select individual fatty acids, g/d								
C4:0	42.3	45.3	46.7	47.6	2.19	<0.01	0.32	0.81
C6:0	31.9	32.8	32.1	30.6	1.79	0.16	0.08	0.78
C8:0	21.1	21.3	19.8	18.1	1.18	<0.001	0.03	0.41
C10:0	60.4	56.9	49.9	42.8	3.17	<0.001	0.08	0.42
C12:0	76.8	68.5	59.2	49.0	3.62	<0.001	0.41	0.99
C14:0	211	202	186	165	7.28	<0.001	0.03	0.77
C16:0	569	484	418	364	17.2	<0.001	0.01	0.78
cis-9 C16:1	35.0	28.9	24.8	21.5	1.15	<0.001	<0.01	0.54
C18:0	105	143	169	188	8.09	<0.001	0.02	0.66
trans -6 to -8 C18:1	4.15	7.69	11.6	14.6	0.33	<0.001	0.30	0.36
trans-9 C18:1	3.22	5.05	6.85	8.05	0.21	<0.001	0.01	0.25
trans-10 C18:1	10.4	13.9	18.1	18.3	1.81	<0.001	0.04	0.16
trans-11 C18:1	12.6	14.7	15.4	15.9	0.90	<0.001	0.07	0.55
cis-9 C18:1	241	371	471	562	10.1	<0.001	<0.01	0.40
cis-9, *cis*-12 C18:2	36.4	35.3	32.1	28.9	1.11	<0.001	<0.01	0.19
cis-9, *cis*-12, *cis*-15 C18:3	5.76	6.22	6.51	6.54	0.22	<0.001	0.08	0.86

[1]Experimental diets fed to 24 cows in replicated 4 × 4 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).
[2]Inclusion level of HOSB, %DM
[3]Greatest SEM.
[4]Contrasts correspond to the linear, quadratic, and cubic effects of increasing HOSB inclusion.

Table 24. Milk fatty acid content for cows fed treatment diets (n=24)[1].

Variable	Treatment[2]				SEM[3]	Contrast[4]		
	0%	8%	16%	24%		Linear	Quadratic	Cubic
Summation by source, g/100 g								
De novo	28.8	26.6	24.1	21.6	0.43	<0.001	0.41	0.89
Mixed	37.1	30.5	25.9	22.7	0.40	<0.001	<0.001	0.47
Preformed	34.0	42.9	50.0	55.6	0.69	<0.001	<0.001	0.86
Selected individual fatty acids, g/100 g								
C4:0	2.64	2.67	2.73	2.81	0.06	<0.01	0.39	0.98
C6:0	1.98	1.93	1.86	1.75	0.06	<0.001	0.25	0.87
C8:0	1.31	1.26	1.15	1.04	0.04	<0.001	0.11	0.37
C10:0	3.74	3.39	2.86	2.46	0.11	<0.001	0.52	0.07
C12:0	4.75	4.03	3.38	2.84	0.11	<0.001	0.03	0.85
C14:0	13.2	12.2	11.0	9.70	0.13	<0.001	0.25	0.73
C16:0	34.9	28.7	24.4	21.4	0.39	<0.001	<0.001	0.53
cis-9 C16:1	2.19	1.74	1.49	1.29	0.07	<0.001	<0.001	0.13
C18:0	6.54	8.57	9.90	11.3	0.28	<0.001	0.01	0.11
trans -6 to -8 C18:1	0.26	0.48	0.70	0.86	0.03	<0.001	0.06	0.19
trans-9 C18:1	0.20	0.31	0.41	0.46	0.01	<0.001	<0.001	0.14
trans-10 C18:1	0.69	0.93	1.26	1.35	0.17	<0.001	0.35	0.36
trans-11 C18:1	0.80	0.86	0.89	0.93	0.04	<0.001	0.52	0.66
cis-9 C18:1	15.5	22.7	28.3	32.7	0.49	<0.001	<0.001	0.72
cis-9, *cis*-12 C18:2	2.36	2.14	1.92	1.69	0.04	<0.001	0.49	0.83
cis-9, *cis*-12, *cis*-15 C18:3	0.36	0.38	0.39	0.39	0.01	<0.001	0.09	0.88

[1]Experimental diets fed to 24 cows in replicated 4 × 4 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).
[2]Inclusion level of HOSB, %DM
[3]Greatest SEM.
[4]Contrasts correspond to the linear, quadratic, and cubic effects of increasing HOSB inclusion.

Table 25. Plasma metabolites for cows fed treatment diets (n=24)[1].

	Treatment[2]				SEM[3]	Contrast[4]		
Variable	0%	8%	16%	24%		Linear	Quadratic	Cubic
Insulin, µg/L	0.97	0.92	0.95	0.88	0.04	0.08	0.74	0.18
Blood urea nitrogen, mg/dL	13.2	12.4	11.5	10.4	0.25	<0.001	0.42	0.96

[1]Experimental diets fed to 24 cows in replicated 4 × 4 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).
[2]Inclusion level of HOSB, %DM
[3]Greatest SEM.
[4]Contrasts correspond to the linear, quadratic, and cubic effects of increasing HOSB inclusion.

Table 26. Ingredient and nutrient composition of treatment diets.

	Treatment			
	CON	RST	RAW-D	RAW-U
Ingredient, % DM				
Corn Silage	45.1	44.8	44.6	44.7
Alfalfa Silage	8.06	8.02	7.98	8.00
Ground Corn	11.3	11.2	11.2	11.2
Vitamin and Mineral Mix[2]	2.00	1.99	1.98	1.99
Lactation Mix[3]	4.17	4.14	4.13	4.13
DCAD	0.43	0.43	0.43	0.43
Soybean Hulls	10.4	6.51	6.43	6.48
Soybean Meal	18.6	6.12	6.32	-
By-Pass Protein[4]	-	-	-	6.24
Roasted HOSB[5]	-	16.7	-	-
Raw HOSB[5]	-	-	16.9	16.8
Nutrient Composition[6], % DM				
NDF	31.3	29.7	29.7	29.9
Forage NDF	21.6	21.6	21.6	21.6
CP	17.2	17.2	17.2	17.2
Starch	27.7	28.1	27.9	28.1
FA	2.82	4.98	5.10	5.08

[1]Inclusion levels of HOSB, %DM

[2]Vitamin and mineral mix contained 26% Amino Plus (Ag Processing Inc), 14% calcium carbonate, 13% Caledonia Pass (Papillon) and sodium sesquicarbonate, 9% calcium phosphate, 6% urea, 5% white salt, 4% magnesium oxide, 3% MIN AD (Min Ad Inc) and potassium chloride red, 2% D&D Ion Pak, 1% selenium, and <1% ferrous sulfate, Micro 5, Smartamine (Adisseo), vitamin D, vitamin E, and vitamin A.

[3]Lactation supplement contained 50% ground corn, 26% sodium sesquinate, 12% calcium carbonate, 7% calcium phosphate, 2.5% urea, 1.7% Smartamine (Adisseo).

[4]AminoPlus (Ag Processing Inc; Omaha, NE)

[5]Plenish Soybean (Pioneer; Johnston, IA, USA). FA profile composed of: (g/100g) 5.44 of C16:0, 3.40 of C18:0, 81.0 of *cis*-9 18:1, 3.51 of *cis*-9, *cis*-12 18:2, and 1.47 of *cis*-9, *cis*-12, *cis*-15 18:3; 20.3% DM of total FA.

[6]Expressed as a percent of as fed.

Table 27. Production responses of cows fed treatment diets (n=36)[1].

Variable	Treatment[2]				SEM[3]	*P*-value[4]	Contrast[4]		
	CON	RST	RAW-D	RAW-U		Trt x Week	CON vs SOY	RST vs RAW	PRTN
DMI, kg/d	28.4	29.0	29.1	29.1	0.48	-	0.04	0.91	0.90
Yield, kg/d									
Milk	42.3	45.9	42.2	43.7	0.62	0.01	<0.01	<0.001	<0.01
3.5% FCM[5]	43.6	49.4	45.9	46.6	0.67	0.02	<0.001	<0.001	0.13
ECM[6]	44.0	49.0	45.6	46.4	0.66	0.01	<0.001	<0.001	0.07
Fat	1.56	1.83	1.71	1.70	0.03	0.02	<0.001	<0.001	0.85
Protein	1.40	1.47	1.37	1.41	0.02	<0.01	0.21	<0.001	0.01
Lactose	2.06	2.26	2.08	2.15	0.03	0.01	<0.001	<0.001	<0.01
Milk composition, %									
Fat	3.73	4.03	4.06	3.95	0.05	0.05	<0.001	0.23	<0.01
Protein	3.34	3.21	3.26	3.23	0.03	<0.001	<0.001	<0.01	0.03
Lactose	4.86	4.92	4.91	4.92	0.02	<0.01	<0.001	0.48	0.55
MUN (ug/mL)	12.9	10.5	11.6	11.0	0.23	<0.01	<0.001	<0.01	<0.01
ECM/DMI	1.49	1.62	1.57	1.61	0.03	-	<0.01	0.25	0.24
BW, kg	727	727	718	723	11.5	0.76	<0.01	<0.001	<0.01
BW change, kg/d	0.44	0.25	0.50	0.39	0.09	-	0.60	0.08	0.40
BCS	3.06	3.06	3.10	3.04	0.03	-	0.76	0.78	0.03
BCS change	0.05	0.02	0.08	0.01	0.02	-	0.73	0.32	0.04

[1]Experimental diets fed to 36 cows in replicated 4 × 2 truncated Latin squares with 35-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 30 to 35).
[2]CON = no HOSB supplementation; RST = 16% DM roasted and ground HOSB; RAW-D = 16% DM raw and ground HOSB; RAW-U = 16% DM raw and ground HOSB + by-pass protein
[3]Greatest SEM.
[4]Contrasts are: CON vs SOY = CON vs 1/3 (RST + RAW-D + RAW-U); RST vs RAW = RST vs ½ (RAW-D + RAW-U); PRTN = RAW-D vs RAW-U.

Table 27 (cont'd)
[5]Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)] (NRC, 2001).
[6]Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein) (Tyrrell and Reid, 1965).

Table 28. Milk fatty acid yield for cows fed treatment diets (n=36)[1].

Variable	Treatment[2]				SEM[3]	Contrast[4]		
	CON	RST	RAW-D	RAW-U		CON vs SOY	RST vs RAW	PRTN
Summation by source, g/d								
de novo	402	408	404	396	11.3	0.94	0.42	0.47
Mixed	562	466	475	463	13.6	<0.001	0.75	0.38
Preformed	446	772	723	729	18.7	<0.001	0.01	0.79
Selected individual fatty acids, g/d								
C4:0	36.5	46.9	44.2	44.1	1.30	<0.001	0.02	0.97
C6:0	28.0	33.5	31.6	31.8	1.06	<0.001	0.07	0.84
C8:0	17.7	20.2	19.4	19.2	0.67	<0.01	0.14	0.84
C10:0	52.2	51.7	52.1	50.5	1.80	0.62	0.80	0.41
C12:0	66.9	58.8	60.8	58.3	2.00	<0.001	0.65	0.25
C14:0	184	184	184	179	4.89	0.64	0.63	0.35
C16:0	534	445	455	443	13.2	<0.001	0.72	0.38
cis-9 C16:1	27.8	20.6	20.4	20.0	0.71	<0.001	0.48	0.55
C18:0	92.5	174	181	179	5.89	<0.001	0.21	0.79
trans -6 to -8 C18:1	2.75	7.64	5.68	5.78	0.24	<0.001	<0.001	0.68
trans-9 C18:1	2.17	4.82	3.73	3.74	0.15	<0.001	<0.001	0.96
trans-10 C18:1	6.36	8.42	5.79	5.82	0.44	0.36	<0.001	0.95
trans-11 C18:1	7.61	12.2	6.60	7.02	0.49	0.04	<0.001	0.46
trans-12 C18:1	4.44	7.70	5.53	5.55	0.25	<0.001	<0.001	0.94
cis-9 C18:1	219	440	405	406	10.1	<0.001	<0.01	0.88
cis-9, *cis*-12 C18:2	32.3	32.9	27.4	27.6	0.81	<0.01	<0.001	0.81
cis-9, *cis*-12, *cis*-15 C18:3	6.11	7.49	6.31	6.54	0.19	<0.01	<0.001	0.32

[1]Experimental diets fed to 36 cows in replicated 4 × 2 truncated Latin squares with 35-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 30 to 35).
[2]CON = no HOSB supplementation; RST = 16% DM roasted and ground HOSB; RAW-D = 16% DM raw and ground HOSB; RAW-U = 16% DM raw and ground HOSB + by-pass protein

Table 28 (cont'd)
[3]Greatest SEM.
[4]Contrasts are: CON vs SOY = CON vs 1/3 (RST + RAW-D + RAW-U); RST vs RAW = RST vs ½ (RAW-D + RAW-U); PRTN = RAW-D vs RAW-U.

Table 29. Milk fatty acid concentration for cows fed treatment diets (n=36)[1].

Variable	Treatment[2]				SEM[3]	Contrast[4]		
	CON	RST	RAW-D	RAW-U		CON vs SOY	RST vs RAW	PRTN
Summation by source, g/100 g								
de novo	28.5	24.7	25.3	24.9	0.31	<0.001	0.16	0.20
Mixed	39.5	28.1	29.6	29.1	0.45	<0.001	<0.01	0.25
Preformed	31.6	47.4	45.6	45.9	0.46	<0.001	<0.001	0.38
Selected individual fatty acids, g/100 g								
C4:0	2.65	2.86	2.74	2.79	0.05	<0.01	0.02	0.30
C6:0	1.99	2.01	1.97	2.00	0.03	0.79	0.20	0.27
C8:0	1.26	1.22	1.21	1.20	0.02	0.01	0.45	0.73
C10:0	3.71	3.11	3.26	3.17	0.07	<0.001	0.08	0.19
C12:0	4.75	3.53	3.81	3.67	0.09	<0.001	0.01	0.1
C14:0	12.9	11.1	11.5	11.2	0.14	<0.001	0.14	0.16
C16:0	37.5	26.9	28.2	27.8	0.44	<0.001	<0.01	0.25
cis-9 C16:1	1.97	1.25	1.27	1.27	0.04	<0.001	0.46	0.79
C18:0	6.54	10.7	11.6	11.9	0.26	<0.001	<0.001	0.28
trans -6 to -8 C18:1	0.20	0.48	0.35	0.36	0.01	<0.001	<0.001	0.26
trans-9 C18:1	0.16	0.30	0.23	0.23	0.01	<0.001	<0.001	0.67
trans-10 C18:1	0.44	0.54	0.37	0.36	0.03	0.41	<0.001	0.76
trans-11 C18:1	0.52	0.76	0.42	0.44	0.03	0.63	<0.001	0.61
trans-12 C18:2	0.32	0.47	0.34	0.35	0.01	<0.001	<0.001	0.70
cis-9 C18:1	15.4	27.0	25.2	25.5	0.30	<0.001	<0.001	0.31
cis-9,*cis*-12 C18:2	2.27	2.01	1.71	1.76	0.04	<0.001	<0.001	0.26
cis-9,*cis*-12,*cis*-15 C18:3	0.42	0.45	0.39	0.41	0.01	0.83	<0.001	0.04

[1]Experimental diets fed to 36 cows in replicated 4 × 2 truncated Latin squares with 35-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 30 to 35).
[2]CON = no HOSB supplementation; RST = 16% DM roasted and ground HOSB; RAW-D = 16% DM raw and ground HOSB; RAW-U = 16% DM raw and ground HOSB + by-pass protein
[3]Greatest SEM.

Table 29 (cont'd)
[4]Contrasts are: CON vs SOY = CON vs 1/3 (RST + RAW-D + RAW-U); RST vs RAW = RST vs ½ (RAW-D + RAW-U); PRTN = RAW-D vs RAW-U.

Table 30. Plasma metabolites for cows fed treatment diets (n=36)[1].

Variable	Treatment[2]				SEM[3]	Contrast[4]		
	CON	RST	RAW-D	RAW-U		CON vs SOY	RST vs RAW	PRTN
Insulin, µg/L	1.13	0.95	0.93	0.97	0.07	0.03	0.94	0.70
BUN[5], mg/dL	14.1	10.5	12.4	11.8	0.30	<0.001	<0.001	0.03

[1]Experimental diets fed to 24 cows in replicated 4 × 4 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).
[2]Inclusion level of HOSB, %DM
[3]Greatest SEM.
[4]Contrasts are: CON vs SOY = CON vs 1/3 (RST + RAW-D + RAW-U); RST vs RAW = RST vs ½ (RAW-D + RAW-U); PRTN = RAW-D vs RAW-U.
[5]Blood urea nitrogen

APPENDIX B: LIST OF FIGURES

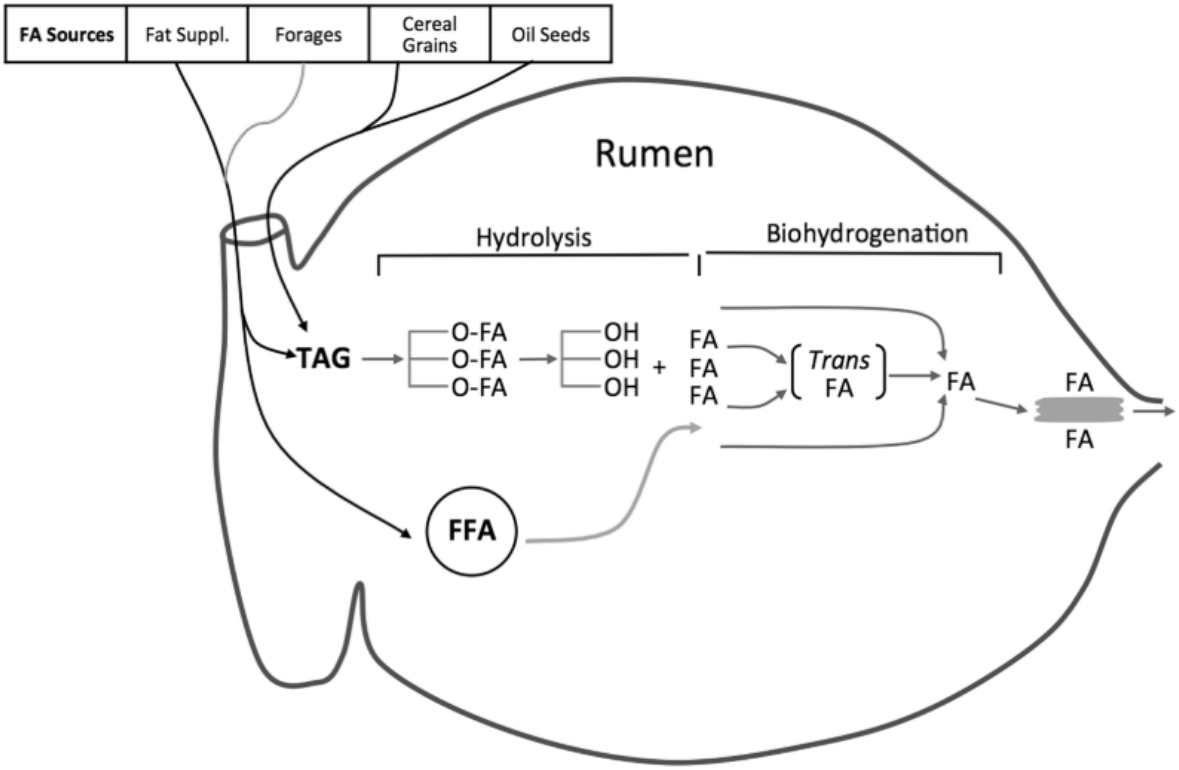

Figure 1. Metabolism of dietary lipids in the rumen. Triacylglycerides (TAG), free fatty acids (FFA), trans fatty acids (trans FA), and mixture of fatty acids (FAs), and volatile fatty acids (VFA). Adapted from Lock et al., 2006.

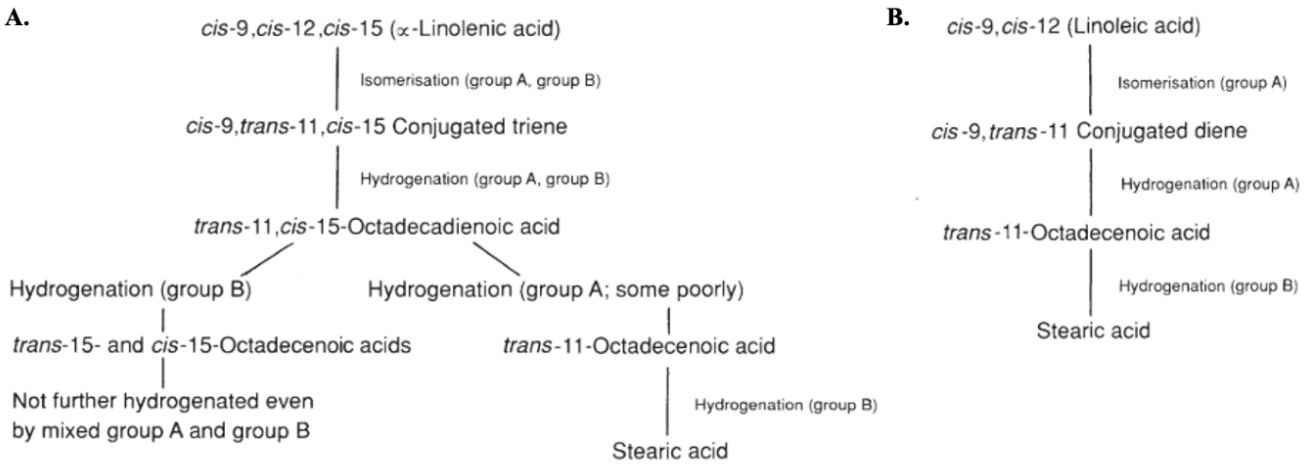

Figure 2. Scheme for the biohydrogenation of (A) linolenic acid and (B) linoleic acid. Group A and group B refer to the two classes of biohydrogenation bacteria. Adapted from Palmquist et al., 2005.

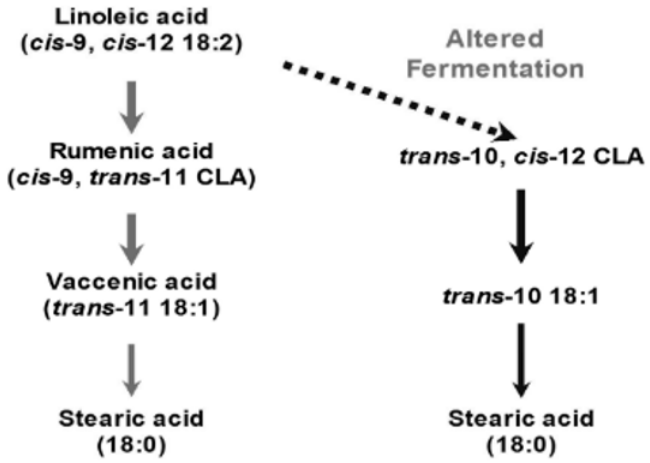

Figure 3. Biohydrogenation pathways of dietary lipids in the rumen. Adapted from Bauman et al., 2003.

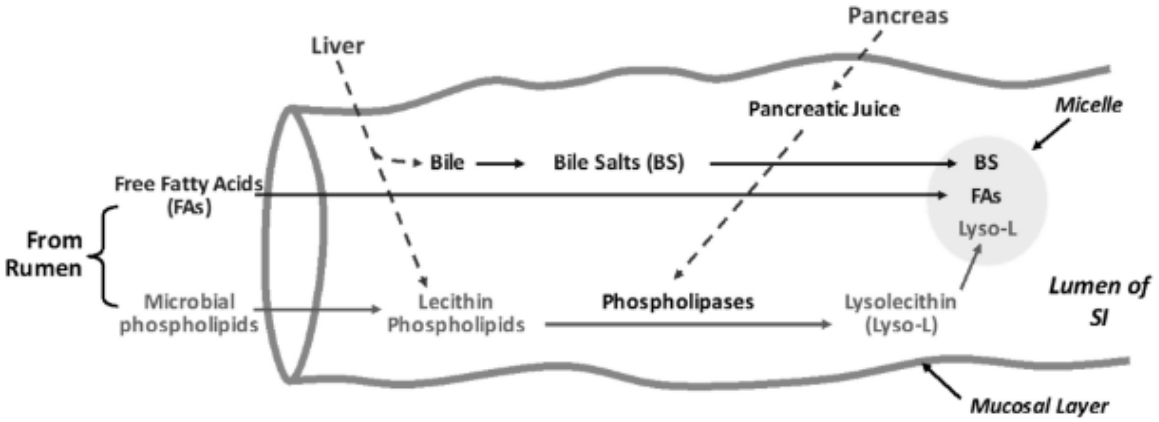

Figure 4. Fat digestion in the small intestine of ruminants. Adapted from Lock et al., 2006.

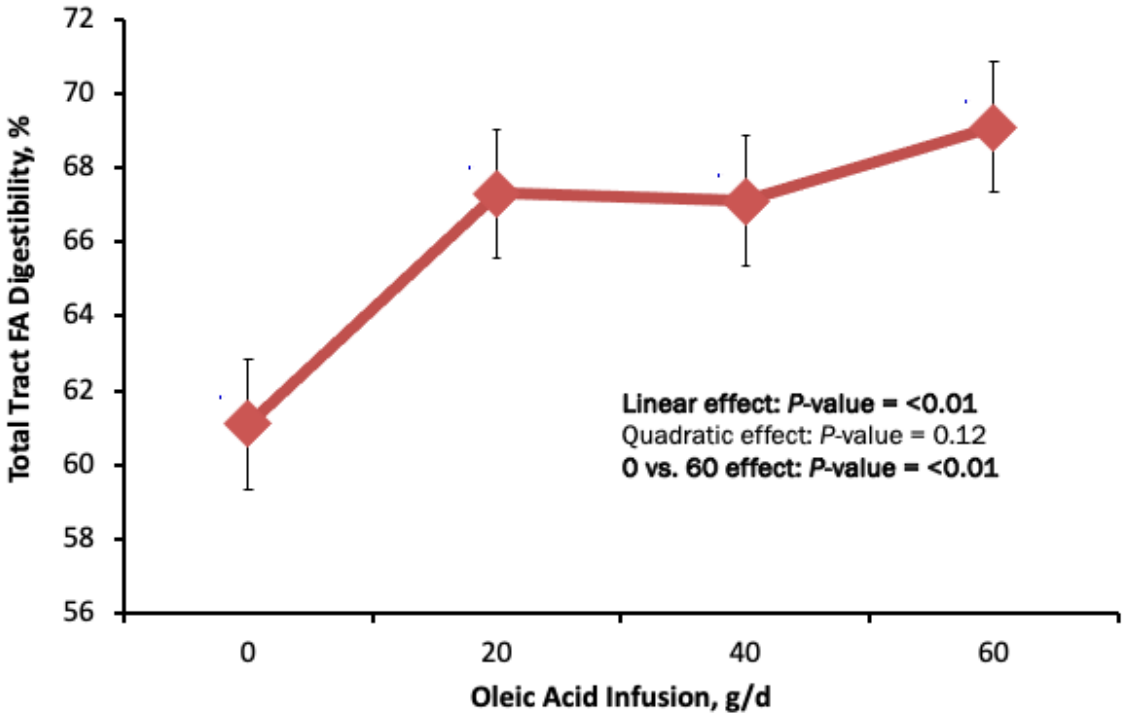

Figure 5. Total FA digestibility with increasing abomasal infusion of *cis*-9 C18:1. Adapted from Prom et al. (2021). Increasing abomasal infusion of up to 60 g/d of *cis*-9 C18:1 increased total FA digestibility by ~8 percentage units.

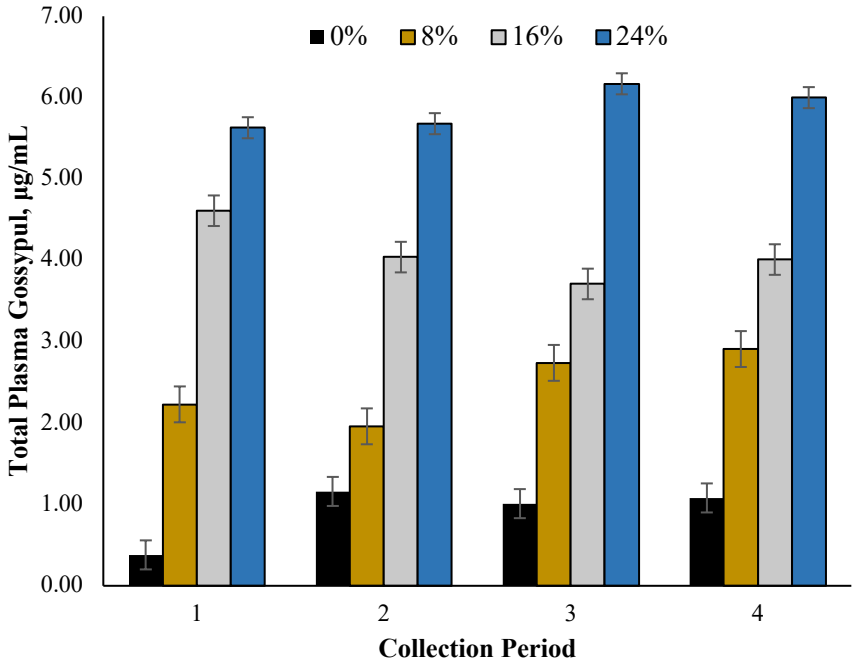

Figure 6. Treatment by period interactions were observed for plasma gossypol with increasing inclusion of WCS. Treatments were 0%, 8%, 16%, and 24% DM inclusion of WCS. Error bars represent SEM used for each individual treatment. Three orthogonal contrasts were used to test the linear, quadratic, and cubic effect of increasing WCS inclusion. A treatment by period interaction (P =0.03) was detected, as the 0% WCS had the lowest gossypol level in period 1 compared with periods 2, 3, and 4 (P=0.03, P=0.07, and P=0.05, respectively) suggesting a carry-over effect of gossypol in plasma across periods, although there were no other period differences within WCS treatments.

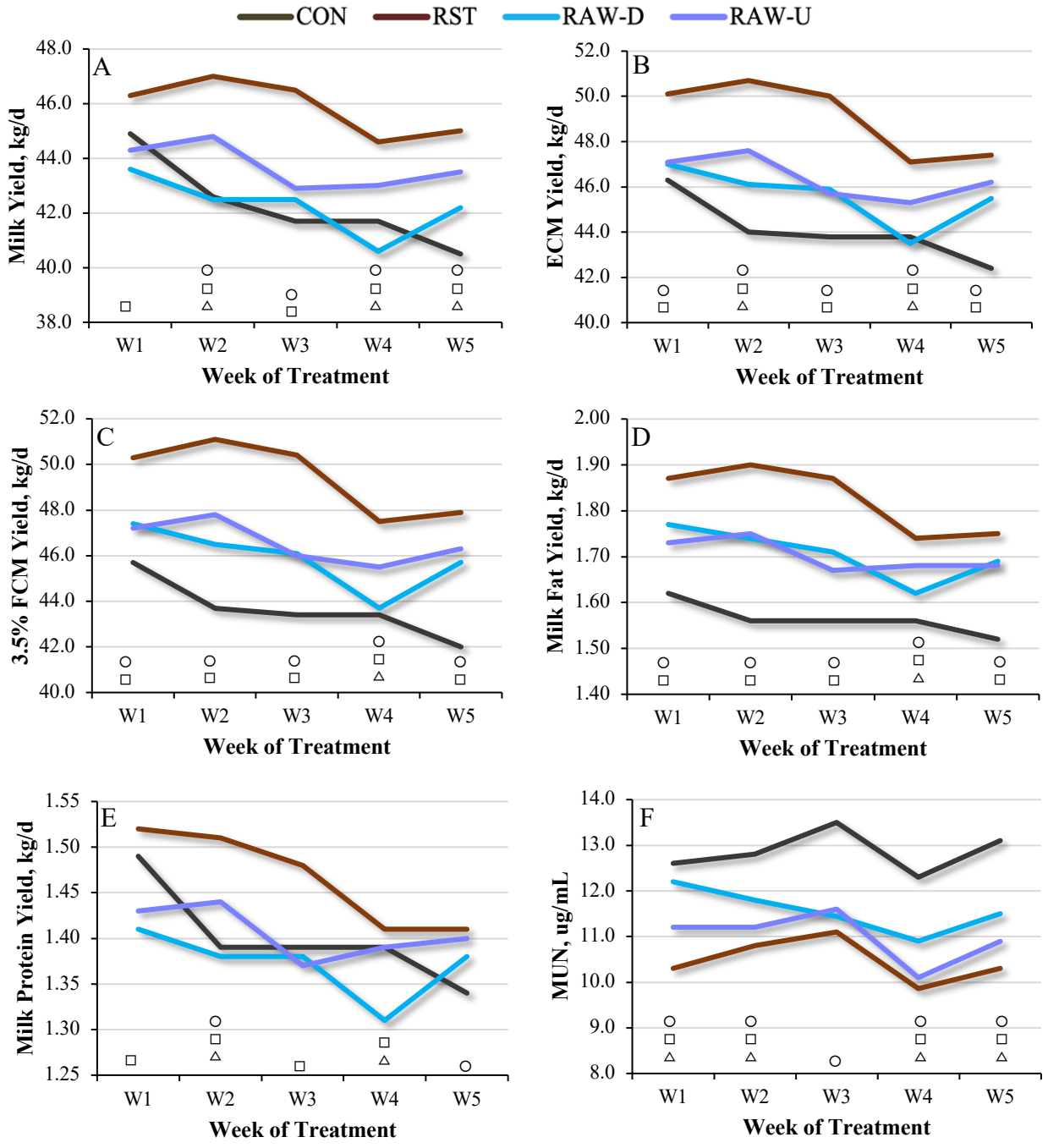

Figure 7. Effects of treatments on yields of milk (A), ECM (B), 3.5% FCM (C), milk fat (D), milk protein (E), and MUN content (F) of high-producing dairy cows. Treatments were as follows: CON = no HOSB supplementation, RAW = 16% DM roasted and ground HOSB, RAW-D = 16% DM raw and ground HOSB, and RAW-U = 16% DM raw and ground HOSB + additional by-pass protein. Significant interactions between treatment and week were detected for yields of milk (P=0.01), ECM (P=0.01), 3.5% FCM (P=0.02), milk fat (P=0.02), and milk protein (P<0.01), and MUN content (P<0.01). Contrasts are: CON vs SOY (○) = CON vs 1/3

Figure 7 (cont'd)
(RST + RAW-D + RAW-U); RST vs RAW (□) = RST vs ½ (RAW-D + RAW-U); PRTN (△) = RAW-D vs RAW-U; significance was determined at $P \leq 0.05$ and tendencies were determined at $P \leq 0.10$.