12-2020

Rumen Fermentation Responses to Purified Palmitic, Stearic, or Oleic Fatty Acids and the Impact of a Palmitic Acid-Enriched Supplement on Animal Performance

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RUMEN FERMENTATION RESPONSES TO PURIFIED PALMITIC, STEARIC, OR OLEIC FATTY ACIDS AND THE IMPACT OF A PALMITIC ACID-ENRICHED SUPPLEMENT ON ANIMAL PERFORMANCE

by

Austin Paul Sears

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Animal, Dairy, and Veterinary Sciences

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UTAH STATE UNIVERSITY
Logan, Utah

2020

ABSTRACT

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by

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Utah State University, 2020

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Supplemental fats in ruminant diets have been shown to impact fiber digestion and animal performance in varying degrees, with the type of fatty acids supplemented being the major factor influencing the response. Our research examined the responses of palmitic, stearic and oleic fatty acids on neutral detergent fiber (**NDF**) digestibility and rumen fermentation and the impact of a palmitic acid-enriched supplement on animal performance. In the first research chapter, we evaluated the effect of purified palmitic, stearic, or oleic fatty acids on NDF digestibility and rumen fermentation. Eight continuous culture fermenters were used in a 4 × 4 Latin square design with four treatments: control, palmitic acid (PA), stearic acid (SA), and oleic acid (OA). Data were analyzed using a mixed model including the fixed effect of treatment, and the random effect of period and fermenter. Three contrasts were used to evaluate (1) the effect of the combination of palmitic, stearic, and oleic acids treatment means compared with control [Control vs. (PA+SA+OA)/3]; (2) the effect of palmitic acid compared with oleic acid

(PA vs. OA); and (3) the effect of stearic acid compared with oleic acid (SA vs. OA). Palmitic acid increased NDF digestibility (46.6% vs. 36.1%) and total production of volatile fatty acids (168.9 mmol vs. 146.7 mmol) compared with oleic acid. Bacterial fatty acid composition was altered by palmitic acid, increasing *anteiso*-C15:0 (7.34% vs. 6.31%), C18:2n6 (3.86% vs. 2.68%), and C18:3n3 (0.30% vs. 0.23%) fatty acids compared with oleic acid. Compared with control, total protein expression in chemiluminescence units (C.U.) of the fatty acid synthase (0.035 vs. 0.068 C.U.) and acetyl CoA carboxylase (0.033 vs. 0.044 C.U.) enzymes were decreased by the combination of palmitic, stearic, and oleic fatty acids. No treatment effect was observed for ammonia and cellulase activity. In conclusion, palmitic acid compared with oleic acid can be utilized in ruminant diets to improve NDF digestibility and total VFA production, providing more energy and nutrients for the host animal. Furthermore, the combination of palmitic, stearic and oleic acid decreased total protein expression of the fatty acid synthase and acetyl CoA carboxylase enzymes compared with control indicating that palmitic, stearic and oleic acid can be utilized by the bacteria and inhibit the ACC and FAS enzymes in the fatty acid synthesis pathway. In the second research chapter, the impact of a palmitic acid-enriched supplement (82.5% palmitic acid) on production responses and nitrogen metabolism of Jersey and Holstein cows was evaluated. Eighty mid-lactation dairy cows, 40 Jersey and 40 Holstein were used in a complete block design with a split-plot arrangement with two treatments: control and palmitic acid supplementation. Data were analyzed using a mixed model with the random effect of block and cow nested within breed, and the fixed effect of treatment, breed, time and their interactions. Compared with control, palmitic acid increased milk fat yield (1.36 vs.

1.26 kg/d), NDF digestibility (46% vs. 42%), and dry matter digestibility (68% vs. 66%) and tended to increase 3.5% fat-corrected milk (35.6 vs. 34.0 kg/d) and energy-corrected milk (35.7 vs 34.1 kg/d). Compared with Holstein cows, Jersey cows had greater dry matter intake as % body weight (**BW**; 4.90 vs. 3.37% of BW), and tended to have greater milk fat yield (1.36 vs. 1.26 kg/d) but had lower milk production (29.6 vs. 32.7 kg/d) and milk lactose yield (1.42 vs. 1.58 kg/d). There was a breed effect on BW change, as Holstein cows gained 0.385 kg/d during the experiment while Jersey cows gained 0.145 kg/d. Jersey cows had lower nitrogen intake (636 vs. 694 g/d), blood urea nitrogen (12.6 vs. 13.8 mg/dL), urine total nitrogen (125 vs. 145 g/d), and urine total nitrogen as a percent of nitrogen intake (19.5 vs. 21.1%) compared with Holstein cows. Overall, feeding a palmitic acid-enriched supplement increased milk fat yield as well as dry matter and fiber digestibility in both Holstein and Jersey cows, but did not have any major effects on nitrogen metabolism.

(90 pages)

PUBLIC ABSTRACT

Rumen Fermentation Responses to Purified Palmitic, Stearic, or Oleic Fatty Acids and the Impact of a Palmitic Acid-Enriched Supplement on Animal Performance

Austin Paul Sears

Our research examined the responses of palmitic, stearic and oleic fatty acids on neutral detergent fiber (**NDF**) digestibility and rumen fermentation and the impact of a palmitic acid-enriched supplement on animal performance. In the first research chapter, we evaluated the effect of purified palmitic, stearic, and oleic fatty acids on NDF digestibility and rumen fermentation. Palmitic acid increased NDF digestibility and total production of volatile fatty acids (**VFA**) compared with oleic acid. Bacterial fatty acid composition was altered by palmitic acid, increasing *anteiso*-C15:0, C18:2n6 and C18:3n3 fatty acids compared with oleic acid. Compared with control, total protein expression in chemiluminescence units (C.U.) of the fatty acid synthase and acetyl CoA carboxylase enzyme was decreased by the combination of palmitic, stearic, and oleic fatty acids. No treatment effect was observed for ammonia and cellulase activity. In conclusion, palmitic acid compared with oleic acid can be utilized in ruminant diets to improve NDF digestibility and total VFA production, providing more energy and nutrients for the host animal. Furthermore, palmitic, stearic and oleic acid decreased total protein expression of the fatty acid synthase and acetyl CoA carboxylase enzyme compared with control indicating that palmitic, stearic and oleic acid can be utilized by the bacteria and inhibit the ACC and FAS enzymes in the fatty acid synthesis pathway.

In the second research chapter, we determined the impact of a palmitic acid-enriched supplement on production responses and nitrogen metabolism of Jersey and Holstein cows. Compared with control, palmitic acid increased milk fat yield, NDF and dry matter digestibility and tended to increase 3.5% fat-corrected milk and energy-corrected milk. Compared with Holstein cows, Jersey cows had greater intake as percentage of body weight and tended to have greater milk fat yield, but had lower milk production, and milk lactose yield. There was a breed effect on body weight change as Holstein cows gained 0.385 kg/d during the experiment while Jersey cows gained 0.145 kg/d. Jersey cows had lower nitrogen intake, blood urea nitrogen, urine total nitrogen and urine total nitrogen as a percent of nitrogen intake. Feeding a palmitic acid-enriched supplement increased milk fat yield as well as dry matter and fiber digestibility in both Holstein and Jersey cows. Palmitic acid did not have any major effects on nitrogen metabolism. Overall, our results indicate that palmitic acid can improve fiber digestion, rumen fermentation, and animal performance.

ACKNOWLEDGMENTS

First and foremost, I would like to express my sincere gratitude to my advisor and mentor, Dr. Fernanda Batistel for giving me the opportunity of a lifetime to be her first graduate student and to work in her laboratory. She encouraged and supported me throughout my degree, seeing my potential and pushing me to achieve it. I have learned from her that great effort yields great success and appreciate the time that she has taken to make me feel important and become successful in my endeavors of graduate school and for her kindness toward my family.

I give special thanks to my committee members, Dr. Allen Young and Dr. Clay Isom for the hours of mentorship and advice over the years and for their encouragement to pursue this degree. I am honored to call you my friends. My lab mates, Anthony Alberto and Osvaldo Gonzalez deserve a big “Muchas Gracias” for their help with my projects and their great friendship. I appreciate the support and friendship of Jonas de Souza and for his help in training me to perform important laboratory procedures for my research and for the great conversations we had in the lab. I thank Perdue Agribusiness and Utah Agriculture Experiment Station for partial support of the experiments. I want to thank Dr. Robert Ward from the Nutrition, Dietetics and Food Sciences here at USU for running my fatty acid analysis and taking the time to help interpret the results. I appreciate the help received in setting up the fermenters from Dr. Benjamin Wenner from The Ohio State University.

Thanks to my parents and family for their endless support throughout my education and for encouraging me to “be something great”. By far the one who deserves the biggest thank you is my wife, Carianne. She is the only one who knows the efforts,

discouragements, joys and successes of my education. She has been my support and strength and stood by me every step of the way and I love her very much for that. My 3 daughters Elsie, Rebecca, and Oakley deserve a thanks too, as they have been patiently waiting for dad to be done with school.

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CHAPTER 1

REVIEW OF LITERATURE

Ruminants have the ability to convert low quality feed that is rich in fiber into high quality protein in the form of meat and milk; this is possible through rumen fermentation. The rumen is a fermentation site with an environment that supports anaerobic microbial life which is sensitive to pH, oxygen, and temperature. Rumen pH can vary by diet in a range from 5.7 to 7.3 (Shriver et al., 1986, Yang et al., 2001). An anaerobic environment is necessary to keep the microbes alive and the temperature of the rumen is maintained at 39°C. The microbial population contained in the rumen is substantial and contains bacteria, protozoa, fungi and archaea (Church, 1993, Kothari et al., 2018). Bacteria are the primary microbes within the rumen and make up several classification groups such as fibrolytic, amylolytic, proteolytic, and lipolytic (**Table 1-1**). These different groups help to digest both structural and non-structural carbohydrates (**CHO**), proteins and lipids, respectively.

Table 1-1. End products of fermentation of principal rumen bacteria.

Microorganisms	Fermentation end products	Reference
Cellulolytic bacteria		
Fibrobacter succinogenes	Acetate	(Ivan et al., 2012)
Prevotella spp.	Acetate, Butyrate	(Kurita-Ochiai et al., 1995)
Amylolytic bacteria		
Succinomonas amylolitica	Acetate, Propionate	(Cotta, 1992)
Proteolytic bacteria		
Bacteroides amylophilus	Acetate, Propionate	(Miura et al., 1980)
Butyrivibrio fibrisolvens	Acetate, Butyrate	(Cotta and Hespell, 1986)
Lipolytic bacteria		
Anaerovibrio lipolytica	Acetate, Propionate	(Fuentes et al., 2009)
Megasphaera elsdenii	Acetate, Propionate	(Klieve et al., 2003)

In ruminant nutrition, CHO are categorized into two main types, non-structural and structural. Non-structural CHO are found inside the cellular contents of the plant tissues in the form of storage saccharides such as glucose, fructose, sucrose and starch. The most common form of non-structural CHO fed in dairy cow diets is starch, which is the way that most plants store energy, such as grain seeds. Starch exists in organized structures of amylose and amylopectin held together by α-1,4 bonds with amylopectin also containing α-1,6 bonds (**Figure 1-1)**, forming reserve CHO for the plant (Zobel, 1988). Amylose is a linear chain of linked glucose molecules, while amylopectin contains branches off the straight chain. Starch is digested by enzymes called α-amylases which are synthesized by amylolytic bacteria. Structural CHO compose plant cell walls and include cellulose, pectin, and hemicellulose (Casper et al., 1990) and are responsible for maintaining the plant's rigid structure. Cellulose is the main structural CHO found in fibrous plants and is an unbranched polymer of glucose linked together through β-1,4 bonds (**Figure 1-1**). This type of bond allows the glucose to form long, straight chains (Van Es, 1981). Animals are not able to digest β bonds and thus digestion of cellulose is made possible through the fibrolytic bacteria that synthesize the enzyme β-1,4 cellulase (Miron and Ben-Ghedalia, 1993). After the glucose has been liberated from the CHO in the rumen, specialized CHO fermenting bacteria utilize it for energy and production of fermentation compounds.

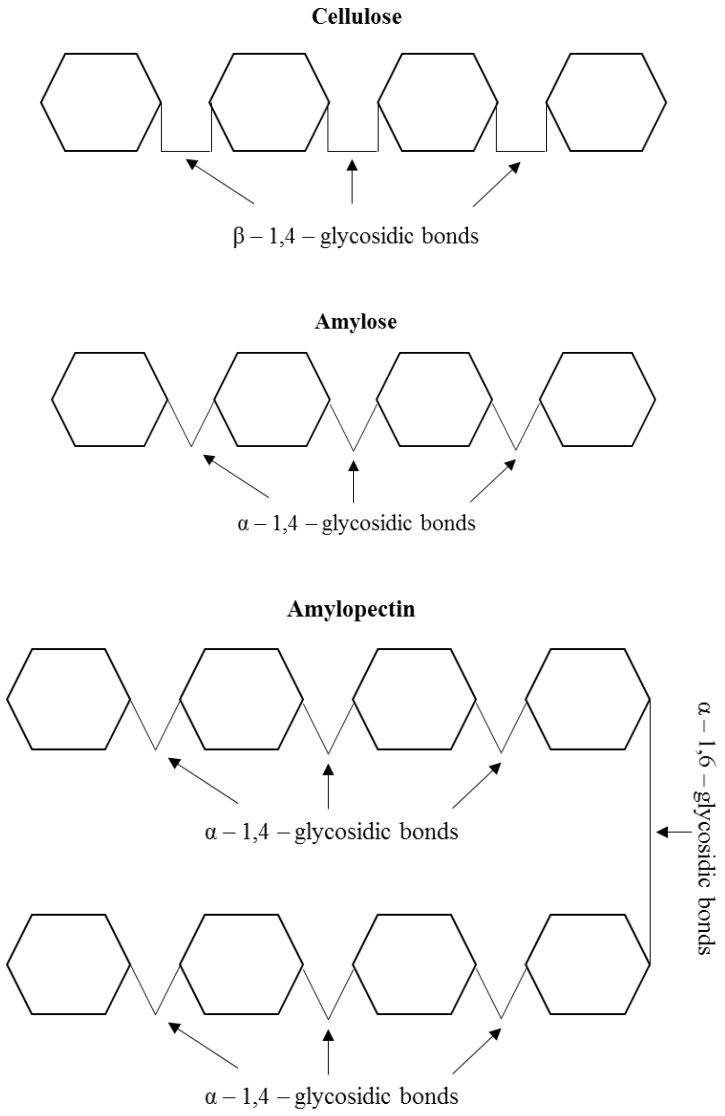

Figure 1-1. Chemical structure of cellulose, amylose, and amylopectin.

Rumen fermentation of CHO provides energy for the growth of the microbes and nutrients to the host animal by utilizing fermentation compounds. The main intermediate compound formed by CHO fermentation is pyruvate, and it is also the central substrate used to form the main end products of fermentation: acetate, butyrate and propionate which are called volatile fatty acids (**VFAs**) (Wolin, 1979, Russell and Hespell, 1981).

Two molecules of pyruvate, a three-carbon molecule, can be converted to acetyl CoA as an intermediate which then can be utilized to produce acetate (2 carbons) or butyrate (4 carbons). Pyruvate can also form succinate or lactate, which can be further used to form propionate (3 carbons) either by the succinate or acrylate pathways (Ungerfeld and Kohn, 2006). The VFAs are removed from the rumen through diffusion across the rumen wall and provide energy for the host animal (**Figure 1-2**). Acetate is used for energy production and synthesis of short (4-8 carbons) and medium chain (10-14 carbons) fatty acids in the mammary gland and adipose tissue and butyrate is mainly utilized by the rumen epithelium for energy and growth of those cells (Bergman, 1990). Propionate is used for gluconeogenesis in the liver. Because of its 3 carbon structure it can be synthesized to glucose by being converted to succinyl-CoA and entering the tricarboxylic acid (**TCA**) cycle (VanSoest, 1994).

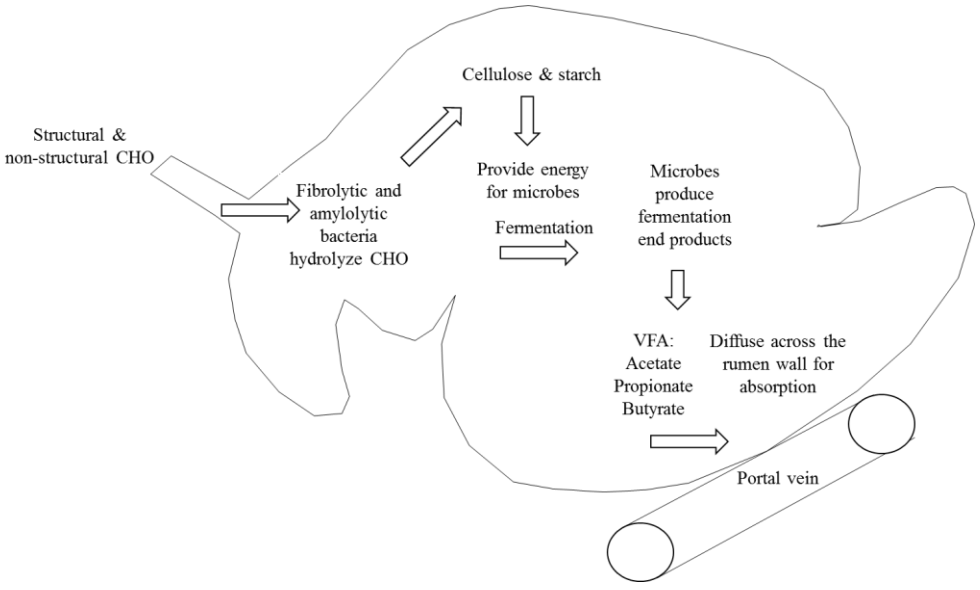

Figure 1-2. Carbohydrate metabolism in the rumen.

The dietary protein fed to ruminants is classified in two fractions, rumen degradable protein (**RDP**) and rumen un-degradable protein (**RUP**). Rumen degradable protein is composed of protein and non-protein nitrogen (**NPN**) sources, whereas the RUP is composed mainly by protein (Santos et al., 1998). In the rumen, protein is broken down into amino acids (**AA**) and peptides by proteolytic bacteria (Blackburn and Hobson, 1962). These AA can be utilized by the bacteria for microbial protein synthesis and the excess can escape the rumen and be absorbed in the small intestine (Kung and Rode, 1996). Non-protein nitrogen is hydrolyzed by bacteria to ammonia (**NH_3**) (Waldo, 1968, Abdoun et al., 2006) which provides a nitrogen (**N**) source for the rumen microbes to synthesize protein for maintenance and growth (Tillman and Sidhu, 1969) (**Figure 1-3**). Excess NH_3 can diffuse across the rumen wall and enter the portal vein where it travels to the liver to be converted to urea to prevent toxicity (Bartley et al., 1981). Blood urea can be recycled back to the rumen and saliva or can be excreted in the milk and urine (Harmeyer and Martens, 1980). Microbial protein consists of protein from dead rumen microbes that are continually flowing out of the rumen and are digested within the abomasum allowing for the AA to be absorbed in the small intestine (Moran, 2005). Microbial protein is important to ruminants because it provides a protein source with a complete AA profile (Atasoglu et al., 1998) and has greater digestibility compared to RUP sources (Schingoethe, 1991, Schwab, 1994) . Rumen un-degradable protein avoids further degradation and enters the small intestine for absorption without being modified by the rumen microbial population.

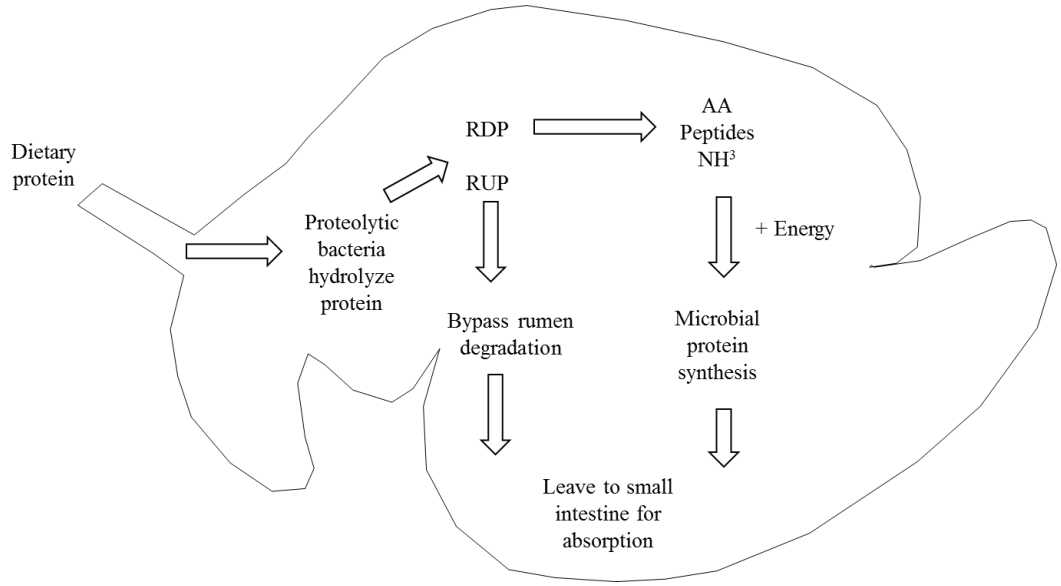

Figure 1-3. Protein metabolism in the rumen.

Fats in the diet can be obtained through forages, fat supplements and seed oils. The main form of fats in the diet are triglycerides (**TAG**) which have a glycerol backbone with three fatty acids (**FA**) attached to it and glycolipids which are similar in structure to TAG except that one of the FA attached to the glycerol backbone is replaced with a sugar molecule (Drackley, 2004). Triglycerides are found in cereal grains, animal fats and oil seeds, while glycolipids are mainly found in forages and legumes (Wattiaux and Grummer, 1991). The FA attached to the glycerol backbone of the TAG or glycolipid can either be saturated (**SFA**) which have no double hydrogen bonds or unsaturated (**UFA**) which do contain double bonds (Vander Wal, 1964). Lipolytic rumen bacteria are responsible for fat hydrolysis using lipases to free the FA from the glycerol backbone (Henderson and Hodgkiss, 1973). Once liberated, UFA are subject to biohydrogenation (**BH**) by rumen bacteria because they seem to impose a toxic effect on specific microbial populations, the end-product of the BH being the SFA stearic acid (Palmquist and

Jenkins, 1980a, Shingfield and Wallace, 2014). However, not all UFA are completely biohydrogenated, with BH efficiency ranging from 70-95% (Jenkins et al., 2008). The reasons for this incomplete BH can be the amount of UFA present in the rumen, increased passage rate of digesta and decreased rumen pH from high concentrate diets (Latham et al., 1972, Jenkins and Harvatine, 2014). Continual flow of digesta from the rumen allows for BH fatty acids to leave the rumen and be absorbed in the small intestine (**Figure 1-4**).

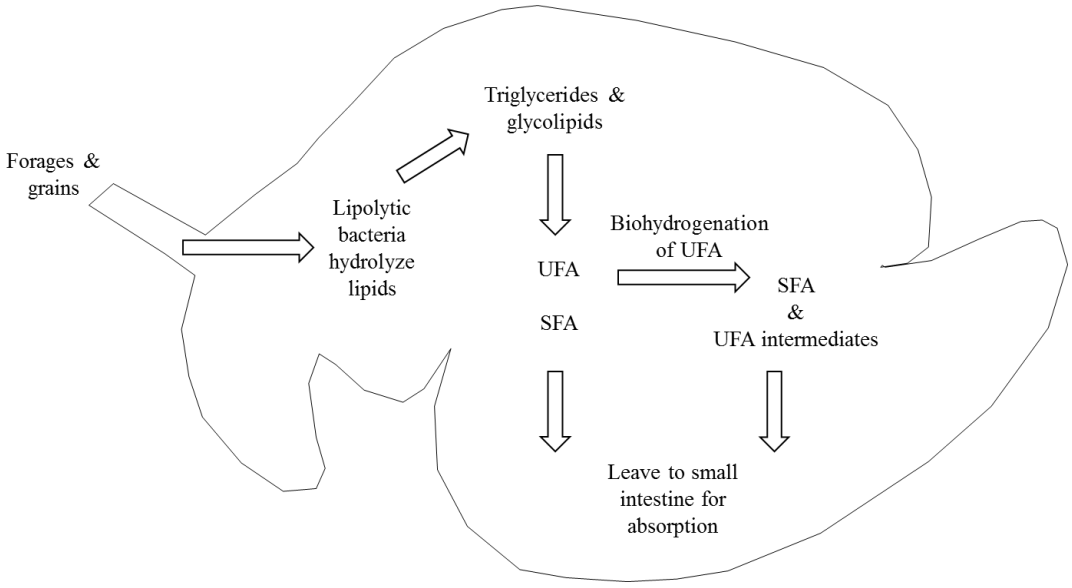

Figure 1-4. Lipid metabolism in the rumen.

Rumen Bacteria Membrane Fatty Acid Composition

Bacteria are the most abundant microbe in the rumen with about 10^{10}-10^{11} cells per milliliter of rumen fluid (McSweeney, 2012). The bacterial composition consists of gram-positive (**G+**) and gram-negative (**G-**) bacteria. The cell wall of gram-positive bacteria contains a unique cell membrane, while gram-negative bacteria contain a cell membrane and an outer membrane (Beveridge and Graham, 1991). The cell membrane is

composed of a phospholipid bilayer that has a polar head group and two hydrophobic FA tails allowing for their tails to turn toward the interior of the cell and expose their hydrophilic heads to water, creating a barrier (Alberts et al., 2002). These bilayers contain a wide variety of FA which can be modulated by the bacteria to maintain fluidity (Silhavy et al., 2010). Maintaining membrane fluidity of bacteria is critical in regulating the passage of solutes between the cell and the outer environment, keeping essential metabolites and nutrients inside the cell, preventing the entry of toxic compounds from the environment, and are of special importance for the energy transduction of the cell (Sikkema et al., 1995). This can be important for fiber digestion because the bacteria can be more efficient at maintaining homeostasis and using the cell energy and nutrients to grow, digest and ferment.

The cell membranes of mixed rumen bacteria are rich in palmitic (C16:0), stearic (C18:0) and oleic acids (C18:1) (Or-Rashid et al., 2007), and these FA can be synthesized through the FA synthesis pathway or can be obtained from the environment (Erwin, 1973). Fatty acid synthesis is an energy intensive reaction using seven adenosine triphosphate (**ATP**) to synthesize a 16 carbon FA chain (palmitic acid) (Berg et al., 2002). Synthesis begins in the cytosol with acetyl CoA being carboxylated to form malonyl CoA; this is done by the acetyl CoA carboxylase (**ACC**) enzyme. The enzyme responsible for the synthesis of long chain fatty acids (**LCFA**; >16 carbons) from malonyl CoA is the fatty acid synthase (**FAS**). The FAS elongates the FA chain by 2 carbons until the formation of palmitic acid (C16:0); further elongation and insertion of double bonds are carried out by other enzyme systems (Berg et al., 2002). Rumen bacterial membrane maintenance could also occur through incorporation of free fatty

acids from the environment, e.g., dietary FA. Silbert et al. (1968) observed that when omega-7 monounsaturated or vaccenic acids were added to *Escherichia coli*, there was a 33% increasing in growth rate and direct incorporation of both fatty acids into the phospholipid membrane. The incorporation of FA from the environment could be advantageous to the rumen bacteria by allowing them to spare ATP energy from membrane FA synthesis and potentially grow and reproduce more efficiently.

Impact of Dietary Fat on Fiber Digestion

Fats have been utilized in the diets of ruminants to increase diet energy density, milk production and milk fat, decrease heat stress and increase reproductive performance; yet it has been recognized that fat can negatively impact fiber digestion. Many studies done in the 1940-1950's looked at fat supplementation and its effect on fiber digestion (Lucas and Loosli, 1944, Swift et al., 1947). One particular study done by Brooks et al. (1954) fed corn oil and lard at two levels (32 g and 64 g) to sheep, and observed that both treatments decreased fiber digestibility when compared to a control diet without supplemental fat. Studies like this were key in allowing for the wide acceptance of the idea that supplemental fat can negatively impact fiber digestion. However, not all fats are created equal and recent evidences show that the SFA palmitic acid and stearic acid can enhance neutral detergent fiber (**NDF**) digestibility (Piantoni et al., 2015, de Souza et al., 2018). Neutral detergent fiber is the common measure of fiber used in ruminant diets and measures the majority of the structural CHO from plants (Van Soest et al., 1991). An increase in NDF digestibility efficiency can improve animal performance. Oba and Allen (1999) showed that a 1-unit increase in forage rumen NDF digestion corresponds to a

0.17 kg increase in DMI and just over a 0.25 kg increase in 4% fat corrected milk per cow per day, indicating the advantages of increasing NDF digestion. Supplementation of SFA is one strategy that has been shown to increase NDF digestibility. de Souza and Lock (2018a) observed a 5% increase in NDF digestibility when a palmitic acid enriched fat supplement was fed to lactating Holstein dairy cows at 1.5% diet dry matter (**DM**) when compared to a control diet with no supplemental fat. A meta-analysis done by Weld and Armentano (2017) that analyzed the effects of different fat supplementation on dry matter intake (**DMI**) and NDF digestibility, found that feeding a SFA combination of palmitic and stearic increased NDF digestibility but did not affect DMI. Additional studies that fed palmitic acid supplements observed varying NDF digestibility improvements. Piantoni et al. (2013a) observed a 3% increase in NDF digestibility with a palmitic acid supplement fed at 2% diet DM, and de Souza et al. (2017) saw a 2% increase in NDF digestibility at the same inclusion rate of 2% diet DM. Rico et al. (2017b) observed a 3% increase in NDF digestibility in low milk producing cows (< 38 kg/d) and 2% increase in high producing (>40 kg/d) when palmitic acid was fed at 1.5% diet DM. The reasons for this improvement in NDF digestibility are still not understood and need further investigation. One postulated reason is that palmitic acid's effects on microbial populations could incorporate palmitic acid into rumen bacterial membranes, sparing ATP, which may favor bacterial growth, possibly increasing NDF digestibility.

Impact of Dietary Fat on Milk Synthesis

Milk synthesis occurs in the mammary gland, creating a product that includes fat and protein. Milk fat can be noticeably influenced by the diet and is the most variable component in milk, being impacted by many factors, including genetics, lactational stage,

and diet (Palmquist et al., 1993). There are two sources of FA for milk fat synthesis: the *de-novo* synthesis of FA in the mammary gland and preformed fatty acids that are incorporated from blood circulation derived from either diet or mobilized body fat (Smith and Abraham, 1975). *De-novo* FA are short-chain (4 to 8 carbons) and medium-chain (10-14 carbons) FA that are synthesized in the mammary gland through the FAS pathway, while long-chain FA (>16 carbons) are incorporated from TAG in the blood and 16 carbon FA can be derived from both sources (Bauman and Griinari, 2003). The major carbon sources for *de-novo* fat synthesis come from β-hydroxybutyrate (**BHB**) and acetate (Bauman and Griinari, 2003). Acetate is converted to acetyl CoA and then to malonyl CoA which then can enter the fatty acid synthesis pathway and BHB is used as a primer for the first 4 carbons of a FA chain that will be synthesized by FAS (Larson, 1969). Preformed lipids are TAG present in circulating chylomicrons and low density lipoproteins within the blood (Mansbridge and Blake, 1997) and account for about 40% of milk fatty acids, and *de-novo* synthesis of fatty acids within the mammary gland make up the rest (Bickerstaffe et al., 1974). Acetate provides 80% of the FA carbons for *de-novo* synthesis while BHB contributes 20% of the carbons (McCarthy and Smith, 1969). Preformed FA are imported to the mammary gland for milk fat synthesis from plasma lipoprotein lipase (**LPL**) or non-esterified fatty acids (**NEFA**) bound to albumin (Clegg et al., 2001).

The amount and type of proteins in milk is largely determined by the genetics of the animal and is not easily altered through nutrition. However, due to the high energy requirement for protein synthesis, the milk protein yield can be impacted by the energy content in the diet (Reynolds et al., 1994) along with the availability of AA for protein

synthesis in the mammary gland (Bequette et al., 1998). Studies evaluating the effect of increasing dietary protein in rations have failed to show a consistent positive effect on milk protein synthesis (DePeters and Cant, 1992). The overall process of protein synthesis within the mammary gland consists of several integrated functions such as AA uptake, transcription of DNA to mRNA, translation of mRNA to protein, and post translational modifications of the protein before it is secreted (Maas et al., 1997). Amino acids are transported across mammary cell membranes by specific AA transporters within the membrane and facilitate the transport of single AA into the cell (Christensen, 1990). These AA are then used to make proteins. Mammary proteins are secreted by the alveolar cell through exocytosis where secretory vesicles are carried to the cell membrane, and their contents are secreted into the extracellular environment (Maas et al., 1997).

Supplementing fat in dairy cow rations has been extensively evaluated over the past few decades, and results have indicated that the type of supplemental fat fed can have variable results on milk production. Palmitic acid supplementation has shown to consistently increase the yield of milk fat (Lock et al., 2013b, Mathews et al., 2016b). It has been proposed that the uptake of palmitic acid in the mammary gland is higher than that of other fatty acids. Enjalbert et al. (1998) postulated that higher plasma concentrations of supplemented palmitic acid enhance the activity of mammary LPL, resulting in greater extraction of FA from the blood TAG. de Souza et al. (2018) observed that supplemented palmitic acid increases milk energy output and energy partitioning towards milk fat synthesis, suggesting that palmitic acid can partition energy toward the mammary gland, although the specific mechanism is unknown.

Conclusions

Neutral detergent fiber digestion is crucial in providing the energy necessary for ruminants to grow and be productive. It has been shown that certain dietary fatty acids can alter NDF digestibility but the exact mechanisms on how this happens has not been fully researched. Understanding this phenomenon can help ruminant nutritionists create specific diets that can further improve digestibility, allowing for more efficient animals. Palmitic acid supplementation has been shown to increase the yield and concentration of milk fat. However, to our knowledge the effects of dietary palmitic acid supplementation has only been evaluated with Holstein cows. Therefore, determining the impact of palmitic acid supplementation on Jersey cows is of particular importance because breed differences can impact milk fat synthesis. These subsequent research chapters help to increase our knowledge of how specific dietary fatty acids can impact rumen digestion, fermentation and animal performance.

References

Abdoun, K., F. Stumpff, and H. Martens. 2006. Ammonia and urea transport across the rumen epithelium: a review. Anim Health Res Rev 7(1-2):43-59.

Alberts, B., A. Johnson, and J. Lewis. 2002. The Lipid Bilayer in Molecular Biology of the Cell. 4th ed. Garland Science, New York.

Bartley, E. E., T. B. Avery, T. G. Nagaraja, B. R. Watt, A. Davidovich, S. Galitzer, and B. Lassman. 1981. Ammonia toxicity in cattle. V. Ammonia concentration of lymph and portal, carotid and jugular blood after the ingestion of urea. J Anim Sci 53(2):494-498.

Bauman, D. E. and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annual Review of Nutrition 23(1):203-227.

Bequette, B. J., F. R. C. Backwell, and L. A. Crompton. 1998. Current Concepts of Amino Acid and Protein Metabolism in the Mammary Gland of the Lactating Ruminant. Journal of Dairy Science 81(9):2540-2559.

Berg, J. M., J. L. Tymoczko, and L. Stryer. 2002. Biochemistry. Fatty Acids Are Synthesized and Degreaded by Different Pathways. W H Freeman, New York.

Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol Rev 70(2):567-590.

Beveridge, T. J. and L. L. Graham. 1991. Surface layers of bacteria. Microbiol Rev 55(4):684-705.

Bickerstaffe, R., E. F. Annison, and J. L. Linzell. 1974. The metabolism of glucose, acetate, lipids and amino acids in lactating dairy cows. The Journal of Agricultural Science 82(1):71-85.

Blackburn, T. H. and P. N. Hobson. 1962. Further studies on the isolation of proteolytic bacteria from the sheep rumen. J Gen Microbiol 29:69-81.

Brooks, C. C., G. B. Garner, C. W. Gehrke, M. E. Muhrer, and W. H. Pfander. 1954. The Effect of Added Fat on the Digestion of Cellulose and Protein by Ovine Rumen Microorganisms. Journal of Animal Science 13(4):758-764.

Casper, D. P., D. J. Schingoethe, and W. A. Eisenbeisz. 1990. Response of early lactation dairy cows fed diets varying in source of nonstructural carbohydrate and crude protein. J Dairy Sci 73(4):1039-1050.

Christensen, H. N. 1990. Role of amino acid transport and countertransport in nutrition and metabolism. Physiol Rev 70(1):43-77.

Church, D. C. 1993. Ruminant Animal: Digestive Physiology and Nutrition. Prentice-

Hall, Englewood Cliffs, NJ.

Clegg, R. A., M. C. Barber, L. Pooley, I. Ernens, Y. Larondelle, and M. T. Travers. 2001. Milk fat synthesis and secretion: molecular and cellular aspects. Livestock Production Science 70(1):3-14.

de Souza, J., J. L. Garver, C. L. Preseault, and A. L. Lock. 2017. Short communication: Effects of prill size of a palmitic acid enriched fat supplement on the yield of milk and milk components, and nutrient digestibility of dairy cows. Journal of Dairy Science 100(1):379-384.

de Souza, J. and A. L. Lock. 2018. Long-term palmitic acid supplementation interacts with parity in lactating dairy cows: Production responses, nutrient digestibility, and energy partitioning. Journal of Dairy Science 101(4):3044-3056.

de Souza, J., C. L. Preseault, and A. L. Lock. 2018. 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. Journal of Dairy Science 101(1):172-185.

Demchick, P. and A. L. Koch. 1996. The permeability of the wall fabric of Escherichia coli and Bacillus subtilis. J Bacteriol 178(3):768.

DePeters, E. J. and J. P. Cant. 1992. Nutritional factors influencing the nitrogen composition of bovine milk: a review. J Dairy Sci 75(8):2043-2070.

Drackley, J. K. 2004. Overview of Fat Digestion and Metabolism in Dairy Cows. University of Illinios, Urbana, Urbana IL.

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. The Journal of Nutrition 128(9):1525-1532.

Erwin. 1973. Lipids and Biomembranes of Eukaryotic Microorganisms. . Academic Press, London and San Diego.

Harmeyer, J. and H. Martens. 1980. Aspects of urea metabolism in ruminants with reference to the goat. J Dairy Sci 63(10):1707-1728.

Henderson, C. and W. Hodgkiss. 1973. An Electron Microscopic Study of Anaerovibrio lipolytica (Strain 5s) and Its Lipolytic Enzyme. Microbiology 76(2):389-393.

Jenkins, T. C. and K. J. Harvatine. 2014. Lipid Feeding and Milk Fat Depression. Veterinary Clinics of North America: Food Animal Practice 30(3):623-642.

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(2):397-412.

Kothari, R. K., N. M. Nathani, C. Mootapally, J. K. Rank, H. B. Gosai, B. P. Dave, and C. G. Joshi. 2018. Chapter 11 - Comprehensive Exploration of the Rumen Microbial Ecosystem With Advancements in Metagenomics. Pages 215-229 in Metagenomics. M. Nagarajan, ed. Academic Press.

Kung, L. and L. M. Rode. 1996. Amino acid metabolism in ruminants. Animal Feed Science and Technology 59(1):167-172.

Larson, B. L. 1969. Biosynthesis of Milk. Journal of Dairy Science 52(5):737-747.

Latham, M. J., J. E. Storry, and M. E. Sharpe. 1972. Effect of low-roughage diets on the microflora and lipid metabolism in the rumen. Appl Microbiol 24(6):871-877.

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. Journal of Dairy Science 96(10):6650-6659.

Lucas, H. L. and J. K. Loosli. 1944. The Effect of Fat Upon the Digestion of Nutrients by Dairy Cows. Journal of Animal Science 3(1):3-11.

Maas, J. A., J. France, and B. W. McBride. 1997. Model of Milk Protein Synthesis. A Mechanistic Model of Milk Protein Synthesis in the Lactating Bovine Mammary Gland. Journal of Theoretical Biology 187(3):363-378.

Mai-Prochnow, A., M. Clauson, J. Hong, and A. B. Murphy. 2016. Gram positive and Gram negative bacteria differ in their sensitivity to cold plasma. Scientific Reports 6(1):38610.

Mansbridge, R. J. and J. S. Blake. 1997. Nutritional factors affecting the fatty acid composition of bovine milk. Br J Nutr 78 Suppl 1:S37-47.

Mathews, A. T., J. E. Rico, N. T. Sprenkle, A. L. Lock, and J. W. McFadden. 2016. Increasing palmitic acid intake enhances milk production and prevents glucose-stimulated fatty acid disappearance without modifying systemic glucose tolerance in mid-lactation dairy cows. Journal of Dairy Science 99(11):8802-8816.

McCarthy, S. and G. H. Smith. 1969. Beta-hydroxybutyrate as a precursor of milk fat in the ruminant. Proc Nutr Soc 28(1):28a-29a.

McSweeney, C. 2012. Micro-organisms and ruminant digestion: state of knowledge, trends and future prospects.

Miron, J. and D. Ben-Ghedalia. 1993. Digestion of cell-wall monosaccharides of ryegrass and alfalfa hays by the ruminal bacteria Fibrobacter succinogenes and Butyrivibrio fibrisolvens. Can J Microbiol 39(8):780-786.

Moran, J. 2005. How the Rumen Works. in Tropical Dairy Farming: Feeding Managment for Small Holder Dairy Farmers in the Humid Tropics. L. Press, ed.

Oba, M. and M. S. Allen. 1999. Evaluation of the Importance of the Digestibility of Neutral Detergent Fiber from Forage: Effects on Dry Matter Intake and Milk Yield of Dairy Cows. Journal of Dairy Science 82(3):589-596.

Or-Rashid, M. M., N. E. Odongo, and B. W. McBride. 2007. Fatty acid composition of ruminal bacteria and protozoa, with emphasis on conjugated linoleic acid, vaccenic acid, and odd-chain and branched-chain fatty acids1. Journal of Animal Science 85(5):1228-1234.

Palmquist, D. L., A. Denise Beaulieu, and D. M. Barbano. 1993. Feed and Animal Factors Influencing Milk Fat Composition1. Journal of Dairy Science 76(6):1753-1771.

Palmquist, D. L. and T. C. Jenkins. 1980. Fat in Lactation Rations1, 2: Review. Journal of Dairy Science 63(1):1-14.

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. Journal of Dairy Science 96(11):7143-7154.

Piantoni, P., A. L. Lock, and M. S. Allen. 2015. Milk production responses to dietary stearic acid vary by production level in dairy cattle. Journal of Dairy Science 98(3):1938-1949.

Reynolds, C. K., D. L. Harmon, and M. J. Cecava. 1994. Absorption and delivery of nutrients for milk protein synthesis by portal-drained viscera. J Dairy Sci 77(9):2787-2808.

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 cottonseed1. Journal of Animal Science 95(1):436-446.

Russell, J. B. and R. B. Hespell. 1981. Microbial Rumen Fermentation. Journal of Dairy Science 64(6):1153-1169.

Salton, M. R. J. 1967. Structure and Function of Bacterial Cell Membranes. Annual Review of Microbiology 21(1):417-442.

Santos, F. A., J. E. Santos, C. B. Theurer, and J. T. Huber. 1998. Effects of rumen-undegradable protein on dairy cow performance: a 12-year literature review. J Dairy Sci 81(12):3182-3213.

Schingoethe, D. J. 1991. Protein quality and amino acid supplementation in dairy cattle. . Pages 101-106 in Proc. Southwest Nutrition Managment Conference. Department of

Animal Science University of Arizona, Tempe, AZ.

Schwab, C. G. 1994. Optimizing amino acid nutrition for optimum yields of milk and milk protein. Pages 114-129 in Proc. Southwest Nutrition and Managment Conference Department of Animal Science University Arizona, Phoenix, AZ.

Shingfield, K. J. and R. J. Wallace. 2014. Chapter 1 Synthesis of Conjugated Linoleic Acid in Ruminants and Humans. Pages 1-65 in Conjugated Linoleic Acids and Conjugated Vegetable Oils. The Royal Society of Chemistry.

Shriver, B. J., W. H. Hoover, J. P. Sargent, R. J. Crawford, and W. V. Thayne. 1986. Fermentation of a High Concentrate Diet as Affected by Ruminal pH and Digesta Flow1. Journal of Dairy Science 69(2):413-419.

Sikkema, J., J. A. de Bont, and B. Poolman. 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiol Rev 59(2):201-222.

Silbert, D. F., F. Ruch, and P. R. Vagelos. 1968. Fatty acid replacements in a fatty acid auxotroph of Escherichia coli. J Bacteriol 95(5):1658-1665.

Silhavy, T. J., D. Kahne, and S. Walker. 2010. The bacterial cell envelope. Cold Spring Harb Perspect Biol 2(5):a000414-a000414.

Smith, S. and S. Abraham. 1975. The Composition and Biosynthesis of Milk Fat. Pages 195-239 in Advances in Lipid Research. Vol. 13. R. Paoletti and D. Kritchevsky, ed. Elsevier.

Swift, R. W., E. J. Thacker, and et al. 1947. Digestibility of rations for ruminants as affected by proportions of nutrients. J Anim Sci 6(4):432-444.

Tillman, A. D. and K. S. Sidhu. 1969. Nitrogen Metabolism in Ruminants: Rate of Ruminal Ammonia Production and Nitrogen Utilization by Ruminants—A Review. Journal of Animal Science 28(5):689-697.

Ungerfeld, E. and R. Kohn. 2006. Ruminant physiology.

Van Es, A. J. H. 1981. Feeds rich in cellulose in ruminant and non-ruminant nutrition. Agriculture and Environment 6(2):195-204.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. Journal of Dairy Science 74(10):3583-3597.

Vander Wal, R. J. 1964. Triglyceride Structure. Pages 1-16 in Advances in Lipid Research. Vol. 2. R. Paoletti and D. Kritchevsky, ed. Elsevier.

VanSoest, P. J. 1994. Nutritional Ecology of the Ruminant. 2nd ed. Cornell University

Press.

Vollmer, W., D. Blanot, and M. A. De Pedro. 2008. Peptidoglycan structure and architecture. FEMS Microbiology Reviews 32(2):149-167.

Waldo, D. R. 1968. Nitrogen Metabolism in the Ruminant1. Journal of Dairy Science 51(2):265-275.

Wattiaux, M. A. and R. R. Grummer. 1991. Lipid Metabolism in Dairy Cows.

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(3):1766-1779.

Wolin, M. J. 1979. The Rumen Fermentation: A Model for Microbial Interactions in Anaerobic Ecosystems. Pages 49-77 in Advances in Microbial Ecology: Volume 3. M. Alexander, ed. Springer US, Boston, MA.

Yang, W. Z., K. A. Beauchemin, and L. M. Rode. 2001. Effects of grain processing, forage to concentrate ratio, and forage particle size on rumen pH and digestion by dairy cows. J Dairy Sci 84(10):2203-2216.

Zhang, G., T. C. Meredith, and D. Kahne. 2013. On the essentiality of lipopolysaccharide to Gram-negative bacteria. Current Opinion in Microbiology 16(6):779-785.

Zobel, H. F. 1988. Molecules to Granules: A Comprehensive Starch Review. Starch - Stärke 40(2):44-50.

CHAPTER 2

PALMITIC, STEARIC, OR OLEIC FATTY ACIDS DIFFERENTLY ALTER NDF DIGESTIBILITY IN A CONTINOUS CULTURE SYSTEM

Abstract

The objective of this study was to evaluate the effect of palmitic, stearic and oleic acid on neutral detergent fiber (**NDF**) digestibility and rumen fermentation. Continuous culture fermenters (n = 8) were used in a replicated 4 × 4 Latin square design with 7 days of adaptation and 4 days of sampling. Treatments were: 1) control diet without fatty acids; 2) control diet plus 1.5% of palmitic acid (**PA**) (99% C16:0); 3) control diet plus 1.5% of stearic acid (**SA**) (99% C16:0); and 4) control diet plus 1.5% oleic acid (**OA**) (99% *cis*-9 C18:1). The control diet (60 g dry matter/day) was a 50:50 orchard grass hay: concentrate mixture that provided 8.7 g crude protein (**CP**), 21 g NDF, 11 g starch, and 1.5 g fatty acids fed twice daily. The fatty acid treatments maintained the same nutrient input into the fermenters as the control except for fatty acids. Data were analyzed using a mixed model including the fixed effect of treatment, and the random effects of period and fermenter. Data are reported as least squares means with contrast differences declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$. Three contrasts were used to evaluate (1) the effect of the combination of palmitic, stearic, and oleic acids treatment means compared with control [Control vs. (PA+SA+OA)/3]; (2) the effect of palmitic acid compared with oleic acid (PA vs. OA); and (3) the effect of stearic acid compared with oleic acid (SA vs. OA). The combination of palmitic, stearic and oleic acids did not affect NDF digestibility compared with control (42.54% vs. 42.05%). However, compared with oleic acid,

palmitic acid increased NDF digestibility (46.6% vs. 36.1%) whereas no effect was observed for stearic. Compared with control, total production of volatile fatty acids (**VFA**) was not affected by the combination of palmitic, stearic and oleic fatty acids. Palmitic acid increased total VFA production compared with oleic acid (168.9 mmol vs. 146.7 mmol), but stearic acid did not. Ammonia concentration for control was not affected by the combination of palmitic, stearic or oleic acids, nor was palmitic acid or stearic acid compared with oleic acid respectively. No effect of treatment was observed for cellulase enzyme activity. Bacterial membrane FA composition of all FA tested was not altered by the combination of palmitic, stearic or oleic fatty acids when compared with control. Compared with oleic acid, palmitic acid increased *anteiso*-C15:0 (7.34% vs. 6.31%), C18:2n6 (3.86% vs. 2.68%), and C18:3n3 (0.30% vs. 0.23%) fatty acids. Stearic acid increased C18:2n6 (3.74% vs. 2.68%) and tended to increase C18:0 (4.92% vs. 3.25%), C18:3n3 (0.28% vs. 0.23%), and C20:0 (0.09% vs. 0.05%) when compared with oleic acid. All other FA were not affected by the contrasted treatments. Compared with control, total protein expression in chemiluminescence units (C.U.) of the fatty acid synthase (0.035 vs. 0.068 C.U.) and acetyl CoA carboxylase (0.033 vs. 0.044 C.U.) enzymes were decreased by the combination of palmitic, stearic, and oleic fatty acids and respectively. In conclusion, palmitic acid increased NDF digestibility and total VFA production compared with oleic acid and the combination of palmitic, stearic and oleic acid decreased total protein expression of the fatty acid synthase and acetyl CoA carboxylase enzyme compared with control.

Introduction

Ruminants have the ability to convert low quality feeds that are rich in fiber, into

high quality protein, e.g., meat and milk, and this is possible through rumen fermentation. Rumen fermentation occurs as the vast population of rumen bacteria and other microbes break down the fiber tissues to yield the by-products, mainly VFAs, needed for the host animal's energy. Increasing NDF digestibility can improve animal production. In a review done by Oba and Allen (1999), the authors stated that an increase in 1 unit of NDF digestibility resulted in greater dry matter intake (**DMI**) and 4% fat corrected milk (**FCM**). Furthermore, environmental impact of ruminants can be reduced by increasing their energy utilization from forages. White (2016) indicated a 3.4 % reduction in methane (**CH_4**) and nitrogen excretion, and 6.9 % reduction of water and land use when high quality forages with greater digestibility and higher protein content were fed to dairy cattle.

Fats have been used in the diet of ruminants to increase the diet energy density, yield of milk and milk components (Rabiee et al., 2012), improve reproductive performance (Lammoglia et al., 2000, Williams and Stanko, 2000) and reduce metabolic heat production (Holter and Urban, 1992); yet it has been recognized that dietary fat can negatively impact fiber digestion in ruminants (Brooks et al., 1954, Jenkins, 1993). However, not all fats are created equal and recent experiments indicate that specific fatty acids, e.g. palmitic, enhance NDF digestibility (Piantoni et al., 2013a, Rico et al., 2017b, de Souza and Lock, 2018a). In a meta-analysis, de Souza et al. (2016) observed that palmitic acid enriched supplements (>80% palmitic acid) increased NDF digestibility in average by 3% compared with a control diet with no added fat.

Cellular membrane integrity is crucial for cell survival and can be impacted by the fatty acid composition of the phospholipid membrane (Silhavy et al., 2010). Cell fluidity

is maintained by adaptation of the phospholipids within the membrane and is fundamental in maintaining membrane structure (Hazel and Eugene Williams, 1990). Mixed rumen bacteria cell membranes are composed of palmitic, stearic and oleic acids in the highest amount (Or-Rashid et al., 2007), indicating their importance to the microbes. These fatty acids can be synthesized in the fatty acid synthesis pathway or can be directly incorporated in the membrane from free fatty acids in the environment (Erwin, 1973). The fatty acid synthesis pathway requires a significant amount of energy in the form of 7 adenosine triphosphate (**ATP**) to synthesize the end product, palmitic acid (C16:0). Within the pathway are two enzymes, the acetyl CoA carboxylase (**ACC**) enzyme and the fatty acid synthase (**FAS**) enzyme, and if enough fatty acids are present in the cytosol of the cell, the fatty acid synthesis pathway is inhibited and the energy that would have been required for the synthesis of fatty acids is spared. Silbert et al. (1968) observed this in *Escherichia coli* when purified >99% palmitoleic and oleic fatty acids were added to the media. Their results indicated a 33% increase in growth rate of the bacteria and direct incorporation of both fatty acids into the phospholipid membrane. Incorporation of fatty acids from the environment into the membrane would allow the bacteria to spare energy and partition that energy to grow and be more efficient at fermenting and digesting nutrients. Thus, our objective was to evaluate the effect of palmitic, stearic, and oleic acids on NDF digestibility and rumen fermentation and uncover the mechanisms behind the increase in NDF digestibility. We hypothesized that supplementing palmitic, stearic, or oleic fatty acids would increase fiber digestibility by allowing the mixed rumen bacteria to incorporate the supplemental fatty acids from the environment into their membranes, sparing energy.

Materials and Methods

Continuous Culture Operation

The Institutional Animal Care and Use Committee at Utah State University, Logan, Utah approved all experimental procedures (Protocol #10145). Eight continuous culture fermenters, designed according to Teather and Sauer (1988), were used in a 4 × 4 Latin square design with 4 periods of 11 days,7 days for adaptation and the last 4 days for sampling. Rumen content was collected from lactating cannulated Holstein cows fed a diet of approximately 54% forage and 46% concentrate mix. The rumen contents were squeeze-filtered through double layered, grade 60 cheese cloth into pre-warmed 39°C containers. Filtered rumen content was inoculated 1:1 with 39°C artificial saliva (Weller and Pilgrim, 1974) and transferred into the fermenters. Continuous stirring of fermenter contents was achieved by a central paddle set at a speed of 50 rpm, CO_2 was administered at a fixed rate of 20 mL/min to displace O_2 and a circulating water bath maintained the temperature of the fermenters at 39°C. Artificial saliva was prepared as described by Weller and Pilgrim (1974) and delivered continuously using a peristaltic pump to maintain a 10%/hour fractional dilution rate. The fermenters' pH was maintained at a range from 5.8 to 6.6.

Treatment diets

Experimental diet consisted of a 50:50 orchard grass hay and concentrate mixture (60 g DM/day) that provided 8.7 g CP, 21 g NDF, 11 g starch, and 1.5 g fatty acids (**Table 2-1**) fed twice daily (0800 and 1600). Treatments were: 1) control diet without fatty acids; 2) control diet plus 1.5% diet dry matter of palmitic acid (99% C16:0) (#P0500, Sigma Aldrich, St. Louis, MO); 3) control diet plus 1.5% diet dry matter of

stearic acid (99% C18:0) (#S4751, Sigma Aldrich); and 4) control diet plus 1.5% diet dry matter of oleic acid (99% *cis*-9 C18:1) (#O1008, Sigma Aldrich).The fatty acid treatments maintained the same nutrient input into the fermenters as the control except for fatty acids.

Data and Sample Collection and Analysis

On day 8, 9, 10, 11, of each period, outflow effluent was collected on ice to prevent further fermentation. Subsamples of the effluent were collected as described below for analyses.

A subsample of 400 mL of effluent per fermenter was frozen at -20° and then freeze dried (FreeZone 12, Labconco, Kansas City, MO). Dried effluent was composed by period and treatment and ground through a 1-mm screen with a Wiley mill (Arthur H. Thomas, Philadelphia, PA) and analyzed for NDF (Van Soest et al., 1991) using the Ankom^{200} Fiber Analyzer (Ankom Technology, Macedon, NY).

A subsample of 20 mL of effluent was added to a bottle containing 1 mL of 6 *N* HCl to stop fermentation and then frozen at -40°C for VFA analysis. Samples were analyzed according to Eun and Beauchemin (2007).

For ammonia concentration, 50 mL of effluent was acidified with 3 mL of 6 *N* HCl and stored at -40°C until analysis. Samples were analyzed according to the colorimetric method described by Weatherburn (1967).

Effluent samples for cellulase activity were stored at -40°C and analyzed using the procedure of Elolimy et al. (2018).

Bacteria from the effluent were isolated immediately through centrifugation according to Siddons et al. (1982) with modifications that include initial centrifugation at

3500 rpm for 5 min at 14°C and final centrifugation at 12000 rpm for 30 min at 14°C. The pellet was then lyophilized (FreeZone 12, Labconco, Kansas City, MO) and 500 mg were used to extract the fatty acids as stated in Folch et al. (1957). Lipid extract was dried under N_2 and weighed to obtain total lipid amount, then reconstituted in 0.5 mL of hexane: methyl tert-butyl ether: acetic acid solution. The phospholipids were extracted according to Bateman and Jenkins (1997) using a vacuum manifold chamber (Teknokroma, Sant Cugat del Valles, Barcelona) and HyperSep aminopropyl SPE columns (#03-251-281, Fisher Scientific, Waltham, MA). Fatty acids were determined using a one-step transesterification method (Sukhija and Palmquist, 1988) with modifications of (Lock et al., 2013a). Briefly, 1 mg of *cis*-10 C17:1, diluted in toluene, was used as an internal standard. Fatty acid methyl esters (**FAME**) were then prepared by adding 5% methanolic sulfuric acid to the samples. Samples were incubated for 10 minutes (50°C) and cooled at room temperature for 7 minutes then incubated for 10 minutes (80°C) and neutralized with a 5% sodium chloride solution. The solvent layer was washed with a 6% aqueous potassium bicarbonate solution and dried over anhydrous sodium sulfate. The FAME was filtered through anhydrous sodium sulfate, solvents removed under nitrogen flux at 37°C, the fatty acid methyl esters weighed, and a 1% solution with n-hexane prepared on a weight basis. Fatty acid methyl esters were determined by GLC using a Shimadzu GC2010 gas chromatograph with flame ionization detection (Shimadzu Corporation, Columbia, MD). Fatty acids were separated using a CP Sil 88 column (100 m X 0.25 mm, 0.2 µm of film thickness, Agilent Technologies, Santa Clara, CA). Hydrogen was used as the carrier gas at a constant rate of 1 mL/min. The temperature of the GLC oven was maintained at 45°C fro 4 min, increased at 13°C/min to

175°C and held for 27 min, and increased at the rate of 4°C/min to a final temperature of 215°C and held for 35 min. Peaks were identified by the comparison of retention times with fatty acid methyl ester standards (GLC no. 463, no. UC-59-M, Nu-Check-Prep Inc., Elysian, MN) and bacterial acid methyl ester standard (#47080-U, Sigma Aldrich). Fatty acid composition was expressed as a percentage of total fatty acids.

A subsample of the isolated bacteria was used for protein expression. Protein was obtained by homogenizing (Bead Ruptor 4, Omni International, Kennesaw, GA) 50 mg of lyophilized bacterial pellet with 0.4 g of sterile glass beads in a lysis buffer prepared according to Snelling and Wallace (2017). Extracted protein concentrations were determined using the Qubit protein assay kit (Fisher Scientific). Protein expression was determined by western blots (WB) using the WES technology (ProteinSimple, San Jose, CA) following the manufacturer's recommendations for a 25-well plate protocol using a 66-440 kDa kit. Briefly, purified rumen bacterial proteins at a concentration of 0.5 mg/mL was used. Target proteins were immune-probed with primary antibodies, fatty acid synthase (#3189S, Cell Signaling Technologies, Danvers, MA) and acetyl CoA carboxylase (#3676S, Cell Signaling Technologies) followed by HRP-conjugated secondary antibodies provided in the WES kit. All antibodies were diluted using an antibody diluent (Protein Simple) at a 1:50 or 1:10 ratio respectively. Glyceraldehyde 3-phospahte dehydrogenase (GAPDH) (#125247 Abcam, Cambridge, MA) was used as a positive control at a 1:50 dilution. Digital image was analyzed with Compass software (Protein Simple), and a sample was considered positive by WB if immunodominant bands: 290 kDa and 280 kDa, was present, as these bands are known to be associated with the FAS and ACC complex respectively.

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS v.9.4 (SAS Institute, Inc. Cary, NC) according to the following model:

$$Y_{ijk} = \mu + p_i + f_j + T_k + e_{ijk,}$$

where Y_{ijk} = variable of interest, μ = overall mean, p_i = random effect of period (i = 1 to 4), f_j = random effect of fermenter (j = 1 to 8), T_k = fixed effect of treatment (k = Control, Palmitic, Stearic, and Oleic), e_{ijk} = residual error. Significance was determined at $P \leq 0.05$ and tendencies at $P \leq 0.10$. Three contrasts were used to evaluate (1) the effect of the combination of palmitic, stearic, and oleic acids treatment means compared with control [Control vs. (PA+SA+OA)/3]; (2) the effect of palmitic acid compared with oleic acid (PA vs. OA); and (3) the effect of stearic acid compared with oleic acid (SA vs OA).

Results

NDF digestibility & fermentation

The combination of palmitic, stearic and oleic acids did not affect NDF digestibly when compared with control (P = 0.86). However, compared with oleic acid, palmitic acid increased NDF digestibility (46.6% vs. 36.1%, P = 0.01) whereas no effect was observed for stearic acid (P = 0.12). Compared with control, total production of VFA was not affected (P > 0.10) by the combination of palmitic, stearic, and oleic acids. Palmitic acid increased total VFA production compared with oleic acid (161.4 mmol vs. 146.7 mmol, P = 0.03) but stearic acid did not (P = 0.66). Ammonia concentration for control was not affected by the combination of palmitic, stearic or oleic acids (P = 0.43), nor for palmitic acid or stearic acid compared with oleic acid (P = 0.51, P = 0.27) respectively

(**Table 2-2).**

Enzyme activity

The combination of palmitic, stearic, and oleic fatty acids had no effect on cellulase enzyme activity when compared with control ($P = 0.32$), neither did palmitic acid ($P = 0.47$) or stearic acid ($P = 0.70$) when compared with oleic acid (**Table 2-2**).

Mixed rumen bacteria membrane FA composition

Mixed rumen bacteria membrane FA composition of all FA tested was not altered by the combination of palmitic, stearic or oleic fatty acids when compared with control, except that C18:2n6 tended to decrease with FA supplementation (3.84% vs 3.43%, $P = 0.08$). Compared with oleic acid, palmitic acid increased *anteiso*-C15:0 (7.34% vs. 6.31%, $P = 0.01$), C18:2n6 (3.86% vs. 2.68%, $P < 0.01$), and C18:3n3 (0.30% vs. 0.23%, $P = 0.01$) fatty acids. Stearic acid increased C18:2n6 (3.74% vs. 2.68%, $P = 0.01$) and tended to increase C18:0 (4.92% vs. 3.25%, $P = 0.10$), C18:3n3 (0.28 vs. 0.23, $P = 0.08$), and C20:0 (0.09% vs. 0.05%, $P = 0.07$) compared with oleic acid. All other FA where not affected by the treatments (**Table 2-3**).

FAS and ACC protein expression

Compared with control, total protein expression of the FAS (0.035 vs. 0.068 C.U., $P = 0.01$) and ACC (0.033 vs. 0.044 C.U., $P = 0.02$) enzymes by the combination of palmitic, stearic, and oleic fatty acids were decreased. Compared with palmitic acid, oleic acid had lower protein expression of ACC (0.025 vs. 0.040 C.U., $P < 0.01$) and tended to

have lower protein expression of the FAS (0.020 vs. 0.048 C.U., $P = 0.07$). Compared with stearic acid, oleic acid had lower protein expression of ACC (0.025 vs. 0.036 C.U., $P = 0.03$), but no differences were observed for FAS ($P = 0.29$) (**Figure 2-1**).

Discussion

This study measured the effect of palmitic, stearic and oleic fatty acids on NDF digestibility, rumen fermentation, mixed rumen bacteria membrane FA composition, and bacterial protein expression of the ACC and FAS enzymes. As fats have been utilized in the diets of ruminants, it has been observed that fiber digestibility can be negatively impacted with high amounts of unsaturated fats (Brooks et al., 1954, Jenkins, 1993). Unsaturated fats have been shown to negatively impact fiber digestion and there are postulated mechanisms that may explain why including: 1) a “coating” effect on the fiber tissue that inhibit microbial fermentation; and 2) decreased microbial activity due to membrane disruption (Palmquist and Jenkins, 1980). Unsaturated fats become liquefied within the rumen temperature and coat the plant particles, interrupting the ability for the bacteria to adhere to the particles and reducing fiber digestion (Devendra and Lewis, 1974). Decreased microbial activity due to membrane disruption can occur with unsaturated fatty acids as the double bonds alter the shape of the membrane lipid, causing these kinks to disrupt the membrane, negatively impacting the cell (Maia et al., 2007). However, recent studies have demonstrated an improvement in fiber digestibility with highly enriched palmitic acid supplements (Warntjes et al., 2008, Rico et al., 2017, de Souza and Lock, 2018) indicating that the fatty acid profile of supplemental fat is a major factor affecting fiber digestibility. Saturated fatty acid (e.g. palmitic acid) has been shown

to have little to no inhibition on specific species of rumen bacteria as shown by Maczulak et al. (1981), where they found that among five strains of *Butyrivibrio fibrisolvens*, a fiber-digesting bacteria, growth was not inhibited with palmitic acid concentrations supplied at 0.001% of culture media. This supports the theory that saturated fatty acids do not inhibit the rumen bacteria population in the way that unsaturated fats do, giving clear benefits for their use in ruminant nutrition. Saturated fats, in our case palmitic acid, increased NDF digestibility similar to results of Riestanti et al. (2020) where they observed an increase in dry matter digestibility and organic matter digestibility *in vitro* when a 96% palmitic acid supplement was fed at 2% diet dry matter. Cellulase enzyme activity in the rumen plays a critical part in efficient rumen carbohydrate metabolism and therefore it was important to investigate if this played a role in the increase in NDF digestibility. However, there was no effect observed for cellulase activity indicating that the supplemented FA did not improve bacteria enzyme production efficiency in our study.

Volatile fatty acids are the end products of rumen fermentation and their concentrations can be affected or manipulated by the diet (Srinivas and Gupta, 1997). In our study, total production of VFA increased with palmitic acid in compared with oleic acid. This information coincides with that previously discussed above, where saturated and unsaturated fats differently impact the rumen microorganisms, conveying the idea that the increase in total VFA relates to the increase in NDF digestibility, as fermentation improves so do the end products of that fermentation. These changes may be linked to the alteration in the rumen bacteria community, a recent dose-response study conducted with continuous culture fermenters indicated that palmitic acid enhances the prevalence of some fibrolytic bacteria (e.g. *Prevotella* spp.) (Wenner and St-Pierre, personal

communication). However, the specific effect of purified fatty acids on bacterial communities deserves further investigation.

Rumen NH_3 is required for synthesis of microbial protein to aid in the growth of the microorganism. It has been shown by Vargas-Bello-Perez et al. (2016) that a diet supplemented with hydrogenated palm oil (2.7% diet DM; 47% palmitic acid and 43% stearic acid) increased total ruminal bacteria compared with a non-fat control diet, indicating that dietary saturated fatty acids can alter microbial population and favor microbial growth, it could be expected to see better utilization of rumen NH_3 as it is required for synthesis of microbial protein to aid in the growth of rumen bacteria. However, our results indicated that supplementation of palmitic, stearic or oleic fatty acids did not affect NH_3 concentrations *in vitro*, suggesting that the mechanism by which NH_3 is utilized by the rumen microbes is not altered by the supplementation of these three fatty acids.

Mixed rumen bacteria plasma membranes are composed of a phospholipid bilayer. This phospholipid bilayer consists of membrane lipids that have a polar head group and two hydrophobic FA tails allowing for their tails to turn toward interior of the cell and expose their hydrophilic heads to water, creating a barrier (Alberts et al., 2002). These bilayers contain a wide variety of FA which can be modulated by the bacteria to maintain fluidity (Silhavy et al., 2010). It has been shown that membrane fatty acids can be altered within the cell to maintain normal cell physiological function (Shibuya, 1992). To maintain membrane homeostasis, membrane composition is continuously changed through a mechanism called homeoviscous adaptation. This process was demonstrated in *Escherichia coli* by the observation that fluidity of the membrane remains relatively

constant at various temperatures (Sinensky, 1974). In our study, no major effect of treatment was observed on bacteria membrane FA composition. However, this could mean that the fatty acids were modified after uptake. It is essential for bacterial cells to retain the integrity and function of their membranes in response to their environment. The bacteria environment, e.g. pH, did not differ and therefore could be the reason that we did not observe any changes with palmitic, stearic, or oleic acids amounts in the phospholipid membrane fatty acid composition.

Bacterial fatty acid synthesis occurs within the cell to synthesize the needed fatty acids for membrane maintenance. Within the fatty acid synthesis pathway are two enzymes called the fatty acid synthase and the acetyl CoA carboxylase enzyme. These enzymes are rate limiting, meaning that they can be inhibited or stop synthesizing fatty acids if there are enough fatty acids already present within the cell. Brinster et al. (2009), found that FAS shuts down completely in presence of exogenous fatty acids in *Lactobacillales*, allowing this family of bacteria to synthesize phospholipids entirely from exogenous fatty acids. Exogenous fatty acids cause a dose dependent repression of endogenous fatty acid synthesis in these bacteria, including production of malonyl-CoA, the rate-determining intermediate required for endogenous fatty acid synthesis (Parsons et al., 2011). If the fatty acid synthesis pathway is inhibited the energy that would have been required for the synthesis of fatty acids is spared and can be utilized for other physiological processes within the cell. Bacterial total protein expression of ACC and FAS enzymes was decreased in the combination of palmitic, stearic, and oleic acids compared with control. This coincides with that previously discussed above, as exogenous fatty acids are supplied, the bacteria utilize them for membrane support and

reduce the need for *de-novo* synthesis of fatty acids. Total protein expression of FAS tended to decrease with oleic acid when compared with palmitic acid. This difference observed could be explained by the type of fatty acid supplied, it has been explained that saturated and unsaturated fatty acids are taken into the cell by different mechanisms. Black and DiRusso, (1994) explained that long-chain fatty acids require facilitated transport to enter the cells, while medium-chain fatty acids can enter the cell by free diffusion. Overall, in our experiment the supply of fatty acid in the diet decreased the protein expression of the enzymes ACC and FAS. The ACC enzyme is the first-rate limiting step in the fatty acid synthesis pathway and has been shown to be regulated differently in varying species of bacteria. Parsons et al., (2011) observed different biochemical regulation of ACC by exogenous fatty acids in *Staph aureus* and *Streptococcus pneumoniae*. Different bacteria utilize exogenous fatty acids differently and could be a possible explanation to the differences seen between our treatments. Overall, the ACC and FAS total protein expression was decreased by the combination of palmitic, stearic, and oleic fatty acids compared with control while oleic acid decreased ACC protein expression compared with palmitic acid and stearic acid suggesting that the supplied fatty acids were utilized by the bacteria and that the need to synthesize fatty acids from the fatty acid synthesis pathway was reduced.

Conclusion

This study shows that purified palmitic acid improved NDF digestibility and total VFA production compared with oleic acid. Additionally, supplemental palmitic, stearic and oleic fatty acids seem to be utilized by rumen bacteria since ACC and FAS enzymes were suppressed.

Tables and Figures

Table 2-1. Ingredient and nutrient composition of treatment diets delivered to continuous culture fermenters.

	Treatments			
Ingredient, % DM	CTR	PA	SA	OA
Orchard grass hay	45.9	45.9	45.9	45.9
Ground corn	16.3	16.3	16.3	16.3
Canola meal	16.4	16.4	16.4	16.4
Beat pulp	4.90	4.90	4.90	4.90
Robot pellet	14.7	14.7	14.7	14.7
Palmitic acid 99%	-	1.50	-	-
Stearic acid 99%	-	-	1.50	-
Oleic acid 99%	-	-	-	1.50
Mineral and vitamin mix	1.80	1.80	1.80	1.80
Nutrient Composition, % DM				
NDF	35.0	35.0	35.0	35.0
Crude protein	14.5	14.5	14.5	14.5
Starch	18.3	18.3	18.3	18.3
Fatty acids	2.50	4.00	4.00	4.00

Table 2-2. Neutral detergent fiber digestibility, rumen fermentation, and cellulase activity responses to four treatments in continuous culture fermenters.

Item	Treatments				SEM	Contrasts *P*-value*		
	Control	Palmitic	Stearic	Oleic		Control vs Fatty Acids	Oleic vs Palmitic	Oleic vs Stearic
NDF Digestibility %	42.54	46.66	43.35	36.14	3.13	0.86	0.01	0.12
Total VFA, mmol/d	161.4	168.9	151.0	146.7	10.2	0.47	0.03	0.66
Acetate, mmol/mol	58.74	59.44	58.17	59.04	2.82	0.87	0.71	0.43
Butyrate, mmol/mol	7.532	7.210	7.734	8.180	0.49	0.73	0.12	0.47
Isobutyrate, mmol/mol	1.010	0.956	1.012	0.912	0.10	0.51	0.63	0.29
Isovalerate, mmol/mol	3.762	3.824	3.845	3.004	0.51	0.66	0.16	0.15
Propionate, mmol/mol	27.57	27.16	27.80	27.93	2.30	0.95	0.59	0.92
Valerate, mmol/mol	1.383	1.401	1.430	0.923	0.30	0.63	0.16	0.14
Ac. Pr. ratio[1], mmol/mol	2.264	2.292	2.159	2.209	0.27	0.71	0.53	0.71
Ammonia mg/dL	17.61	16.47	17.51	15.18	1.55	0.43	0.51	0.27
Cellulase activity liberated glucose µm/h/mL	2547	2927	3183	3467	662	0.32	0.47	0.70

[1]Acetate:Propionate ratio is the difference between acetate and propionate mmol/mol concentrations.
*$P \leq 0.05$ (significant), $P \leq 0.10$ (tendency).

Table 2-3. Fatty acid composition of mixed rumen bacteria phospholipids of the four treatments in continuous culture fermenters.[1]

Fatty Acid, %	Treatments				SEM	Contrasts *P*-value[*]		
	Control	Palmitic	Stearic	Oleic		Control vs Fatty Acids	Oleic vs Palmitic	Oleic vs Stearic
C10:0	0.06	0.05	0.07	0.07	0.03	0.95	0.46	0.88
C12:0	2.11	1.95	2.15	2.22	0.43	0.98	0.51	0.87
anteiso-C13:0	0.05	0.06	0.06	0.06	0.01	0.42	0.83	0.49
C13:0	0.56	0.55	0.51	0.56	0.02	0.56	0.65	0.17
C14:0	5.81	5.17	5.06	5.47	0.52	0.12	0.50	0.37
anteiso-C15:0	6.83	7.34	6.57	6.31	0.41	0.74	0.01	0.40
C15:0	6.36	6.31	6.24	6.31	0.25	0.78	0.98	0.82
C16:0	28.0	27.9	27.5	28.2	0.76	0.84	0.54	0.24
C16:1n7	0.78	0.85	0.75	0.87	0.10	0.63	0.89	0.28
C18:0	3.72	2.64	4.92	3.25	0.88	0.88	0.53	0.10
C18:1n7	5.89	5.30	4.86	5.54	0.98	0.23	0.71	0.31
C18:1n12	0.25	0.24	0.21	0.21	0.03	0.46	0.54	0.91
C18:1t7	0.30	0.29	0.35	0.35	0.05	0.52	0.25	0.93
C18:1	0.77	0.83	0.80	0.83	0.11	0.55	0.99	0.81
C18:2n6	3.84	3.86	3.74	2.68	0.54	0.08	0.01	0.01
C18:3n3	0.29	0.30	0.28	0.23	0.03	0.45	0.01	0.08
C19:0	0.10	0.10	0.11	0.10	0.05	0.30	0.61	0.13
C20:0	0.06	0.04	0.09	0.05	0.01	0.93	0.73	0.07

[1]The data are expressed as the percentage of total fatty acids.
[*]$P \leq 0.05$ (significant), $P \leq 0.10$ (tendency)

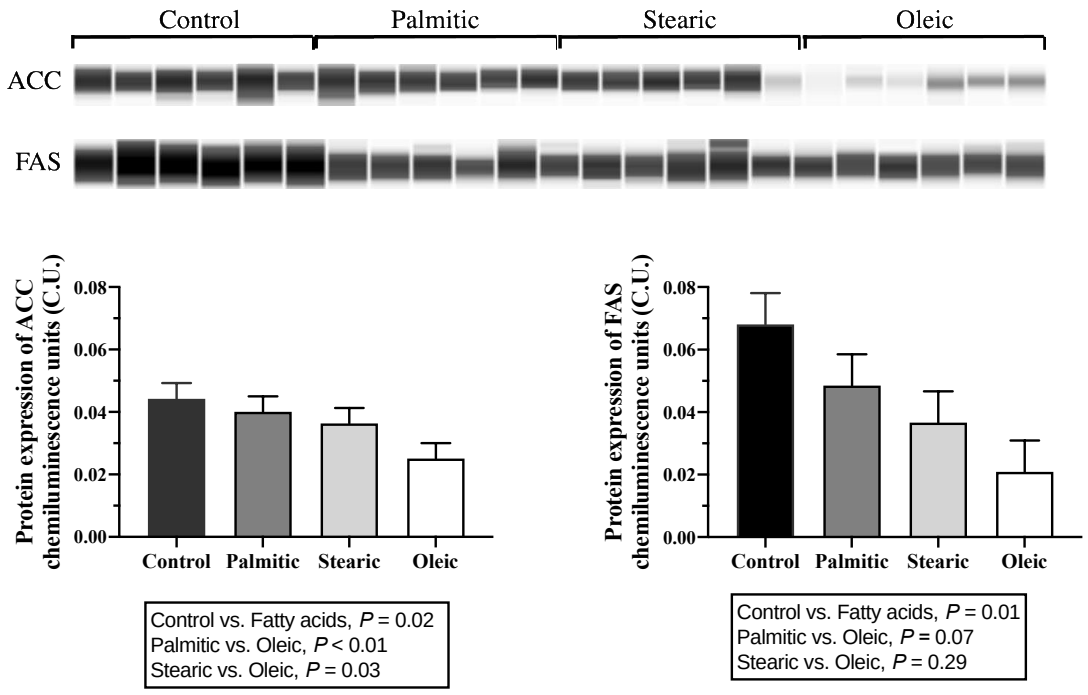

Figure 2-1. Protein expression of acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS) in chemiluminescence units of control, palmitic acid, stearic acid, and oleic acid treatments in continuous culture fermenters.

References

AOAC. 2000. Official Methods of Analysis. Association of Official Analytical Chemists. 17 ed., Arlington, VA.

Alberts, B., A. Johnson, and J. Lewis. 2002. The Lipid Bilayer. in Molecular Biology of the Cell. 4th ed. Garland Science, New York.

Bateman, H. G. and T. C. Jenkins. 1997. Method for Extraction and Separation by Solid Phase Extraction of Neutral Lipid, Free Fatty Acids, and Polar Lipid from Mixed Microbial Cultures. Journal of Agricultural and Food Chemistry 45(1):132-134.

Black, P.N., DiRusso, C.C. 1994. Molecular and biochemical analyses of fatty acid transport, metabolism, and gene regulation in *Escherichia coli*. Biochim Biophys Acta 1210:123-145.

Brandao, V. L. N., L. G. Silva, E. M. Paula, H. F. Monteiro, X. Dai, A. L. J. Lelis, A. Faccenda, S. R. Poulson, and A. P. Faciola. 2018. Effects of replacing canola meal with solvent-extracted camelina meal on microbial fermentation in a dual-flow continuous culture system. Journal of Dairy Science 101(10):9028-9040.

Brinster, S., G. Lamberet, B. Staels, P. Trieu-Cuot, A. Gruss, and C. Poyart. 2009. Type II fatty acid synthesis is not a suitable antibiotic target for Gram-positive pathogens. Nature 458(7234):83-86.

Brooks, C. C., G. B. Garner, C. W. Gehrke, M. E. Muhrer, and W. H. Pfander. 1954. The Effect of Added Fat on the Digestion of Cellulose and Protein by Ovine Rumen Microorganisms. Journal of Animal Science 13(4):758-764.

Brooks, P. D., J. M. Stark, B. B. McInteer, and T. Preston. 1989. Diffusion Method To Prepare Soil Extracts For Automated Nitrogen-15 Analysis. Soil Science Society of America Journal 53(6):1707-1711.

Chalupa, W., B. Rickabaugh, D. Kronfeld, and S. David Sklan. 1984. Rumen Fermentation In Vitro as Influenced by Long Chain Fatty Acids¹. Journal of Dairy Science 67(7):1439-1444.

de Souza, J., R. J. Tempelman, M. S. Allen, and A. L. Lock. 2016. 1312 Production response, nutrient digestibility, and energy partitioning of post-peak dairy cows when palmitic acid-enriched supplements are included in diets: a meta-analysis and meta-regression. Journal of Animal Science 94(suppl_5):632-632.

de Souza, J. and A. L. Lock. 2018. Long-term palmitic acid supplementation interacts with parity in lactating dairy cows: Production responses, nutrient digestibility, and energy partitioning. Journal of Dairy Science 101(4):3044-3056.

de Souza, J., C. L. Preseault, and A. L. Lock. 2018. 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. Journal of Dairy Science 101(1):172-185.

Devendra, C. and D. Lewis. 1974. The interaction between dietary lipids and fibre in the sheep 2. Digestibility studies. Animal Science 19(1):67-76.

Elolimy, A. A., J. M. Arroyo, F. Batistel, M. A. Iakiviak, and J. J. Loor. 2018. Association of residual feed intake with abundance of ruminal bacteria and biopolymer hydrolyzing enzyme activities during the peripartal period and early lactation in Holstein dairy cows. J Anim Sci Biotechnol 9:43-43.

Erwin. 1973. Lipids and Biomembranes of Eukaryotic Microorganisms. . Academic Press, London and San Diego.

Eun, J. S. and K. A. Beauchemin. 2007. Enhancing In Vitro Degradation of Alfalfa Hay and Corn Silage Using Feed Enzymes. Journal of Dairy Science 90(6):2839-2851.

Firkins, J. L., W. P. Weiss, and E. J. Piwonka. 1992. Quantification of intraruminal recycling of microbial nitrogen using nitrogen-15. Journal of Animal Science 70(10):3223-3233.

Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 226(1):497-509.

Hazel, J. R. and E. Eugene Williams. 1990. The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. Progress in Lipid Research 29(3):167-227.

Holter, J. B. and W. E. Urban, Jr. 1992. Water partitioning and intake prediction in dry and lactating Holstein cows. J Dairy Sci 75(6):1472-1479.

Hristov, A. N., P. Huhtanen, L. M. Rode, S. N. Acharya, and T. A. McAllister. 2001. Comparison of the Ruminal Metabolism of Nitrogen from 15N-Labeled Alfalfa Preserved as Hay or as Silage1. Journal of Dairy Science 84(12):2738-2750.

Jenkins, T. C. 1987. Effect of Fats and Fatty Acid Combinations on Ruminal Fermentation in Semi-Continuous In Vitro Cultures. Journal of Animal Science 64(5):1526-1532.

Jenkins, T. C. 1993. Lipid Metabolism in the Rumen. Journal of Dairy Science 76(12):3851-3863.

Lammoglia, M. A., R. A. Bellows, E. E. Grings, J. W. Bergman, S. E. Bellows, R. E. Short, D. M. Hallford, and R. D. Randel. 2000. Effects of dietary fat and sire breed on puberty, weight, and reproductive traits of F1 beef heifers. J Anim Sci 78(9):2244-2252.

Li, D., J. Wang, F. Li, and D. Bu. 2012. Effects of Malic Acid and Unsaturated Fatty Acids on Methanogenesis and Fermentation by Ruminal Microbiota in vitro. Journal of Animal and Veterinary Advances 11:2917-2922.

Maczulak, A. E., B. A. Dehority, and D. L. Palmquist. 1981. Effects of long-chain Fatty acids on growth of rumen bacteria. Appl Environ Microbiol 42(5):856-862.

Maia, M. R. G., L. C. Chaudhary, L. Figueres, and R. J. Wallace. 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. Antonie van Leeuwenhoek 91(4):303-314.

Oba, M. and M. S. Allen. 1999. Evaluation of the Importance of the Digestibility of Neutral Detergent Fiber from Forage: Effects on Dry Matter Intake and Milk Yield of Dairy Cows. Journal of Dairy Science 82(3):589-596.

Or-Rashid, M. M., N. E. Odongo, and B. W. McBride. 2007. Fatty acid composition of ruminal bacteria and protozoa, with emphasis on conjugated linoleic acid, vaccenic acid, and odd-chain and branched-chain fatty acids1. Journal of Animal Science 85(5):1228-1234.

Palmquist, D. L. and T. C. Jenkins. 1980. Fat in Lactation Rations: Review. Journal of Dairy Science 63(1):1-14.

Parsons, J. B., M. W. Frank, C. Subramanian, P. Saenkham, and C. O. Rock. 2011. Metabolic basis for the differential susceptibility of Gram-positive pathogens to fatty acid synthesis inhibitors. Proc Natl Acad Sci U S A 108(37):15378-15383.

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. Journal of Dairy Science 96(11):7143-7154.

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(6):3225-3247

Rico, J. E., M. S. Allen, and A. L. Lock. 2014. 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(2):1057-1066.

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(1):436-446.

Riestanti, L. U., Y. Retnani, and Despal. 2020. Fermentability and Digestibilty Responses of Prill Fat Supplementaion in Dairy Ration. in IOP Conference Series: Earth and Environmental Science. Vol. 411. I. Science, ed. IOP Publishing Ltd, Bali, Indonesia.

Shibuya, I. 1992. Metabolic regulations and biological functions of phospholipids in Escherichia coli. Prog Lipid Res 31(3):245-299.

Siddons, R. C., D. E. Beever, and J. V. Nolan. 1982. A comparison of methods for the estimation of microbial nitrogen in duodenal digesta of sheep. British Journal of Nutrition 48(2):377-389.

Sikkema, J., J. A. de Bont, and B. Poolman. 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiol Rev 59(2):201-222.

Silbert, D. F., F. Ruch, and P. R. Vagelos. 1968. Fatty acid replacements in a fatty acid auxotroph of Escherichia coli. J Bacteriol 95(5):1658-1665.

Silhavy, T. J., D. Kahne, and S. Walker. 2010. The bacterial cell envelope. Cold Spring Harb Perspect Biol 2(5):a000414-a000414.

Sinensky, M. 1974. Homeoviscous adaptation--a homeostatic process that regulates the viscosity of membrane lipids in Escherichia coli. Proc Natl Acad Sci U S A 71(2):522-525.

Snelling, T. J. and R. J. Wallace. 2017. The rumen microbial metaproteome as revealed by SDS-PAGE. BMC Microbiology 17(1):9.

Srinivas, B. and B. N. Gupta. 1997. Rumen fermentation, bacterial and total volatile fatty acid (TVFA) production rates in cattle fed on urea-molasses-mineral block licks supplement. Animal Feed Science and Technology 65(1):275-286.

Teather, R. M. and F. D. Sauer. 1988. A Naturally Compartmented Rumen Simulation System for the Continuous Culture of Rumen Bacteria and Protozoa1. Journal of Dairy Science 71(3):666-673.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. Journal of Dairy Science 74(10):3583-3597.

Varga, G. A. and E. S. Kolver. 1997. Microbial and Animal Limitations to Fiber Digestion and Utilization. The Journal of Nutrition 127(5):819S-823S.

Vargas-Bello-Perez, E., N. Cancino-Padilla, J. Romero, and P. C. Garnsworthy. 2016. Quantitative analysis of ruminal bacterial populations involved in lipid metabolism in dairy cows fed different vegetable oils. Animal 10(11):1821-1828.

Warntjes, J. L., P. H. Robinson, E. Galo, E. J. DePeters, and D. Howes. 2008. Effects of feeding supplemental palmitic acid (C16:0) on performance and milk fatty acid profile of lactating dairy cows under summer heat. Animal Feed Science and Technology 140(3):241-257.

Weatherburn, M. W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Analytical Chemistry 39(8):971-974.

Weller, R. A. and A. F. Pilgrim. 1974. Passage of protozoa and volatile fatty acids from the rumen of the sheep and from a continuous in vitro fermentation system. British Journal of Nutrition 32(2):341-351.

Williams, G. L. and R. L. Stanko. 2000. Dietary fats as reproductive nutraceuticals in beef cattle. Journal of Animal Science 77:1-12.

White, R. R. 2016. Increasing energy and protein use efficiency improves opportunities to decrease land use, water use, and greenhouse gas emissions from dairy production. Agricultural Systems 146:20-29.

Yu, Z. and M. Morrison. 2004. Improved extraction of PCR-quality community DNA from digesta and fecal samples. BioTechniques 36(5):808-812.

CHAPTER 3

IMPACT OF FEEDING A PALMITIC ACID-ENRICHED SUPPLEMENT ON PRODUCTION RESPONSES AND NITROGEN METABOLISM OF MID-LACTATING HOLSTEIN AND JERSEY COWS

Abstract

This study evaluated the effect of feeding a palmitic acid-enriched supplement on production responses and nitrogen metabolism of mid-lactating Holstein and Jersey cows. Eighty mid-lactating dairy cows, 40 Holstein and 40 Jersey, were used in a randomized complete block design with a split-plot arrangement; the main plot was breed and subplot the fatty acid treatment. Cows within each breed were assigned to one of the two treatments: a) control diet with no fat supplement or b) control diet plus a palmitic acid-enriched supplement dosed at 1.5% of diet dry matter (**DM; PA treatment**). The treatment period was 6 weeks with the final 3 weeks used for data and sample collection. There was no treatment × breed interactions for the variables analyzed. Compared with control, PA treatment increased milk fat yield (1.36 vs. 1.26 kg/d) and tended to increase 3.5% fat-corrected milk (**FCM**; 35.6 vs. 34.0 kg/d) and energy-corrected milk (**ECM**; 35.7 vs. 34.1 kg/d). There was no effect of PA treatment on dry matter intake (**DMI**), milk yield, milk protein yield, milk lactose yield, body condition score (**BCS**), body weight (**BW**) change, nitrogen intake and variables related with nitrogen metabolism and excretion. Compared with Holstein cows, Jersey cows had greater DMI as % BW (4.90 vs. 3.37 % of BW), lower milk production (29.6 vs. 32.7 kg/d) and milk lactose yield

(1.42 vs. 1.58 kg/d) and tended to have greater milk fat yield (1.36 vs. 1.26 kg/d). There was a breed effect on BW change: Holstein cows gained 0.385 kg/d during the experiment while Jersey cows gained 0.145 kg/d. Jersey cows had lower nitrogen intake (636 vs. 694 g/d), blood urea nitrogen (**BUN**; 12.6 vs. 13.8 mg/dL), urine total nitrogen (125 vs. 145 g/d), and urine total nitrogen as a percent of nitrogen intake (19.5 vs. 21.1%). Overall, feeding a palmitic acid-enriched supplement increased milk fat yield as well as dry matter and fiber digestibility in both Holstein and Jersey cows. The PA treatment did not have any major effects on nitrogen metabolism in both Holstein and Jersey cows. In addition, our results indicate that Jersey cows had lower urinary nitrogen excretion than Holstein cows.

Introduction

Fat supplements are commonly fed to dairy cow diets as a strategy to increase dietary energy density, the yields of milk and milk fat, and to improve energy balance (Rabiee et al., 2012). Recent research indicated that the fatty acid profile of supplemental fat is a major factor affecting dairy cow performance (de Souza et al., 2018). Palmitic acid supplementation has shown to consistently increase the concentration and yield of milk fat (Lock et al., 2013, Mathews et al., 2016), dry matter digestibility and feed efficiency (Rico et al., 2017, de Souza et al., 2018) when compared with diets without supplemental fat. From an economic standpoint, with the component pricing system most commonly used for milk sales, milk fat increases can result in greater monetary return for the producer. However, to our knowledge the effects of dietary palmitic acid supplementation has only been evaluated with Holstein cows, and it is well established

that milk composition is largely affected by breed. Therefore, determining whether the effects of palmitic acid supplementation is consistent across different dairy cow breeds is of particular importance.

Jersey cows have greater milk fat content and a greater proportion of short and medium-chain milk fatty acids in their milk than Holstein cows (Beaulieu and Palmquist, 1995, Palladino et al., 2010). Additionally, breed differences may affect the utilization and partitioning of supplemental fat as well as milk fat synthesis. Beaulieu and Palmquist (1995) observed that feeding incremental levels of calcium salts of palm fatty acid distillate increased the ratio of oleic:stearic acids in Holstein cows' milk, but no effects were observed in Jersey cows, suggesting a different mechanism of regulating milk fat synthesis. In most studies, feeding palmitic acid enhanced milk fat yield due to greater incorporation of 16-carbon fatty acids (Rico et al., 2014, de Souza and Lock, 2018). However, changes in the yield of some short and long-chain fatty acids (e.g., butyric and oleic acids) have also been reported (Rico et al., 2014, de Souza and Lock, 2019). When compared with Holstein, Jersey cows contain a greater proportion of *de novo* fatty acids and palmitic acid in milk fat (Beaulieu and Palmquist, 1995). Breed differences may exist for fat utilization since feeding an exogenous source of fatty acids may impact *de novo* synthesis of milk fat and its regulation may differ across breeds (Beaulieu and Palmquist, 1995, Palmquist, 2006). Overall, the regulation of milk fat synthesis seems to be different among breeds and since feeding palmitic acid can affect it, it is important to understand how palmitic acid supplementation affects milk fat yield and composition in Holstein and Jersey cows.

Nitrogen release into the environment from animal agriculture has become an increasing area of concern. Nitrogen escape from urine and feces of dairy cows can be reduced with better diet combinations and management practices (Powell et al., 2006). Much interest has been given to investigating specific ways to reduce nitrogen losses, without affecting the production performance of cows. It is widely accepted that increasing the carbohydrate availability will decrease ammonia production by incorporating nitrogen into microbial protein (Russell et al., 1983). Palmitic acid could have an indirect effect on nitrogen metabolism in the rumen, since previous experiments reported an increase in fiber and DM digestibility (Piantoni et al., 2013, de Souza and Lock, 2018) suggesting that dietary fatty acids can affect microbial population and energy utilization. Vargas-Bello-Perez et al. (2016) reported that a diet supplemented with hydrogenated palm oil (2.7% diet DM; 47% palmitic acid and 43% stearic acid) increased total ruminal bacteria compared with a non-fat control diet and soybean oil indicating a potential role of the fatty acid profile on rumen microbial population. Therefore, if dietary saturated fatty acids can alter microbial population and favor microbial growth, it could be expected to see a better utilization of nitrogen in the rumen.

The objective of this experiment was to determine the effects of feeding a palmitic acid-enriched supplement compared with soyhulls on milk yield and milk components, milk fatty acid composition, nutrient digestibility, and nitrogen metabolism on Holstein and Jersey cows. We hypothesized that Jersey cows would have a greater fat yield response to the palmitic acid supplement when compared with Holstein cows, and that both breeds would have lower nitrogen excretion with the addition of palmitic acid.

Materials and Methods

Animal Housing and Care

The Institutional Animal Care and Use Committee at Utah State University, Logan, Utah approved all experimental procedures (Protocol # 10145). All cows were housed in a free-stall system with sand bedding and water being available ad libitum. Two Lely Astronaut A4® automatic milking systems (Lely Industries NV, Maassluis, the Netherlands) were used. Cows were milked by free choice and the average daily milking per cow during the experiment was 2.5. The cows had free access to the milking system for 22 h/d, with the remaining time dedicated to daily cleaning cycles. To ensure a minimum twice-daily milking frequency, cows that had not voluntarily entered the automatic milking system in more than 10 h since the previous milking were fetched to be milked. Standard reproduction, health herd checks and breeding practices were maintained during this study.

Design and treatment diets

Eighty multiparous mid-lactating cows, 40 Holstein and 40 Jersey, from the Utah State University Caine Dairy Research Farm (Wellsville, UT) were used in a randomized complete block design with a split-plot arrangement, where the main plot was breed and subplot the fatty acid treatment. All animals received a common diet during the 14-days preliminary period to obtain baseline values. Experimental diets were fed for 6 weeks including 3 weeks for diet adaptation and 3 weeks for sample collection. Cows were blocked by milk production during the preliminary period, and the blocks were balanced by parity and DIM (**Supplementary Table 3-1**). At the start of the experiment, the cows

were at 145 ± 31 days in milk (DIM) and had 3.0 ± 1.4 (mean ± SD) parities. Cows within each breed were assigned to one of the two treatments. Treatment diets were a control diet with no fat supplement or the control diet plus a palmitic acid-enriched supplement (Spectrum Fusion®, Purdue AgriBusiness LLC, Salisbury, MD, dosed at 1.5% of diet DM (supplement contained in g/100g FA, 0.45 C14:0, 82.5 C16:0, 4.55 C18:0, 9.85 *cis*-9 C18:1). Cows received the control or treatment diets as a concentrate mix fed individually in the automatic milking system. The amount of concentrate mix offered daily was 3.60 and 4.0 kg/d of DM for Jersey and Holstein cows, respectively. The concentrate mix amount was calculated assuming a predicted intake of 22.5 and 25.0 kg/d for Jersey and Holstein cows, respectively, according to NRC (NRC, 2001). All cows received the same partial mixed ration (**PMR**), that was mixed daily in a vertical-mixer and delivered once a day (0630 h) at ~120% of the expected intake. The concentrate mix and PMR represented 16% and 84% of predicted total DMI. The ingredient and nutrient composition of the diets fed are reported in **Table 1**. All rations were formulated to meet cow predicted requirements according to NRC (NRC, 2001).

Data and Sample Collection and Analysis

Weekly samples of feed ingredients, PMR, concentrate mix, and feed refusals were collected and dried at 65°C for 72 h, ground through a 1-mm screen with a Wiley mill (Arthur H. Thomas, Philadelphia, PA), and analyzed for DM (AOAC, 2000), NDF (Van Soest et al., 1991), crude protein (AOAC, 2000), starch (Hall, 2009), and fatty acids (Sukhija and Palmquist, 1988) with modifications of Lock et al., (2013). Fatty acids were determined using a one-step transesterification method. Briefly, 1 mg of *cis*-10 C17:1,

diluted in acetone, was used as an internal standard. Fatty acid methyl esters (FAME) were then prepared by adding 5% methanolic sulfuric acid to the samples. Samples were incubated overnight (50°C), and they were allowed to cool down and neutralized with a 5% sodium chloride solution. The solvent layer was washed with a 6% aqueous potassium bicarbonate solution and dried over anhydrous sodium sulfate. The FAME was filtered through silica gel and charcoal, solvents removed under nitrogen flux at 37°C, the FAME weighed, and a 1% solution with n-hexane prepared on a weight basis, which was used for GLC analysis. FAME were determined by GLC equipped with a split injector (1:100 split ratio) using a CP8827 WCOT fused-silica column (30 m × 0.32 mm i.d. × 0.25 μm film thickness; Varian Inc.). Injector and detector temperature was kept at 270°C. The oven program was as follows: initial temperature of 140°C and held for 1 min, programmed at 5°C/min to 225°C, then programmed at 50°C/min to 250°C held for 5.5 min.

Milk production was recorded daily, and two consecutive milk samples per individual cow were collected once a week, composited based on milk yield and stored with preservative at 4°C before analysis of fat, protein, lactose, SNF, and MUN by mid-infrared procedures (Rocky Mountain DHIA Laboratory, Logan, UT). Based on milk analysis, the ECM and 3.5% FCM were calculated as follows: ECM = [12.82 × fat yield (kg)] + [7.13 × protein yield (kg)] + [0.323 × milk yield (kg)], and FCM = (0.4324 × kg of milk yield) + (16.216 × kg of milk fat). Milk protein nitrogen in g/d was calculated by [(milk protein yield (kg) x 1000) / 6.38], milk nitrogen efficiency was obtained by dividing the amount of nitrogen in the milk by the daily nitrogen consumed.

Milk samples used for fatty acid composition were composited based on milk fat yield by individual cow and stored at -20°C until milk lipids were extracted and fatty acid composition determined by gas chromatograph according to (Hara and Radin, 1978) with modifications (Lock et al., 2013a). Briefly, composite samples (~50 mL) were centrifuged (30 min, 17,800 × g at 4°C), and the fat cake was collected. Total lipids from the fat cake were extracted using n-hexane/isopropanol (3:2, vol/vol). Fatty acid methyl esters (FAME) were prepared by shaking a mixture of 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. After centrifugation (5 min, 6,000 × g), the supernatant containing the FAME was used for GLC analysis. Fatty acid composition was determined by GLC (Weiss et al., 2013) using a CP-SIL88 capillary column (100 m × 0.25 mm × 0.2 μm film thickness; Varian Inc., Palo Alto, CA). Instrument conditions were an injector temperature of 250°C, a flame-ionization detector temperature of 255°C, hydrogen carrier gas at 0.8 mL/min, detector makeup gas (N_2) at 20 mL/min, and an injector split ratio of 75:1. The initial column temperature was 100°C, programmed at 6°C/min to 170°C for 50 min, and then 8°C/min to 220°C for 20 min. The yield of individual fatty acids (g/d) in milk fat was calculated by correcting for glycerol content according to (Schauff et al., 1992), and other milk lipid classes according to (Glasser et al., 2007). The transfer efficiency was calculated according to as (de Souza and Lock, 2019): [(FA yield for treatment diet – FA yield for control diet) / (FA intake for treatment diet – FA intake for control diet)] ×100.

Body weight was recorded daily by the automatic milking system, and BCS was recorded twice, at the first and last day of the experiment. Body weight change was

calculated using a linear regression applied to BW measurements of individual cows, and the slope of the fitted line represented the cow's BW change (Hardie et al., 2015). Body condition score was scored by two trained investigators using a 5-point scale, where 1 = thin and 5 = fat (Wildman et al., 1982). Rumination time and activity data were collected by electronic rumination detection loggers on the neck collar of each cow in 2-h intervals (Lely Qwes-HR collars, Lely Industries N.V.). The collection of rumination data using these collars has been validated by (Schirmann et al., 2009).

In order to calculate total DMI (concentrate mix intake + PMR intake), concentrate mix intake was daily measured and PMR intake was estimated from total fecal excretion and feed digestibility using external and internal markers once at the end of the trial. To estimate total fecal excretion, capsules containing 10 g of titanium dioxide (**TiO_2**) were administered twice daily at 0600 and 1700 h with balling guns throughout the last 15 days of the experiment to estimate DMI as previously described by (Batistel et al., 2017). Fecal grab samples were collected from each cow during the last 5 days of the experiment with 15 h of interval, and frozen at –20°C until further analysis. Samples were thawed and composed by cow, weighed, dried in a 65°C forced air oven for 48-72 h for DM determination and ground through a 1-mm screen with a Wiley mill. TiO_2 concentrations were used to estimate fecal output according to Myers et al. (2004). To estimate *in vivo* feed digestibility, indigestible NDF was used as a marker; fecal and feed samples were incubated in triplicate and indigestible NDF was estimated as NDF residue after 288-h in vivo fermentation (Bender et al., 2016). Total fecal excretion, fecal excretion coming from the concentrate mix (**FEC**), PMR intake and total DMI intake

were calculated as follows: 1) total fecal excretion = Amount of TiO_2 dosed (g/d)/concentration of TiO_2 in feces (g/kg); 2) FEC = concentrate mix intake (kg/d) × concentrate mix indigestibility (%); 3) PMR intake = total fecal excretion (kg/d) – FEC (kg/d) / PMR indigestibility; Total DMI = concentrate mix intake (kg/d) + PMR intake (kg/d).

To estimate nutrient digestibility iNDF was used as an internal marker to estimate apparent total-tract digestibility of nutrients (Cochran et al., 1986). Fecal samples were analyzed for DM, NDF, and fatty acids. Neutral detergent fiber was determined with heat-stable α-amylase and sodium sulfite method (Van Soest et al., 1991). Fatty acids from feces were extracted with a 2-step methylation procedure, as described by Jenkins (2010). An internal standard (*cis*-10 C17:1) was added to the oven-dried ground fecal samples as described above for feed ingredients and orts.

Blood was sampled from the coccygeal vein or artery at 0900 h once a week during the sampling period. Samples were collected into evacuated tubes (BD Vacutainer, BD and Co., Franklin Lakes, NJ) containing lithium heparin. After blood collection, samples were placed in ice until centrifugation (~40 min). Plasma was obtained by centrifugation at 3,000 × *g* for 15 min at 4°C. Aliquots of plasma were frozen (–40°C) until further analysis. Plasma urea nitrogen concentrations were measured using a colorimetric detection kit (Catalog no. K024-H1, Arbor Assays, Ann Arbor, MI).

Spot urine samples were collected from each cow once a week during the sampling period. Samples were acidified with H_2SO_4 (pH of urine <5) and stored at –20°C until further analysis. Creatinine was analyzed using a colorimetric detection kit

(Catalog no. K002-H1, Arbor Assays) and total nitrogen was analyzed using the Kjeldahl method (AOAC, 1990). Total urine production was calculated by creatinine content of each sample, assuming that excretion of creatinine was constant at 0.213 mmol/kg of BW (Chizzotti et al., 2008). Nitrogen intake was calculated from intakes of concentrate mix and PMR and their respective N contents. Apparent ENU (assuming no retention or mobilization of body N) was calculated for each cow by dividing mean milk N output (milk CP/6.38) by total N intake. Urinary N excretion was calculated from total urine production times its N content.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS v.9.4 (SAS Institute, Inc. Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + b_i + B_j + T_k + P_l + BP_{jl} + TP_{kl} + BTP_{jkl} + e_{ijkl,}$$

where Y_{ijkl} = variable of interest, μ = overall mean, b_i = random effect of block, B_j = fixed effect of breed (j = Holstein or Jersey), T_k = fixed effect of treatment (k = control or palmitic acid), P_l = fixed effect of time, BP_{jl} interaction between breed and time, TP_{kl} interaction between treatment and time, BTP_{jkl} = interaction between breed, treatment and time, e_{ijkl} = residual error. Preliminary milk yield was used as a covariate and when not significant ($P > 0.15$) it was removed from the model. Since total DMI and BW change generated one single estimate per cow, a reduced model was used removing the effect of time and interaction between breed, treatment and time. First-order autoregressive was the covariate structure used for repeated measures analysis because it resulted in the lowest Bayesian information criterion (BIC) for most of the variables measured. The

normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals vs. predicted values. Significance was determined at $P \leq 0.05$ and tendencies at $P \leq 0.10$

Results

Animal production

There was no treatment × breed interaction for any production response measured ($P \geq 0.11$; **Table 3-2**). Compared with control, PA treatment increased milk fat yield (1.36 vs. 1.26 kg/d, $P = 0.03$), milk fat content (4.54% vs. 4.24%, $P = 0.01$), milk protein content (3.61% vs. 3.50%, $P = 0.03$), and tended to increase FCM (35.6 vs. 34.0 kg/d, $P = 0.09$) as well as ECM (35.7 vs 34.1 kg/d, $P = 0.10$) (**Table 3-2**). Compared with control, there were no effects of PA treatment on DMI ($P = 0.47$), DMI as a percent of BW ($P = 0.79$), rumination time ($P = 0.58$), milk yield ($P = 0.68$), milk protein yield ($P = 0.22$), milk lactose yield ($P = 0.74$), BW change ($P = 0.91$), or BCS ($P = 0.76$).

Compared with Holstein cows, Jersey cows had lower DMI (22.7 vs. 25.0 kg/d, $P < 0.01$), milk production (29.6 vs. 32.7 kg/d, $P = 0.02$) and milk lactose yield (1.42 vs. 1.58 kg/d, $P = 0.04$; **Table 3-2**). Jersey cows had a greater DMI as a percent of BW (4.90% vs. 3.37%, $P < 0.01$), milk fat content (4.90% vs. 3.89%, $P < 0.01$), milk protein content (3.83% vs. 3.28%, $P < 0.01$), and tended to have greater milk fat yield (1.36 vs. 1.26 kg/d, $P = 0.08$) when compared with Holstein cows. Holsteins gained 0.385 kg/d during the experiment, while Jerseys gained 0.145 kg/d ($P = 0.03$). There were no effects of breed on milk protein yield ($P = 0.85$), milk lactose content ($P = 0.63$), FCM ($P = 0.97$), ECM ($P = 0.99$), rumination time ($P = 0.50$), and BCS ($P = 0.59$).

Milk fatty acids

There was no treatment × breed interaction for any milk fatty acid measured ($P \geq 0.16$; **Table 3-3 and 3-4**). Compared with control, PA treatment decreased milk concentration of < 16-carbon fatty acids (23.4 vs. 25.0 g/100 g fatty acid; $P = 0.03$) and > 16-carbon fatty acids (35.9 vs. 37.6 g/100 g fatty acid; $P < 0.01$) but increased the concentration of 16-carbon fatty acids (39.8 vs. 36.1 g/100 g fatty acid, $P < 0.01$). The PA treatment increased the concentration of C16:0 ($P < 0.01$) but decreased C12:0 ($P < 0.01$), C14:0 ($P < 0.01$), and *cis*-9 C16:1 ($P = 0.03$) and tended to decrease *cis*-9 C18:1:C18:0 ratio ($P = 0.07$). On a yield basis, PA treatment increased 16-carbon milk fatty acids (502 vs. 422 g/d, $P = 0.01$) with no differences observed for the yields of < 16-carbon fatty acids ($P = 0.95$) and > 16-carbon fatty acids ($P = 0.42$). The PA treatment increased the yield of C8:0 ($P = 0.01$), C10:0 ($P = 0.02$), C16:0 ($P < 0.01$), C18:0 ($P < 0.01$) and *cis*-9 C18:1 ($P < 0.01$) but decreased the yield of C12:0 ($P = 0.01$) and C14:0 ($P < 0.01$), and tended to decrease the yield of *cis*-9 C18:1:C18:0 ratio ($P = 0.07$) compared with control.

Compared to Holsteins, Jerseys had greater concentration of < 16-carbon fatty acids (26.7 vs. 21.7 g/100 g fatty acids, $P < 0.01$) and 16-carbon fatty acids (38.2 vs. 37.7 g/100 g fatty acids, P = 0.01) but a decrease in > 16-carbon fatty acids (39.3 vs. 34.2 g/100 g fatty acids, $P < 0.01$). Jersey cows had a greater concentration of C4:0 ($P < 0.01$), C6:0 ($P < 0.01$), C8:0 ($P < 0.01$), C10:0 ($P < 0.01$), C12:0 ($P < 0.01$), C16:0 ($P < 0.01$), and tended to increase C14:0 ($P = 0.10$) while Holsteins had greater C18:0 ($P < 0.01$), *trans*-6-8 C18:1 ($P = 0.04$), *trans*-9 C18:1 ($P = 0.01$), *cis*-9 C18:1 ($P < 0.01$), *cis*-11

C18:1 ($P < 0.01$), and *cis*-9 C18:1:C18:0 ratio ($P < 0.01$). On a yield basis, Jerseys had greater < 16-carbon fatty acids (335 vs. 251 g/d, $P < 0.01$) and 16-carbon fatty acids (484 vs. 438 g/d, $P = 0.01$) but lower > 16-carbon fatty acids yields ($P = 0.01$) compared with Holstein. Compared with Holstein, Jersey cows had greater yields of C4:0 ($P < 0.01$), C6:0 ($P < 0.01$), C8:0 ($P < 0.01$), C10:0 ($P < 0.01$), C12:0 ($P < 0.01$), C14:0 ($P < 0.01$), C16:0 ($P < 0.01$), *cis*-9 C16:1 ($P < 0.01$), *cis*-9, *cis*-12 C18:2 ($P < 0.01$), *cis*-9, *cis*-12, *cis*-15 C18:3 ($P = 0.04$) and yield of *cis*-9 C18:1:C18:0 ratio ($P < 0.01$). In contrast, Holstein cows had greater yield of C18:0 ($P < 0.01$), *trans*-9 C18:1 ($P = 0.02$), *trans*-10 C18:1 ($P < 0.01$), *cis*-9 C18:1 ($P < 0.01$), *cis*-11 C18:1 ($P < 0.01$).

Nutrient Digestibility

There was no treatment × breed interaction for total-tract digestibility of any feed fraction measured ($P \geq 0.17$; **Table 3-5**). Compared with control, PA treatment increased DM digestibility (68% vs. 66%, $P < 0.01$) and NDF digestibility (46% vs. 42%, $P < 0.01$) but no difference was observed for total fatty acid digestibility ($P = 0.12$).

There were no breed differences for digestibility of DM ($P = 0.42$), NDF ($P = 0.63$), or total fatty acids ($P = 0.19$).

Nitrogen metabolism

There was no treatment × breed interaction for any nitrogen metabolism variables measured ($P \geq 0.17$; **Table 3-6**). Compared with control, PA treatment had no effect on nitrogen intake ($P = 0.48$), nitrogen intake as a percent of BW ($P = 0.45$), BUN ($P = 0.45$), MUN ($P = 0.20$), milk protein nitrogen ($P = 0.24$), milk nitrogen efficiency ($P = 0.16$), urine total nitrogen ($P = 0.20$), urine total nitrogen as a percent of nitrogen intake

(P = 0.18) and urine total nitrogen as a percent of BW (P = 0.97).

Compared with Holstein cows, Jersey cows had lower nitrogen intake (636 vs. 694 g/d, P < 0.01), BUN (12.6 vs. 13.8 mg/dL, P = 0.02), urine total nitrogen (124.5 vs. 145.0 g/d, P < 0.01), and urine total nitrogen as a percent of nitrogen intake (19.5% vs 21.1%, P = 0.04). Jersey cows had greater nitrogen intake as a percent of BW (0.15 vs. 0.09 % of BW, P < 0.01) and urine total nitrogen as a percent of BW (0.027 vs. 0.019 % of BW, P = 0.03) compared with Holstein cows. No breed effect was observed for MUN (P = 0.13), milk protein nitrogen (P = 0.69), or milk nitrogen efficiency (P = 0.23).

Discussion

Recently, the effects of feeding individual fatty acids on nutrient digestibility, metabolism, and production responses of dairy cows have received attention. The effects of palmitic acid supplements on dairy cow performance and metabolism in post-peak (Piantoni et al., 2013, Mathews et al., 2016) and early lactation cows (de Souza and Lock, 2019) have been studied; however, all these studies were conducted using Holstein cows. Breed is an important factor that affects production and milk composition of dairy cows. Jersey cows milk usually contains a greater concentration of milk fat compared with Holstein cows milk, and investigating their responses to palmitic acid would be beneficial to help further the understanding of how it can affect production responses in differing breeds. Furthermore, previous studies reported an increase in DM and fiber digestibility (Piantoni et al., 2013, de Souza and Lock, 2018). Improvements in ruminal nutrient digestibility may increase the nitrogen incorporation into microbial cells and consequently enhance nitrogen utilization. Therefore, the present experiment was

designed to investigate the effects of a palmitic acid-enriched supplement on production responses and nitrogen metabolism of mid-lactation Jersey and Holstein cows.

In our study, adding the palmitic acid supplement increased total FA content and the predicted dietary NE_L, and therefore, partially the effect on production responses related to the PA treatment may be associated with greater FA and energy intake. Additionally, considering the high variability in nutrient digestibility among cows and the potential effect that individual FA may have on the digestibility of other fractions, using energy concentrations predicted from the dietary composition is likely inadequate for calculating energy intake and energy balance (Piantoni et al., 2013). de Souza et al. (2019c) reported in a study feeding palmitic acid to replace soyhulls that the NRC (2001) model estimated an increase in NE_L intake by 1 Mcal/d, whereas the actual measured increase in NE_L intake was 2.5 Mcal/d. This difference was at least in part associated with the observed increase in NDF digestibility with palmitic acid that the NRC (2001) model is not able to account for on its estimation. Therefore, it is important to consider not only the direct caloric effect of individual fatty acids, but also the potential effect on the digestion of other dietary fractions.

Milk yield response to palmitic acid supplementation has been inconsistent, with some studies reporting an increase in milk yield (Mosley et al., 2007, de Souza and Lock, 2018), while others reporting no effect on milk yield (Lock et al., 2013, Rico et al., 2014). In our study, we did not observe differences in milk yield due to palmitic acid supplementation in both Holstein and Jersey cows. As reported by Lock et al., (2013), no effect on milk yield may indicate that different partitioning of energy from the

supplemental fat occurred favoring milk fat synthesis. In the present study, we calculated total DMI using internal and external markers to estimate PMR intake. Although the use of external markers is extensively used in research with grazing cows, their use to estimate intake can generate some error due to methodological and analytical errors, time for marker adaptation and marker recovery. We used TiO_2 as a marker because its utilization has been previously validated in sheep (Glindemann et al., 2009), beef cattle (Titgemeyer et al., 2001) and dairy cows (de Souza et al., 2015). In the present study, DMI was not affected by treatments that agree with most previous studies feeding palmitic acid supplements to Holstein cows (Piantoni et al., 2013, Rico et al., 2014, de Souza and Lock, 2019). The effect of fat supplements on DMI is variable and dependent on the type of fat being fed (Rabiee et al., 2012). Feeding saturated fatty acid supplements (up to 3% of dietary DM) mostly results in no change in DMI (Palmquist and Jenkins, 1980b). Dry matter intake as a percent of body weight was greater in Jersey cows when compared to Holstein cows and similar results were observed by French (2006).

Milk fat is greatly influenced by chemical characteristics of the feed, which affect nutrient supply to the mammary gland, as well as by other factors including breed, lactation stage and seasonal variation (Jensen, 2002). Our results indicate that feeding palmitic acid consistently increased milk fat content and yield in both the Holstein and Jersey breeds when compared with the control diet. In our study, palmitic acid treatment increased milk fat content (+0.30 percentage points) and milk fat yield (+100 g/d) compared with control, which is in agreement with several previous studies that fed

palmitic acid supplements to Holstein cows (Lock et al., 2013, Piantoni et al., 2013, Mathews et al., 2016). From our results, we estimated that transfer efficiency (additional fat yield/additional supplemental fat) was 36 and 21% for Jersey and Holstein cows, respectively. For mid lactating Holstein cows, the transfer efficiency from diet to milk fat ranged from 16 to 24% in studies feeding palmitic acid supplements (Lock et al., 2013, Piantoni et al., 2013), while using duodenal infusions of palmitic acid (Enjalbert et al., 2000) reported an apparent fatty acid yield response (FAYR) of 46.7%. de Souza and Lock, (2019) pointed out that differences in fatty acid digestibility may influence the interpretation of the transfer efficiency when supplemental fat is fed. However, we did not observe treatment or breed differences for fatty acid digestibility in our study. Therefore, differences in the transfer efficiency and milk fat responses between Jersey and Holstein cows are likely explained by differences in mammary gland extraction and incorporation of these fatty acids into milk fat.

Overall, the increase in milk fat associated with the palmitic acid treatment occurred due to an increase in yield of 16-carbon milk fatty acids (derived from both de novo synthesis and extraction from plasma), which is in agreement with previous studies (Rico et al., 2014, de Souza and Lock, 2018). Tzompa-Sosa et al. (2014) suggested that an increase in the availability of palmitic acid for lipid synthesis in mammary cells may increase the activity of glycerol-3-phosphate acyltransferase in the mammary gland, therefore increasing the proportion of this fatty acid acylated at sn-1 at the expense of sn-2 position, while other fatty acids would counterbalance the decrease in the amount of palmitic acid at sn-2. Additionally, in our study milk < 16-carbon fatty acid concentration

was reduced, but not yield when palmitic acid was fed. Feeding palmitic acid only reduced the yield of the medium-chain fatty acids C12:0 and C14:0. Hansen and Knudsen, (1987) proposed that the inhibitory effect of certain fatty acids on the de novo synthesis is that they compete with newly synthesized medium-chain acyl-CoA for the triglyceride backbone during the esterification steps of mammary lipid synthesis. We also observed that *cis*-9 C18:1:C18:0 ratio was greater when expressed as concentration and lower when reported on a yield basis for Holstein compared with Jersey cows. This ratio has been proposed as a proxy for the activity of stearoyl-CoA desaturase that plays a pivotal role in regulating the desaturation of saturated to monounsaturated fatty acids (Palmquist, 2006). Similar to our results, a greater *cis*-9 C18:1:C18:0 ratio when expressed on a concentration basis was reported for Holstein compared with Jersey cows (Beaulieu and Palmquist, 1995), which indicates possibly a different mechanism used by Holstein and Jersey cows to maintain milk fluidity. Altogether, our results with both Jersey and Holstein cows are consistent with previous studies regarding the changes in milk fatty acids when palmitic acid is fed.

Neutral detergent fiber digestibility increased with palmitic acid supplementation in comparison with the control diet in both Holstein and Jersey cows. These results agree with previous studies feeding highly enriched palmitic acid supplements (Warntjes et al., 2008, Rico et al., 2017, de Souza and Lock, 2018). The positive effect on NDF digestibility with palmitic acid supplements may be associated with an increase in retention time driven by a rise in cholecystokinin secretion (Piantoni et al., 2013b), which has previously been reported when saturated fats were fed (Choi et al., 2000).

Additionally, rumen bacteria population and bacteria growth are major factors that impact fiber digestion in the rumen (Varga and Kolver, 1997). A recent study (Vargas-Bello-Perez et al., 2016) reported that a diet supplemented with hydrogenated palm oil (47% C16:0 and 43% C18:0) increased total ruminal bacteria measured as copies of 16S ribosomal DNA and the prevalence of some fibrolytic bacteria (e.g., *Prevotella* spp.) compared with a non-fat control diet and soybean oil, indicating a potential role of the fatty acid profile of supplemental fat on rumen microbes. Alternatively, bacteria typically synthesize palmitic acid to produce phosphatidic acid, the precursor for fatty acid components in membranes of *Butyrivibrio* bacteria (Hackmann and Firkins, 2015). However, if dietary palmitic acid could be incorporated into rumen bacterial membranes, considerable carbon and energy precursors would be spared which may favor bacterial growth (Vlaeminck et al., 2006), potentially increasing NDF digestibility. Although the mechanisms by which palmitic acid increases NDF digestibility are not completely understood, the topic deserves further research.

Our nitrogen metabolism data suggests that palmitic acid, despite its ability to increase NDF digestibility, potentially thereby providing more energy to the rumen microbes, does not enhance nitrogen utilization in the rumen. Higher fermentable carbohydrate loads have been shown to increase nitrogen utilization and incorporation (Cameron et al., 1991). Similar to our results, a recent study (Wenner and St-Pierre, 2019) feeding increasing levels of palmitic acid (0, 1, 2, and 3% of diet DM) in continuous culture fermenters indicated that palmitic acid increased NDF and ADF digestibility but did not affect bacterial N flow and ammonia levels. Additionally,

bacterial N yield per NDF or ADF digested (g/kg) tended to increase with palmitic acid supplementation, which suggests an improvement in microbial efficiency but not in microbial yield. Further research is needed to determine the mechanism by which palmitic acid affects fiber digestion. Although a limited number of studies compared nutrient excretion between Jersey and Holstein cows, previous studies suggested that Jersey cows excrete ~30% less fecal and urinary nitrogen than Holstein cows (Kauffman and St-Pierre, 2001, Knowlton et al., 2010). Although we observed the same trend of response with lower urinary nitrogen excretion than the above-mentioned studies, the magnitude of response was smaller in our study which likely associated with differences in the basal diet and levels of fermentable carbohydrates across different studies. Our results indicate that Jersey cows more efficiently utilized nitrogen with greater nitrogen intake as a % of body weight, lower BUN, and urine total nitrogen. This may deserve future investigation as the need for reduced environmental impact continues to affect dairy production.

Conclusions

This study shows that a palmitic acid-enriched supplement improved milk fat yield and content, and protein content in dairy cows consistently in both Jersey and Holstein cows compared with soyhulls. Although palmitic acid increased fiber digestibility, nitrogen metabolism was not affected by fatty acid supplementation. Our results suggest that Jersey cows had lower urinary nitrogen excretion than Holstein cows, but production responses to a palmitic acid-enriched supplement were similar across breeds. This experiment extends our understanding that dietary supplementation of

palmitic acid can improve milk fat yield of the two most common breeds within the U.S. dairy industry.

Tables

Table 3-1. Ingredient and nutrient composition of treatment diets fed to mid-lactating Jersey and Holstein cows.

	Treatments	
Ingredient, % dry matter	Control	Palmitic acid
Corn silage	21.5	21.5
Alfalfa hay	25.0	25.0
Oat hay	3.47	3.47
Ground corn	2.60	2.60
Steam-flaked corn	12.5	12.5
Wheat midds	6.23	6.23
Soybean meal	3.12	3.12
Canola meal	5.21	5.21
Spectrum AgriBlue[1]	0.78	0.78
Beat pulp	6.98	6.98
Soyhulls	3.47	2.00
Whole cottonseed	5.13	5.13
Molasses	0.50	0.50
Palmitic acid supplement[2]	0.00	1.48
Mineral and vitamin mix	3.47	3.47
Nutrient Composition, % dry matter		
NE_L, Mcal/kg[3]	1.45± 0.01	1.54 ± 0.01
Neutral detergent fiber (NDF)	30.3 ± 0.46	29.8 ± 0.44
Forage NDF	21.3 ± 0.22	21.3 ± 0.23
Crude protein	17.5 ± 0.32	17.5 ± 0.31
Starch	23.1 ± 0. 25	23.1 ± 0.27
Total fatty acids	2.39 ± 0.07	3.84 ± 0.06

[1]Spectrum AgriBlue, amino acid supplement, Perdue AgriBusiness, Salisbury, MD.
[2]Spectrum Fusion, palmitic acid supplement, Perdue AgriBusiness, Salisbury, MD.
[3] Net energy of lactation predicted based on NRC (2001).

Table 3-2. Production responses of mid-lactating Jersey and Holstein cows supplemented with or without palmitic acid.

Item	Jersey		Holstein		SEM	*P*-value		
	Control	Palmitic acid	Control	Palmitic acid		Treatment	Breed	Treatment × Breed
Intake								
Dry matter, kg/d	22.6	22.8	25.3	24.7	0.92	0.47	<0.01	0.34
Dry matter, % of body weight	4.91	4.89	3.40	3.34	0.17	0.79	<0.01	0.89
Rumination time, min/d	448	451	459	453	6.67	0.58	0.50	0.88
Milking Frequency, no./d	2.51	2.54	2.47	2.49	0.10	0.92	0.88	0.94
Milk yield, kg/d								
Milk	29.4	29.7	32.5	32.9	1.95	0.68	0.02	0.91
Energy-corrected milk[1]	34.0	35.8	34.2	35.6	1.82	0.10	0.99	0.77
3.5% fat-corrected milk[2]	33.8	35.7	34.1	35.5	1.86	0.09	0.97	0.79
Milk composition								
Fat, %	4.67	5.10	3.81	3.97	0.18	0.01	<0.01	0.26
Fat, kg/d	1.30	1.42	1.22	1.29	0.06	0.03	0.08	0.60
Protein, %	3.73	3.92	3.26	3.30	0.11	0.03	<0.01	0.20
Protein, kg/d	1.05	1.10	1.06	1.08	0.49	0.23	0.85	0.65
Lactose, %	4.83	4.84	4.83	4.81	0.02	0.78	0.63	0.73
Lactose, kg/d	1.42	1.43	1.57	1.59	0.10	0.74	0.04	0.94
Body weight change, kg/d	0.17	0.12	0.37	0.39	0.10	0.91	0.03	0.71
Body condition score	3.17	3.16	3.17	3.22	0.06	0.76	0.59	0.51

[1] Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)].
[2] Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].

Table 3-3. Milk fatty acid profile of mid-lactating Jersey and Holstein cows supplemented with or without palmitic acid.

Item	Jersey		Holstein		SEM	*P*-value		
	Control	Palmitic acid	Control	Palmitic acid		Treatment	Breed	Treatment × Breed
Summation by source[1], g/100 g fatty acids								
< 16-carbon	27.9	25.4	22.0	21.3	0.44	0.03	<0.01	0.23
16-carbon	36.2	40.1	35.9	39.4	0.14	<0.01	0.01	0.45
> 16-carbon	35.0	33.4	40.2	38.3	0.48	<0.01	<0.01	0.35
Selected individual fatty acids[2], g/100 g fatty acids								
C4:0	4.02	3.76	2.6	3.03	0.18	0.73	<0.01	0.19
C6:0	3.77	3.55	1.6	2.15	0.19	0.56	<0.01	0.16
C8:0	2.23	2.21	1.39	1.55	0.05	0.38	<0.01	0.25
C10:0	3.1	3.12	2.42	2.47	0.07	0.79	<0.01	0.89
C12:0	3.15	2.47	2.72	1.96	0.08	<0.01	<0.01	0.73
C14:0	11.4	10.3	11.3	10.2	0.06	<0.01	0.10	0.94
C16:0	34.6	38.5	34.2	37.8	0.13	<0.01	<0.01	0.50
cis-9 C16:1	1.65	1.61	1.70	1.63	0.02	0.03	0.18	0.49
C18:0	8.64	8.83	9.04	9.20	0.13	0.21	0.01	0.91
trans-6 to 8 C18:1	0.33	0.32	0.51	0.43	0.05	0.52	0.04	0.60
trans-9 C18:1	0.17	0.11	0.28	0.21	0.03	0.10	0.01	0.98
trans-10 C18:1	0.61	0.44	1.01	0.93	0.13	0.07	<0.01	0.33
trans-11 C18:1	1.13	1.01	1.12	1.14	0.08	0.84	0.73	0.40
cis-11 C18:1	0.52	0.65	1.05	1.10	0.15	0.36	<0.01	0.81
cis-9 C18:1	17.1	17.0	18.9	18.7	0.15	0.77	<0.01	0.58
cis-9, *cis*-12 C18:2	2.53	2.53	2.34	2.37	0.12	0.92	0.11	0.91
cis-9, *cis*-12, *cis*-15 C18:3	0.44	0.41	0.35	0.34	0.03	0.74	0.11	0.92
cis-9 C18:1: C18:0 ratio	1.98	1.93	2.09	2.03	0.02	0.07	<0.01	0.92

[1]De novo fatty acids originate from mammary de novo synthesis (< 16 carbons), preformed fatty acids originate from extraction from plasma (> 16 carbons), and 16-carbon fatty acids originate from both sources (C16:0 plus *cis*-9 C16:1).

[2]A total of approximately 70 individual fatty acids were quantified. Only select fatty acids are reported in the table.

Table 3-4. Milk fatty acid yield of mid-lactating Jersey and Holstein cows supplemented with or without palmitic acid.

Item	Jersey		Holstein		SEM	P value		
	Control	Palmitic acid	Control	Palmitic acid		Treatment	Breed	Treatment × Breed
Summation by Source[1], g/100 g FA								
< 16-carbon	334	335	247	256	8.56	0.95	<0.01	0.95
16-carbon	438	530	403	473	2.53	0.01	0.01	0.59
> 16-carbon	423	441	463	459	9.18	0.42	0.01	0.22
Selected Individual FA[2], g/100 g FA								
C4:0	48.5	49.7	29.2	36.4	3.55	0.21	<0.01	0.37
C6:0	45.5	46.8	18.0	25.7	3.68	0.20	<0.01	0.36
C8:0	26.8	29.1	15.8	18.6	0.98	0.01	<0.01	0.75
C10:0	37.4	41.2	27.2	29.5	1.35	0.02	<0.01	0.56
C12:0	38.1	32.6	30.7	23.5	1.47	<0.01	<0.01	0.53
C14:0	138	136	127	122	4.24	0.01	<0.01	0.21
C16:0	419	509	384	454	2.48	<0.01	<0.01	0.22
cis-9 C16:1	20.1	21.4	19.2	19.6	0.35	0.01	<0.01	0.14
C18:0	97	106	109	122	2.08	<0.01	<0.01	0.32
trans-6 to 8 C18:1	3.96	4.25	5.69	5.15	0.86	0.88	0.11	0.61
trans-9 C18:1	2.06	1.49	3.19	2.43	0.53	0.14	0.02	0.83
trans-10 C18:1	7.33	5.89	16.5	11.2	2.32	0.12	<0.01	0.37
trans-11 C18:1	13.7	13.7	12.2	13.7	1.43	0.50	0.54	0.51
cis-9 C18:1	210	225	220	239	2.54	<0.01	<0.01	0.42
cis-11 C18:1	6.33	8.79	11.6	13.2	2.21	0.16	<0.01	0.78
cis-9, *cis*-12 C18:2	30.7	33.4	26.4	28.4	1.82	0.09	<0.01	0.80
cis-9, *cis*-12, *cis*-15 C18:3	5.26	5.47	4.00	4.09	0.65	0.81	0.04	0.92
cis-9 C18:1: C18:0 ratio	2.16	2.12	2.01	1.96	0.03	0.07	<0.01	0.96

[1] De novo fatty acids originate from mammary de novo synthesis (< 16 carbons), preformed fatty acids originate from extraction from plasma (> 16 carbons), and 16-carbon fatty acids originate from both sources (C16:0 plus *cis*-9 C16:1).

[2] A total of approximately 70 individual fatty acids were quantified. Only select fatty acids are reported in the table.

Table 3-5. Nutrient total-tract digestibility of mid-lactating Jersey and Holstein cows supplemented with or without palmitic acid.

Item	Jersey		Holstein		SEM	*P*-value		
	Control	Palmitic acid	Control	Palmitic acid		Treatment	Breed	Treatment × Breed
Total-tract digestibility, %								
Dry matter	67.1	68.4	66.2	68.4	0.32	<0.01	0.42	0.27
Neutral detergent fiber	42.7	45.7	41.4	46.3	0.56	<0.01	0.63	0.17
Fatty acids	73.4	75.0	73.0	73.7	0.82	0.12	0.19	0.31

Table 3-6. Nitrogen metabolism of mid-lactating Jersey and Holstein cows supplemented with or without palmitic acid.

Item	Jersey		Holstein		SEM	*P*-value		
	Control	Palmitic acid	Control	Palmitic acid		Treatment	Breed	Treatment × Breed
Nitrogen intake, g/d	633	638	706	682	21.3	0.48	<0.01	0.33
Nitrogen intake, % of body weight	0.13	0.14	0.09	0.09	0.01	0.45	<0.01	0.21
Blood urea nitrogen, mg/dL	12.6	12.6	13.8	13.8	0.57	0.45	0.02	0.28
Milk urea nitrogen, mg/dL	15.1	14.5	14.5	14.1	0.44	0.20	0.13	0.73
Milk protein nitrogen, g/d	165	173	166	170	7.89	0.24	0.69	0.69
Milk nitrogen efficiency, %	26.0	26.9	23.8	25.8	2.57	0.16	0.23	0.59
Urine total nitrogen, g/d	134	115	144	146	6.11	0.20	<0.01	0.12
Urine total nitrogen, % of nitrogen intake	21.0	17.9	20.5	21.7	1.15	0.18	0.04	0.55
Urine total nitrogen, % of body weight	0.029	0.025	0.019	0.020	0.003	0.97	0.03	0.13

Supplementary Table 3-1. Preliminary data of mid-lactating Jersey and Holstein cows supplemented with or without palmitic acid.

	Jersey		Holstein	
	Control	Palmitic acid	Control	Palmitic acid
Preliminary data				
BW, kg	455 ±35.4	460 ±37.7	728 ±56.9	716 ±54.9
Milk Yield, kg/d	27.6 ±3.69	27.0 ±3.81	34.2 ±5.46	35.0 ±5.12
DIM	143 ±29	146 ±22	154 ±34	159 ±32
Milk Fat, %	4.75 ±0.15	4.69 ±0.16	3.85 ±0.11	3.89 ±0.11
Milk Protein, %	3.60 ±0.10	3.65 ±0.13	3.18 ±0.08	3.13 ±0.08

References

AOAC. 1990. Official Methods of Analysis Vol. I. 15 ed., Arlington, VA.

AOAC. 2000. Official Methods of Analysis. Association of Official Analytical Chemists. 17 ed., Arlington, VA.

Batistel, F., J. de Souza, and F. A. P. Santos. 2017. Corn grain-processing method interacts with calcium salts of palm fatty acids supplementation on milk production and energy balance of early-lactation cows grazing tropical pasture. J Dairy Sci 100(7):5343-5357.

Beaulieu, A. D. and D. L. Palmquist. 1995. Differential Effects of High Fat Diets on Fatty Acid Composition in Milk of Jersey and Holstein Cows¹. Journal of Dairy Science 78(6):1336-1344.

Bender, R. W., D. E. Cook, and D. K. Combs. 2016. Comparison of in situ versus in vitro methods of fiber digestion at 120 and 288 hours to quantify the indigestible neutral detergent fiber fraction of corn silage samples. Journal of Dairy Science 99(7):5394-5400.

Cameron, M. R., T. H. Klusmeyer, G. L. Lynch, J. H. Clark, and D. R. Nelson. 1991. Effects of urea and starch on rumen fermentation, nutrient passage to the duodenum, and performance of cows. J Dairy Sci 74(4):1321-1336.

Chizzotti, M. L., S. d. C. Valadares Filho, R. F. D. Valadares, F. H. M. Chizzotti, and L. O. Tedeschi. 2008. Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. Livestock Science 113(2):218-225.

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(3):159-175.

Cochran, R. C., D. C. Adams, J. D. Wallace, and M. L. Galyean. 1986. Predicting Digestibility of Different Diets with Internal Markers: Evaluation of Four Potential Markers. Journal of Animal Science 63(5):1476-1483.

de Souza, J., F. Batistel, K. C. Welter, M. M. Silva, D. F. Costa, and F. A. Portela Santos. 2015. Evaluation of external markers to estimate fecal excretion, intake, and digestibility in dairy cows. Trop Anim Health Prod 47(1):265-268.

de Souza, J. and A. L. Lock. 2018. Long-term palmitic acid supplementation interacts with parity in lactating dairy cows: Production responses, nutrient digestibility, and energy partitioning. J Dairy Sci 101(4):3044-3056.

de Souza, J. and A. L. Lock. 2019a. Effects of timing of palmitic acid supplementation on production responses of early-lactation dairy cows. J Dairy Sci 102(1):260-273.

de Souza, J. and A. L. Lock. 2019b. Milk production and nutrient digestibility responses to triglyceride or fatty acid supplements enriched in palmitic acid. J Dairy Sci 102(5):4155-4164.

de Souza, J., C. Strieder-Barboza, G.A. Contreras, and A.L. Lock. 2019c. Effects of timing of palmitic acid supplementation during early lactation on nutrient digestibility, energy balance, and metabolism of dairy cows. J. Dairy Sci. 102 (1):274–287.

de Souza, J., C. L. Preseault, and A. L. Lock. 2018. 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. Journal of Dairy Science 101(1):172-185.

Enjalbert, F., M. C. Nicot, C. Bayourthe, and R. Moncoulon. 2000. Effects of duodenal infusions of palmitic, stearic, or oleic acids on milk composition and physical properties of butter. J Dairy Sci 83(7):1428-1433.

French, P. D. 2006. Dry Matter Intake and Blood Parameters of Nonlactating Holstein and Jersey Cows in Late Gestation. Journal of Dairy Science 89(3):1057-1061.

Glasser, F., M. Doreau, A. Ferlay, and Y. Chilliard. 2007. Technical note: Estimation of milk fatty acid yield from milk fat data. J Dairy Sci 90(5):2302-2304.

Glindemann, T., B. M. Tas, C. Wang, S. Alvers, and A. Susenbeth. 2009. Evaluation of titanium dioxide as an inert marker for estimating faecal excretion in grazing sheep. Animal Feed Science and Technology 152(3):186-197.

Hackmann, T. J. and J. L. Firkins. 2015. Electron transport phosphorylation in rumen butyrivibrios: unprecedented ATP yield for glucose fermentation to butyrate. Front Microbiol 6:622.

Hall, M. B. 2009. Determination of starch, including maltooligosaccharides, in animal feeds: comparison of methods and a method recommended for AOAC collaborative study. J AOAC Int 92(1):42-49.

Hansen, H. O. and J. Knudsen. 1987. Effect of exogenous long-chain fatty acids on lipid biosynthesis in dispersed ruminant mammary gland epithelial cells: esterification of long-chain exogenous fatty acids. J Dairy Sci 70(7):1344-1349.

Hara, A. and N. S. Radin. 1978. Lipid extraction of tissues with a low-toxicity solvent. Analytical Biochemistry 90(1):420-426.

Hardie, L. C., L. E. Armentano, R. D. Shaver, M. J. VandeHaar, D. M. Spurlock, C. Yao, S. J. Bertics, F. E. Contreras-Govea, and K. A. Weigel. 2015. Considerations when combining data from multiple nutrition experiments to estimate genetic parameters for feed efficiency. J Dairy Sci 98(4):2727-2737.

Jensen, R. G. 2002. The composition of bovine milk lipids: January 1995 to December 2000. J Dairy Sci 85(2):295-350.

Kauffman, A. J. and N. R. St-Pierre. 2001. The Relationship of Milk Urea Nitrogen to Urine Nitrogen Excretion in Holstein and Jersey Cows¹. Journal of Dairy Science 84(10):2284-2294.

Knowlton, K. F., V. A. Wilkerson, D. P. Casper, and D. R. Mertens. 2010. Manure nutrient excretion by Jersey and Holstein cows. Journal of Dairy Science 93(1):407-412.

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(10):6650-6659.

Mathews, A. T., J. E. Rico, N. T. Sprenkle, A. L. Lock, and J. W. McFadden. 2016. Increasing palmitic acid intake enhances milk production and prevents glucose-stimulated fatty acid disappearance without modifying systemic glucose tolerance in mid-lactation dairy cows. J Dairy Sci 99(11):8802-8816.

Mosley, S. A., E. E. Mosley, B. Hatch, J. I. Szasz, A. Corato, N. Zacharias, D. Howes, and M. A. McGuire. 2007. Effect of varying levels of fatty acids from palm oil on feed intake and milk production in Holstein cows. J Dairy Sci 90(2):987-993.

Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical Note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide1. Journal of Animal Science 82(1):179-183.

NRC. 2001. Nutrient Requirements of Dairy Cattle: Seventh Revised Edition, 2001. The National Academies Press, Washington, DC.

Palladino, R. A., F. Buckley, R. Prendiville, J. J. Murphy, J. Callan, and D. A. Kenny. 2010. A comparison between Holstein-Friesian and Jersey dairy cows and their F(1) hybrid on milk fatty acid composition under grazing conditions. J Dairy Sci 93(5):2176-2184.

Palmquist, D. L. 2006. Milk Fat: Origin of Fatty Acids and Influence of Nutritional Factors Thereon. Pages 43-92 in Advanced Dairy Chemistry Volume 2 Lipids. P. F. Fox and P. L. H. McSweeney, ed. Springer US, Boston, MA.

Palmquist, D. L. and T. C. Jenkins. 1980. Fat in lactation rations: review. J Dairy Sci 63(1):1-14.

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(11):7143-7154.

Powell, J. M., D. B. Jackson-Smith, D. F. McCrory, H. Saam, and M. Mariola. 2006. Validation of Feed and Manure Data Collected on Wisconsin Dairy Farms. Journal of Dairy Science 89(6):2268-2278.

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(6):3225-3247.

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(6):3739-3751.

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(2):1057-1066.

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(1):436-446.

Russell, J. B., C. J. Sniffen, and P. J. Van Soest. 1983. Effect of Carbohydrate Limitation on Degradation and Utilization of Casein by Mixed Rumen Bacteria. Journal of Dairy Science 66(4):763-775.

Schauff, D. J., J. P. Elliott, J. H. Clark, and J. K. Drackley. 1992. Effects of feeding lactating dairy cows diets containing whole soybeans and tallow. J Dairy Sci 75(7):1923-1935.

Schirmann, K., M. A. von Keyserlingk, D. M. Weary, D. M. Veira, and W. Heuwieser. 2009. Technical note: Validation of a system for monitoring rumination in dairy cows. J Dairy Sci 92(12):6052-6055.

Sukhija, P. S. and D. L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. Journal of Agricultural and Food Chemistry 36(6):1202-1206.

Titgemeyer, E. C., C. K. Armendariz, D. J. Bindel, R. H. Greenwood, and C. A. Loest. 2001. Evaluation of titanium dioxide as a digestibility marker for cattle. J Anim Sci 79(4):1059-1063.

Tzompa-Sosa, D. A., G. A. van Aken, A. C. van Hooijdonk, and H. J. van Valenberg. 2014. Influence of C16:0 and long-chain saturated fatty acids on normal variation of bovine milk fat triacylglycerol structure. J Dairy Sci 97(7):4542-4551.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. Journal of Dairy Science 74(10):3583-3597.

Varga, G. A. and E. S. Kolver. 1997. Microbial and animal limitations to fiber digestion and utilization. J Nutr 127(5 Suppl):819S-823S.

Vargas-Bello-Perez, E., N. Cancino-Padilla, J. Romero, and P. C. Garnsworthy. 2016. Quantitative analysis of ruminal bacterial populations involved in lipid metabolism in dairy cows fed different vegetable oils. Animal 10(11):1821-1828.

Vlaeminck, B., V. Fievez, A. R. J. Cabrita, A. J. M. Fonseca, and R. J. Dewhurst. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. Animal Feed Science and Technology 131(3):389-417.

Warntjes, J. L., P. H. Robinson, E. Galo, E. J. DePeters, and D. Howes. 2008. Effects of feeding supplemental palmitic acid (C16:0) on performance and milk fatty acid profile of lactating dairy cows under summer heat. Animal Feed Science and Technology 140(3):241-257.

Weiss, W. P., D. E. Shoemaker, L. R. McBeth, and N. R. St-Pierre. 2013. Effects on lactating dairy cows of oscillating dietary concentrations of unsaturated and total long-chain fatty acids. J Dairy Sci 96(1):506-514.

Wenner, B. A. and N. R. St-Pierre. 2019. Palmitic fatty acid dosed in continuous culture fermeters increases fiber digestibility estimates. American Dairy Science Association Annual Meeting 102:316.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt, Jr., and T. N. Lesch. 1982. A Dairy Cow Body Condition Scoring System and Its Relationship to Selected Production Characteristics. Journal of Dairy Science 65(3):495-501.