IMPROVING MILK FAT SYNTHESIS IN LACTATING DAIRY COWS BY UNDERSTANDING THE RELATIONSHIP BETWEEN DE NOVO AND PREFORMED FATTY ACIDS

By

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ABSTRACT

Milk fatty acids (FA) are derived from two sources. Fatty acids from 4 to 14 carbons are synthesized de novo in the mammary gland using acetate and beta-hydroxybutyrate (BHB) produced during ruminal fermentation or liver ketogenesis. Those with 18 or more carbons are “preformed”, meaning they originate from the diet or adipose tissue. An interdependent relationship was proposed by Glasser et al. (2008) where de novo FA and preformed FA drive the synthesis of one another other to increase their incorporation into milk TG. The first experiment in this thesis evaluated the effect of supplemental palmitic acid (PA) on the molar yields of milk FA sources and individual FA. Molar yields were evaluated to adjust for differences in molecular weight of individual FA and standardize the incorporation of FA into TG. Supplementing diets with PA increased mixed FA yield and shifted FA in favor of increased yields of C4:0 and *cis*-9 C18:1 and decreased yields of FA from C8:0 to C14:0 and C18:0. Additionally, evaluating the relative concentration of FA using molar yields better reflects the contribution of de novo FA to milk fat compared to on a mass basis and highlights the important contribution of de novo FA to milk fat synthesis. The second experiment in this thesis evaluated the interactions between altering the dietary supply of de novo FA and preformed FA to maximize milk fat yield by supplementing sodium acetate (NaAc), PA, and whole cottonseed (WCS). Three-way interactions between NaAc, PA, and WCS were observed for the yields of milk fat, 3.5% fat-corrected milk (FCM), de novo FA, mixed FA, and preformed FA. Diets containing NaAc and PA independently increased milk fat yield whereas diets containing NaAc, PA, and WCS led to the greatest increase in milk fat yield. The results from our two studies emphasize the importance of measuring diet-induced changes in milk fat composition on a molar basis. Additionally, our results emphasize the importance of balancing the supply of FA from all sources to increase milk TG synthesis in the mammary gland.

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LIST OF ABBREVIATIONS

ACC	Acetyl CoA carboxylase
BCS	Body condition score
BHB	Beta-hydroxybutyrate
BW	Body weight
CSPF	Calcium salts of palm fatty acids
CP	Crude protein
DGAT	Diacylglycerol acyltransferase
DM	Dry matter
DMI	Dry matter intake
ECM	Energy-corrected milk
FA	Fatty acid
FAS	Fatty acid synthase
FCM	3.5% fat-corrected milk
GPAT	Glycerol-3-phosphate acyltransferase
LPAT	Lysophosphatidic acid acyltransferase
MFG	Milk fat globules
MFGM	Milk fat globule membrane
MUFA	Monounsaturated fatty acid
MUN	Milk urea nitrogen
NaAc	Sodium acetate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NaHCO	Sodium bicarbonate

NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acid
OA	Oleic acid
PA	Palmitic acid
PA+SA	Supplement containing palmitic acid and stearic acid
PA+OA	Supplement containing palmitic acid and oleic acid
SA	Stearic acid
SFA	Saturated fatty acid
SCD	Stearoyl-CoA desaturase
SFA	Saturated fatty acid
TG	Triglyceride
UFA	Unsaturated fatty acid
VFA	Volatile fatty acid
WCS	Whole cottonseed

CHAPTER ONE:

INTRODUCTION

Increasing milk fat production in dairy cows drives profitability for producers as they are paid based on the yields of milk components. Milk fat is comprised primarily of triglycerides (TG) containing fatty acids (FA) derived from de novo and preformed sources. De novo FA (< 16 carbons) are synthesized in the mammary gland using precursors from volatile FA (VFA) produced during ruminal fermentation of dietary carbohydrates, preformed FA (>16 carbons) originate from dietary sources, and mixed FA (16-carbon) originate from both sources. Glasser et al. (2008) proposed an interdependent relationship between de novo FA and preformed FA where the two sources drive the synthesis of one another. Additionally, a balance of short and long chain FA incorporation into TG is needed for maintenance of milk fat fluidity (Enjalbert et al., 1998). Studies in the literature primarily focus on strategies to increase milk fat yield by enhancing substrate supply for de novo FA synthesis or increasing the dietary FA content to increase preformed FA supply to the mammary gland.

Infusing acetate to mid lactation dairy cows quadratically affected the yields of milk fat and de novo FA with a maximum dose observed at 10 mol/d acetate (Urrutia et al., 2017). Additionally, supplementing diets with sodium acetate (NaAc) to target 10 mol/d acetate increased the yields of milk fat, de novo FA, and mixed FA compared to diets with no NaAc (Urrutia et al., 2019; Matamoros et al., 2022). In addition, increasing the dietary supply of FA using oilseeds and FA supplements increases milk fat yield by increasing preformed FA yield. A meta-analysis conducted by dos Santos Neto et al. (2021b) observed that diets supplemented with PA increased mixed FA yield, decreased de novo FA yield, and had no effect on preformed FA yield. Additionally, a meta-analysis evaluating the effect of feeding ≤ 17% WCS observed it increased the yields of mixed FA and preformed FA but decreased de novo FA compared to control diets with no WCS (dos Santos Neto et al., 2022). These meta-analyzes evaluated changes in milk FA yield on a mass basis, although evaluating changes on a molar basis may be important for better understanding milk fat regulation. Molar yields allow for standardization of FA contribution to milk fat by adjusting for differences in FA molecular weights.

To our knowledge, no studies in the literature have evaluated increasing milk fat yield by enhancing the synthesis of de novo FA and increasing the supply of preformed FA. Rico et al. (2015) and de Souza et al. (2018) evaluated the interactions between supplemental FA and basal diet FA content on production performance, although only the supply of PA and long chain FA were increased. Therefore, the objective of this thesis was to improve milk fat synthesis in lactating dairy cows by understanding the relationship between de novo FA and preformed FA. Chapter Three evaluated the effect of supplemental PA on the molar yields of milk FA to determine the effect of PA on the yields of de novo FA and preformed FA. Secondly, Chapter Four evaluated the effects and interactions of supplementing diets with NaAc, PA, and WCS on milk FA to determine if milk fat yield can be maximized by increasing the substrate and FA supply from all sources. Our results will have direct impact on the industry by improving our understanding of de novo FA and preformed FA allowing for improved dietary strategies for increasing milk fat yield in dairy cows.

CHAPTER TWO:

LITERATURE REVIEW

Importance of Milk Components

In the United States, dairy farmers are paid based on milk component yields, therefore it is advantageous to develop nutritional strategies to increase milk component production. Whether positive or negative, milk fat is more easily manipulated than milk protein, and production of milk fat fluctuates depending on stage of lactation, season, and diet (Jensen, 2002). Often, fatty acid (FA) supplements or high fat feeds are fed to dairy cows to increase the energy density of the diet and increase milk fat production (Rabiee et al., 2012). On a mass basis, approximately one half of the FA in milk fat are preformed that originate from dietary sources whereas the remaining one half of the FA are synthesized de novo in the mammary gland. As shown in Figure 2.1, de novo FA are C4 to C14, mixed FA are 16-carbon FA that originate from both sources, and preformed FA are ≥ C18. Developing strategies that enhance the synthesis of both sources of FA are important for increasing milk fat yield. Therefore, the primary focus of this literature review is on de novo FA, mixed FA, and preformed FA, and their relationship and incorporation into triglycerides (TG).

Dietary Sources of Fatty Acids

Dairy cow diets are primarily comprised of forages and grains that contain palmitic acid (PA), stearic acid (SA), and unsaturated FA (UFA) such as linoleic and linolenic acid. Dietary UFA are subject to biohydrogenation by rumen microbes that hydrolyze TG and saturate FA to predominantly yield SA (Jenkins, 1993). Dairy cow diets are relatively low in FA content compared to other dietary nutrients, thus diets are often supplemented with FA-enriched products containing PA, SA, and oleic acid (OA). Supplemental FA are typically fed in the form of SFA prills or calcium salts containing a combination of SFA and UFA. Additionally, oilseeds such as whole cottonseed (WCS) and soybeans are commonly supplemented in diets because they provide a blend of protein, fiber, and long chain FA (Bernard, 2001).

Effect of Supplemental Fatty Acids on Production Performance

The effects of supplemental FA on dry matter intake (DMI) and the yields of milk and milk components are inconsistent in the literature and vary based on FA profile fed (Allen, 2000). A meta-analysis evaluating the effect of supplemental SFA on production performance observed supplements containing PA and PA+SA had no effect on DMI and increased the yields of milk and milk fat compared to the control diets (dos Santos Neto et al., 2021b). In contrast, a meta-analysis evaluating the effect of calcium salts of palm FA (CSPF) on production performance observed that for each 1-DM percentage unit increase in CSPF in the diet decreased DMI by 0.37 kg/d and increased the yields of milk, fat, and 3.5% FCM (FCM) compared to the control diets (dos Santos Neto et al., 2021a). In agreement, Rabiee et al. (2012) reported supplementing diets with various fat supplements and oilseeds decreased DMI by 0.33 kg/d and increased milk yield by 0.24 kg/d suggesting that feeding supplemental FA to dairy cows improves production efficiency. The positive effect of supplementing PA on milk fat production by increasing mixed FA yield has been consistently reported in the literature (dos Santos Neto et al., 2021b). de Souza and Lock (2018) supplemented PA to primiparous and multiparous during a 10 wk treatment period and observed PA increased the yields of FCM and milk fat by increasing mixed FA yield although the effect of PA on de novo FA and preformed FA varied by parity.

Milk Fat Synthesis in the Mammary Gland

Bovine milk fat is comprised of 97-98% TG (Jensen, 2002) with the most frequent combination of FA esterified to the glycerol backbone being C4:0, C16:0, and C18:1 on a molar basis (Gresti et al., 1993). Milk fat synthesis occurs in three stages: 1) synthesis and absorption of short and long chain FA, 2) TG synthesis, and 3) formation of fat globules for secretion into milk (Shorten et al., 2004). Fatty acids <16-carbons are synthesized de novo in the mammary gland from acetate and BHB produced by ruminal fermentation of dietary fiber and long chain FA >16-carbons originate from dietary sources whereas mixed FA 16-carbons originate from both sources. Synthesis of milk fat in the mammary gland involves the balance of de novo and preformed FA incorporated into TG to maintain milk fat fluidity at body temperature (Barbano and Sherbon, 1980; Jensen, 2002).

De Novo FA Synthesis

Acetate or acetyl CoA is a major source of energy for dairy cows because it can enter the citric acid cycle or be used for de novo FA synthesis (Moore and Christie, 1979). In addition to absorption of acetate by the reticulorumen wall, acetate can be metabolized endogenously from long chain FA or amino acids, although this action is dependent on the nutritional state of the animal (Van Soest, 1994). Unlike non-ruminants who synthesize FA from glucose, ruminants utilize acetate for elongation of short and medium chained FA in the mammary gland (Moore and Christie, 1979). Balmain et al. (1954) incubated slices of sheep mammary tissue with carbon labeled acetate and glucose and found that 97% of the carbons in the synthesized FA originated from acetate and the remaining 3% from glucose. Acetate must be metabolized to acetyl CoA by acetyl CoA synthetase in the cytosol of mammary cells before it can be synthesized into FA (Moore and Christie, 1979; Palmquist, 2006). In addition to acetyl CoA, butyrate metabolized to beta-hydroxybutyrate (BHB) provides 50% of the 4-carbon skeletons for initiation of de novo FA synthesis, whereas 50% is from a condensation of 2-carbon units from acetate (Palmquist et al., 1969; Moore and Christie, 1979).

The production of reduced nicotinamide adenine dinucleotide phosphate (NADPH), a reducing equivalent needed for FA synthesis, originates from multiple sources in the ruminant mammary gland. Similar to non-ruminants, glucose oxidation by the pentose phosphate pathway provides NADPH for FA synthesis, although this production alone is not sufficient for FA synthesis (Moore and Christie, 1979). Secondly, ruminants produce NADPH via the isocitrate cycle when acetate is used to generate citrate that diffuses into the cytosol and is converted to isocitrate by the cytosolic NADP-isocitrate dehydrogenase (Bauman et al., 1970; Moore and Christie, 1979). Thus, reducing equivalents generated from acetate could spare glucose for lactose synthesis (Bauman et al., 1970; Palmquist 2006).

Acetyl CoA carboxylase (ACC) is the rate-limiting step for de novo FA synthesis as it catalyzes the transfer of acetyl CoA to malonyl CoA (Moore and Christie, 1979; Palmquist, 2006). Malonyl CoA enters the fatty acid synthase (FAS) complex to provide 2-carbon units for elongation of the carbon chain until termination by a thioesterase occurs (Palmquist, 2006). Kinsella et al. (1975) proposed that C16:0 was the major FA produced by FAS under optimal concentrations of acetate, malonyl CoA, and NADPH in bovine mammary gland tissue suggesting that C16:0 production or supply is important for milk fat synthesis.

Uptake of Preformed FA

Following biohydrogenation in the rumen and absorption in the small intestine, long chain FA are transported to the mammary gland via the bloodstream for incorporation into milk fat TG (Palmquist, 2006). Cows experiencing negative energy balance (NEB) in the immediate postpartum period mobilize non-esterified fatty acids (NEFA) from adipose tissue to be utilized by other tissues or the mammary gland for milk fat synthesis (Palmquist, 1993). Upon transport of very low-density lipoproteins or chylomicron with TG, lipoprotein lipase hydrolyzes the TG to release free FA for absorption in the mammary gland. Although the transport of FA into the mammary gland is not well understood, it is suggested a FA translocator such as CD36 and FA binding proteins are likely involved (Moore and Christie, 1979; Barber et al., 1997). Fatty acid binding protein has a high affinity for long chain FA, mainly UFA. Recently, acyl-CoA binding protein has been shown to have a greater role in regulating the concentration and uptake of FA into the mammary gland, and activity is regulated by de novo FA synthesis and other acyl-CoA enzymes (Neess et al., 2015).

Stearoyl-CoA desaturase (SCD) is an enzyme that converts SFA to monounsaturated FA (MUFA) as a mechanism to maintain milk fat fluidity (Palmquist, 2006; Feng et al., 2007). The main substrates for SCD are C14:0, C16:0, and C18:0 being converted to *cis*-9 C14:1, *cis*-9 C16:1, and *cis*-9 C18:1, respectively, and SCD can add a *cis*-double bond to *trans*-C18:1 FA. A desaturation index of milk fat can be calculated using the yields of the substrate and the *cis*-double bond product as measurement of SCD activity. The C14 desaturation index is used as a proxy for SCD activity because nearly all C14:0 is synthesized de novo, and a positive relationship between SCD activity and C14 desaturation index has been observed (Jacobs et al., 2012). Approximately 60% of *cis*-9 C18:1 in milk fat is synthesized from C18:0 as this process is important for the melting point of the TG to maintain milk fat fluidity (Bernard et al., 2013).

Triglyceride Synthesis

Triglyceride synthesis involves glycerol-3-phosphate and the incorporation of activated FA acyl-CoA esters by the enzyme acyl-CoA synthetase (Moore and Christie, 1979). Glycerol-3-phospate is generated by glycolysis or produced from free glycerol by glycerol kinase (Christie and Moore, 1979; Palmquist, 2006). The *sn*-glycerol-3-phosphate pathway is the major route for TG synthesis in the bovine mammary gland. The *sn*-1 position is esterified first by the enzyme glycerol-3-phosphate acyltransferase (GPAT) to form lysophosphatidic acid followed by the *sn*-2 position by the enzyme lysophosphatidic acid acyltransferase (LPAT) to form phosphatidic acid. Kinsella and Gross (1973) observed palmityl-CoA was the preferred substrate for GPAT and rate of acylation was up to 10 times higher compared to myristyl-, stearyl-, oleyl-CoA. Lastly, 1,2-diacylglycerol is formed before the *sn*-3 position is esterified by the enzyme diacylglycerol acyltransferase (DGAT), likely being the key regulation step of TG synthesis (Shorten et al., 2004). There is a high degree of specificity during the esterification of FA to the glycerol backbone with specific FA preferentially incorporated at different positions (Shorten et al., 2004). Table 2.1 shows the molar proportion (mol/100mol FA) of individual FA at each position on the milk TG. Short chain FA from C4:0 to C6:0 are predominantly esterified at the *sn*-3 position, whereas medium chain FA from C8:0 to C14:0 are largely esterified at the *sn*-2 position. C16:0 is esterified at the *sn*-1 and *sn*-2 position in equal proportions, C18:0 is largely esterified at the *sn*-1 position, and *cis*-9 C18:1 is equally esterified at the *sn*-1 and *sn*-3 position. Gresti et al. (1993) observed the major TG in milk fat contained C4:0, C16:0, and *cis*-9 C18:1 followed by TG containing C4:0, C16:0, and C16:0 and TG containing C4:0, C14:0, and C16:0.

Formation of Milk Fat Globules

Milk fat globules (MFG) are surrounded by a milk fat globule membrane (MFGM) which is a membrane containing phospholipids and proteins originating from the endoplasmic reticulum (Argov-Argaman, 2019; Keenan and Mather, 2006). These globules comprise about 95% of milk fat lipids by mass and contain about 98% TG that are assembled in the endoplasmic reticulum and released from secretory cells in the mammary epithelial (Moore and Christie, 1979; Keenan and Mather, 2006). Walstra and Borggreve (1966) observed a large range in the size of milk fat globules suggesting that globules containing different TG composition lead to differences in the hardness of FA in the globule. Timmen and Patton (1988) evaluated the relationship between milk fat globule size and the composition of milk TG from lactating dairy cows and did not observe large differences between FA composition and the size of milk fat globules, although they observed smaller globules contained more OA over short chain FA and SA suggesting milk fat globule composition is important for maintaining milk fat fluidity. More recently, studies in the literature have observed that the size of MFG can influence the physical properties of milk fat important for processing dairy products and can be affected by different dietary factors. Wiking et al. (2004) observed that increases in the diameter of MFG were positively correlated with concentrations of C16:0, C18:0, and C18:1 in milk but were not correlated with concentrations of FA from C4:0 to C14:0. Argov-Argaman (2019) concluded the that size of MFG is strongly correlated with milk fat content, although the regulation of the size is dependent on molecular, genetic, and metabolic factors.

Factors Altering De Novo Fatty Acids

De novo FA are short and medium chain FA synthesized in the mammary gland using acetate and BHB produced from rumen fermentation of carbohydrates (Moore and Christie, 1979). Many factors affect de novo FA synthesis such as management and dietary factors that alter rumen fermentation and substrate supply (Palmquist et al., 1993; Woolpert et al., 2017). Barbano et al. (2014) evaluated the relationship between contents of bulk tank milk FA and milk fat across farms in the Northeastern United States and observed increasing the concentration of de novo FA was more positively correlated to increases in milk fat compared to performed FA.

Acetate and butyrate are volatile fatty acids (VFA) produced during ruminal fermentation of carbohydrates and are important precursors for de novo FA synthesis (Moore and Christie, 1979). During fermentation, rumen microbes catabolize carbohydrate polymers to pyruvate via the

Embden-Meyerhof-Parnas pathway (France and Dijkstra, 2005). Pyruvate is converted to acetyl CoA which can be converted to different end products such as acetate and butyrate (Russell and Hespell, 1981). Sutton et al. (2003) observed decreasing the concentrate to forage ratio in dairy diets increased the net production of propionate with no effect on the production rates of acetate or butyrate, and observed the net production for acetate and butyrate were 56.5 and 49.0 mol/d, respectively. In high forage diets, the estimated ratio of acetate to propionate to butyrate is 70:20:10 (France and Dijkstra, 2005). Increasing the dietary content of physically effective neutral detergent fiber (NDF) up to 31% has been shown to increase rumen pH, although this was dependent on the content of dietary starch (Zebeli et al., 2008). Decreases in rumen pH can negatively affect milk fat content by decreasing ruminal motility, microbial yield, and fiber digestion (Allen, 1997). Fukumori et al. (2021) observed a positive relationship between rumen pH and de novo FA content determined using Fourier-transform infrared spectrometry although they observed no relationship between rumen pH and milk fat content.

Infusing acetate and supplementing diets with a neutralized acetate product has been shown to increase de novo FA yield. A meta-analysis conducted by Maxin et al. (2011) observed ruminal infusions of acetate and butyrate additively increased milk fat content and yield, although individual FA were not evaluated. Sheperd and Combs (1998) observed ruminal infusion of 36 mol/d acetate increased milk fat content and yield compared to ruminal infusion of propionate. More recently, Urrutia and Harvatine (2017) observed infusing increasing doses of acetate quadratically effected de novo FA yield with a maximum response at 10 mol/d and linearly increased mixed FA yield up to 15 mol/d. Urrutia et al. (2019) and Matamoros et al. (2022) observed supplementing sodium acetate (NaAc) to target 10 mol/d acetate increased de novo FA yield compared to a control diet.

Effect of Supplemental Long Chain FA

Dorea and Armentano (2017) evaluated the effect of dietary FA on milk FA composition and observed that diets containing long chain FA decreased the content of de novo FA in milk, and the authors proposed the inhibition of de novo FA was compensated by the increase in dietary long chain FA available for milk fat synthesis. Although, the mechanism by which preformed FA inhibits the synthesis of de novo FA is not well understood and not consistently observed in the literature. Two meta-analyzes evaluating the effect of different forms and profiles of supplemental FA observed that diets containing PA+SA and CSPF decreased de novo FA yield whereas diets containing PA had no effect on de novo FA yield compared to a control diet (dos Santos Neto et al., 2021a; 2021b). Palmquist (2006) postulated preformed FA compete for positioning on the TG and diacylglycerol transferase with de novo FA, therefore decreasing their content in milk fat. Although, this theory doesn't explain the effect of long chain FA on short chain FA that are predominantly esterified at the *sn*-3 position.

Drackley et al. (1992) abomasally infused SFA, UFA, and mixture of both and observed that all FA treatments decreased the concentrations of FA from C8:0 to C16:0 in milk fat compared to a control. Although, the FA treatment had no effect on the yields of C8:0, C10:0, or C14:0, but decreased the yields of C12:0 and C16:0 as the unsaturation of the FA in the infusate increased. In agreement, Enjalbert et al. (1998) infused PA, SA, and OA, and observed increasing the molar uptake of FA in milk decreased the mean chain length of synthesized FA but increased the molar mammary balance of C4:0. The authors concluded responses to FA supplementation are different on a molar basis compared to a weight basis, and responses are different for short chain FA (C4:0 to C6:0) and medium chain FA (C8:0 to C14:0) due to the competition of medium and long chain FA for the same positions on the TG. Hansen and Knudsen (1987) introduced PA to mammary epithelial cells and observed increasing the concentration of C16:0 in the cell increased the incorporation of acetate into synthesized C4:0 but had no effect on medium chain FA from C6:0 to C16:0. It is proposed supplementation of long chain FA increases the synthesis of C4:0 to regulate milk fat fluidity (Barbano and Sherbon, 1980).

Mixed Fatty Acids

Mixed fatty acids are 16-carbon FA that can be synthesized in the mammary gland or originate from dietary sources (Moore and Christie, 1979). Kinsella and Gross (1973) first proposed that C16:0 plays an important role in initiation of TG synthesis. They observed that palmityl-CoA was the preferred substrate for GPAT and the rate of acylation was up to 10 times higher compared to myristyl-, stearyl-, oleyl-CoA. In agreement, Jensen (2002) concluded that 44% and 43% of C16:0 is located the at the *sn*-1 and *sn*-2 position, respectively emphasizing the importance of C16:0 for TG synthesis. Tzompa-Sosa et al. (2014) evaluated the positional distribution of FA in TG and observed that the amount of C16:0 in TG affected the positioning and amount of other FA. They observed that increasing the amount of C16:0 in TG was negatively correlated with the amount of C6:0 to C10:0 and *cis*-9 C18:1 at the *sn*-1,3 position and the amount of C14:0 and C18:0 at the *sn*-2 position.

The effect of supplementing PA to dairy cows on milk fat yield has been consistently observed in the literature. A meta-analysis conducted by dos Santos Neto et al. (2021b) observed that diets containing PA-enriched supplements increased milk fat yield by increasing mixed FA yield compared to a control diet as shown in Figure 2.4. Additionally, dos Santos Neto et al. (2021a) observed that supplementing CSPF containing an average of 46% PA and 39% OA increased mixed FA yield compared to a control diet. Rico et al. (2015) conducted a dose response study evaluating the effect of increasing the dietary inclusion of PA in diets containing

WCS or soyhulls. They found PA linearly increased mixed FA yield across both basal diets, and diets containing WCS increased 16-carbon digestibility compared to the diets containing soyhulls.

Increasing de novo synthesized C16:0 is not as well documented in the literature due to the challenges of evaluating differences in preformed and de novo synthesized C16:0 in milk fat. Moore and Christie (1979) concluded that C16:0 was the major FA produced during de novo synthesis at optimal concentration of substrates and reducing equivalents. In agreement, Urrutia and Harvatine (2017) ruminally infused increasing doses of acetate and observed acetate linearly increased mixed FA yield up to 15 mol/d acetate. Urrutia et al. (2019) and Matamoros et al. (2022) supplemented diets with NaAc and observed diets containing NaAc increased mixed FA yield compared to diets without it. In addition, the authors observed NaAc increased mixed FA yield to a greater extent than de novo FA, and postulated the supply of acetate could be a limiting factor in de novo FA synthesis due to its role in providing carbon units and reducing equivalents required for FA elongation.

Preformed Fatty Acids

Preformed FA are long chain FA >18 carbons and some 16-carbon FA that originate from dietary sources such as forages, grains, and oilseeds. The most abundant preformed FA reaching the mammary gland is C18:0 due to it being the end product of biohydrogenation of UFA in the rumen (Bauman and Griinari, 2003). Additionally, FA supplements containing combinations of PA, SA, and OA are often fed to increase the supply of FA to the mammary gland for milk fat synthesis. Whole cottonseed is a common feedstuff fed to dairy cows due to the balance of protein and fibrous outer layer that protects the oilseed from degradation by rumen microbes (Bernard, 2001).

Effect of Oilseeds and Supplemental FA

Few studies in the literature have evaluated the interactions between FA supplements and basal diet FA content. de Souza et al. (2018) fed FA supplements containing PA, PA+SA, and PA+OA in diets containing WCS or soyhulls. They observed diets containing WCS increased preformed FA yield compared to diets containing soyhulls and the FA supplements increased preformed FA yield compared to a control diet, although no interaction between basal diet and FA treatment on preformed FA yield was observed. In agreement, Rico et al. (2015) observed that diets containing WCS increased preformed FA yield compared to diets containing soyhulls, although dose of PA had no effect on preformed FA yield. The authors observed that the diets containing WCS increased 16-carbon digestibility and tended to increase total FA digestibility, thus increasing the supply of long chain FA available for milk synthesis.

Glasser et al. (2008b) evaluated the effect of oilseeds on production performance in a meta-analysis and observed all sources of oilseeds increased the yields of total C18 in milk. In agreement, an unpublished meta-analysis from our lab evaluating the effect of WCS supplemented up to 17% observed diets containing WCS increased the yields of preformed FA and mixed FA as shown in Figure 2.5 (dos Santos Neto et al., 2022). A dose response study conducted in our lab supplementing WCS up to 24% observed WCS quadratically effected milk fat yield with a maximum at 8% and 16%. The 8% and 16% diets had similar milk fat yield, but the 16% diet had higher preformed FA and lower mixed FA compared to the 8%.

Supplemental FA containing combinations of PA, SA, and OA are often fed to dairy cows to increase the energy density of the diet and increase the supply of FA to the mammary gland (Rabiee et al., 2012). dos Santos et al. (2021a) observed diets supplemented with CSPF containing an average of 46% PA and 38% OA increased preformed FA yield compared to a control diet. de Souza et al. (2019) supplemented diets altering in the ratio of PA and OA and observed increasing OA linearly increased the yields of de novo FA and preformed FA in high-producing cows (60 kg/d milk). The effect of supplementing diets with SA on preformed FA yield is not as consistent in the literature. Boerman et al. (2017) supplemented diets with increasing doses of SA up to 2.3% diet DM and observed SA had no effect on preformed FA yield. Increasing the duodenal flow of SA negatively affects FA digestibility (Boerman et al., 2015), which could explain why supplementing SA has no effect on preformed FA yield. Shepardson and Harvatine (2021) observed a SA-enriched supplement containing 93% SA had no effect on preformed FA yield, but a FA supplement containing 45% PA and 49% SA increased preformed FA yield compared to a control diet.

Interdependent Relationship with De Novo FA

Glasser et al. (2008a) proposed an interdependent relationship between preformed FA and de novo FA where the two sources to a certain extent drive the synthesis of each other. They observed that 18-carbon FA yield was increased when the yields of C4:0 to C16:0 were increased as shown in Figure 2.3. In a similar analysis of two experiments, Glasser et al. (2007) observed C4:0 to C16:0 FA yield was the limiting factor for the secretion of C18:0 in milk in low forage diets containing fat supplementation. Toral et al. (2013) observed diet-induced changes in milk FA composition were correlated with the melting point of milk fat in cows, ewes, and goats suggesting the maintenance of fat fluidity is important for milk fat synthesis. Preformed FA and de novo FA are comprised of FA with different degrees of saturation and chain lengths which result in differences in FA melting point (Jensen, 2002). Long chain SFA with higher melting points are predominantly esterified at the *sn*-1 and *sn*-2 position whereas short chain and UFA with low FA melting points are predominantly esterified at the *sn*-3 position (Jensen, 2002).

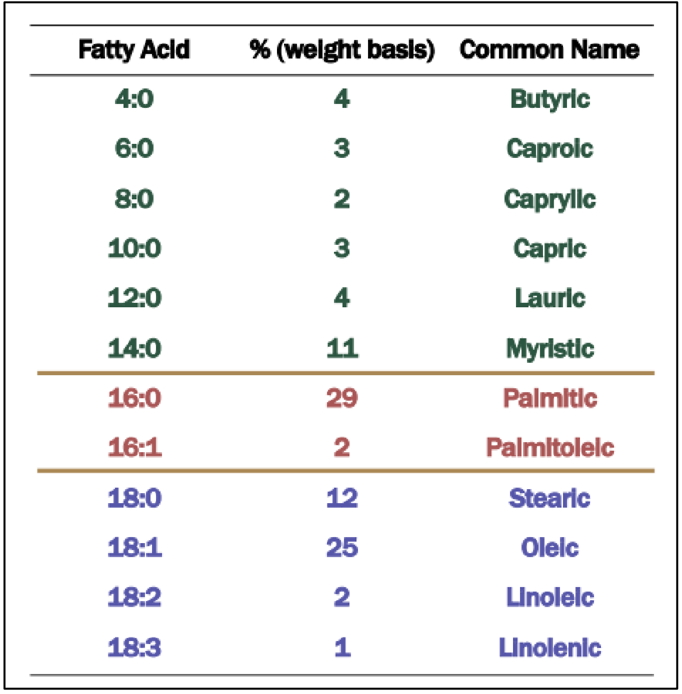
Barbano and Sherbon (1980) proposed the non-random location of short chain FA at the *sn*-3 position is important for regulation of milk fat fluidity and the esterification of such FA at this position could be the limiting factor during TG synthesis.

Conclusion

Often, studies in the literature focus on increasing the production of milk fat by enhancing the production of either de novo FA or preformed FA. However, developing dietary strategies that enhance milk fat production by increasing the yields of all sources of milk fat may be beneficial for increasing TG synthesis and ultimately milk fat production. The goal of Chapter Three was to determine the effects of supplemental PA on the molar yields of individual FA in milk fat. In Chapter Four, our objective was to alter the supply of de novo FA and preformed FA by supplementing diets with NaAc, PA, and WCS to determine if improving the supply of FA to the mammary gland will increase milk fat synthesis. The results from these two studies improve the understanding of the relationship between de novo FA and preformed FA to develop dietary strategies that enhance milk fat production.

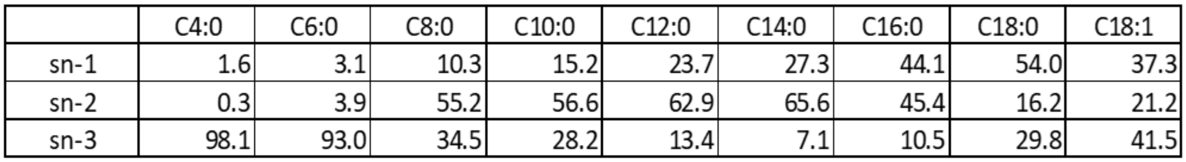
Tables and Figures

Figure 2.1. Common names of fatty acid (FA) and their relative concentration in milk fat on a mass basis. De novo FA shown in green are synthesized in the mammary gland (C4 to C14), mixed FA shown in red originate from both sources (C16), and preformed FA shown in blue originate from the diet (> C18). Figure courtesy of Lock et al. (TSDNC, 2023).



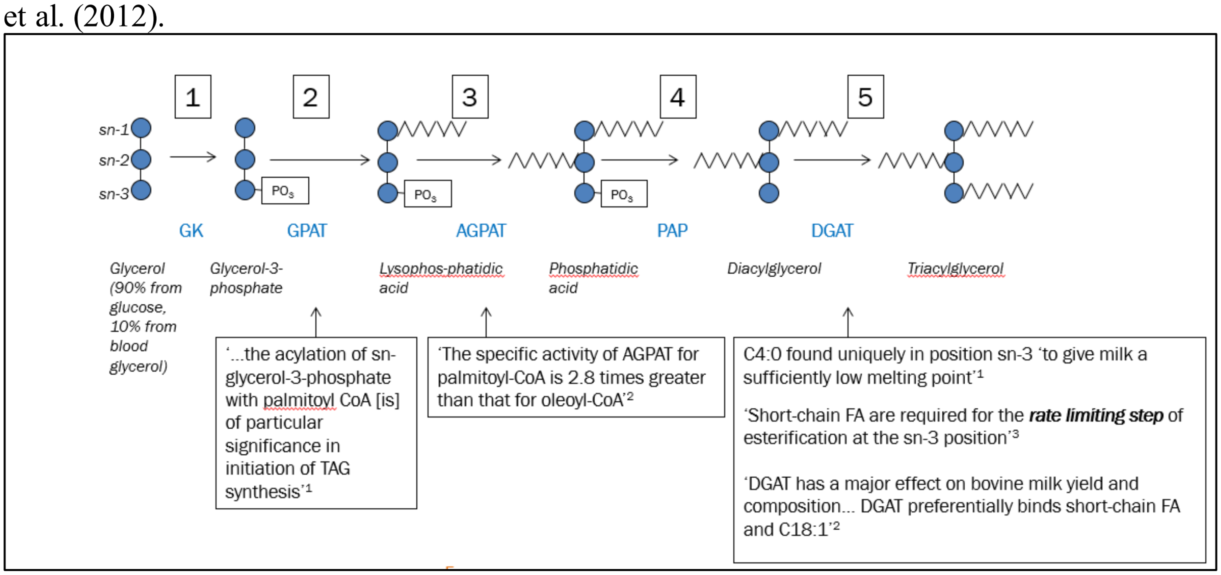
Fatty Acid	% (weight basis)	Common Name
4:0	4	Butyric
6:0	3	Caproic
8:0	2	Caprylic
10:0	3	Capric
12:0	4	Lauric
14:0	11	Myristic
16:0	29	Palmitic
16:1	2	Palmitoleic
18:0	12	Stearic
18:1	25	Oleic
18:2	2	Linoleic
18:3	1	Linolenic

Table 2.1. Stereospecific location of fatty acids (FA) in triglycerides (TG) from bovine milk fat. Fatty acids can be esterified at one of three positions on the TG denoted as *sn*-1, *sn*-2, and *sn*-3. The table represents the molar percentage (mol/100mol FA) of major FA at each of the positions on the TG relative to the individual FA. Table adopted from Jensen (2002).



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

Figure 2.2. Synthesis of bovine milk triglycerides. Figure courtesy of John Newbold, Volac International Ltd and adopted from [1]Moore and Christie (1979), [2]Shorten et al. (2004), and [3]Vyas et al. (2012).



'...the acylation of sn-glycerol-3-phosphate with palmitoyl CoA [is] of particular significance in initiation of TAG synthesis'[1]

'The specific activity of AGPAT for palmitoyl-CoA is 2.8 times greater than that for oleoyl-CoA'[2]

C4:0 found uniquely in position sn-3 'to give milk a sufficiently low melting point'[1]

'Short-chain FA are required for the ***rate limiting step*** of esterification at the sn-3 position'[3]

'DGAT has a major effect on bovine milk yield and composition... DGAT preferentially binds short-chain FA and C18:1'[2]

Figure 2.3. Relationship between milk C18 yield (g/d) and milk C4 to C16 yield (g/d). Glasser et al. (2008a) proposed an interdependent relationship between de novo FA and preformed FA where the two sources drive the synthesis of each other.

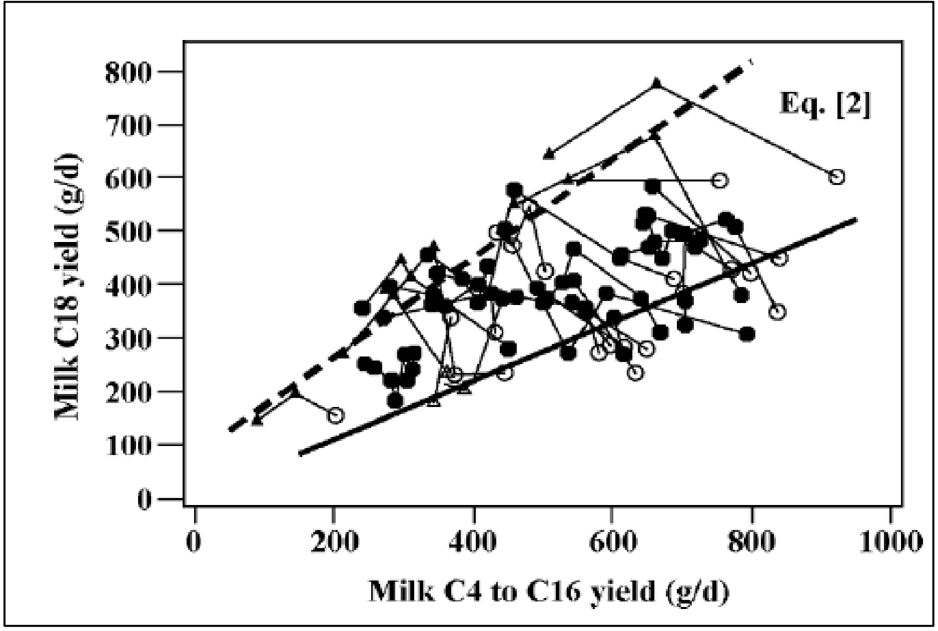


Figure 2.4. Milk fatty acid (FA) yield difference (g/d) between diets containing whole cottonseed (WCS) and a control diet. Studies included in this meta-analysis fed a control diet without WCS and a treatment diet containing ≤ 17% WCS. Adapted from dos Santos Neto et al. (2022, ADSA Abstract).

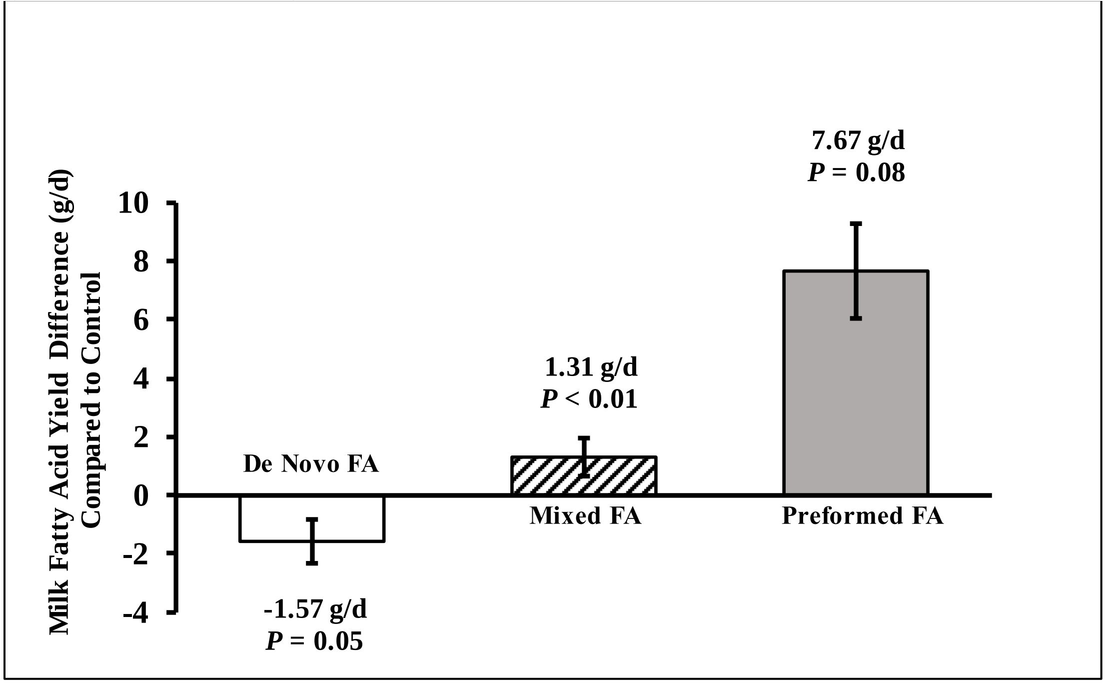
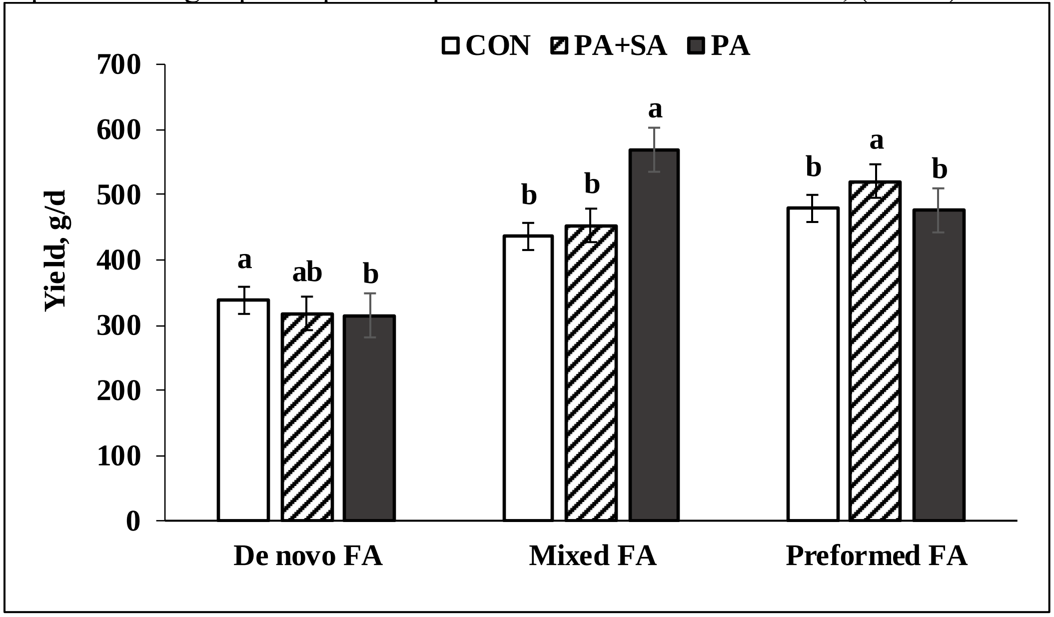


Figure 2.5. Meta-analysis evaluating the effect of supplementing diets with PA and PA+SA on milk fatty acid (FA) yield. Studies included a control diet with no FA supplementation, a diet containing a FA supplemented enriched in PA and SA (≥ 80%) fed at ≤ 3% diet DM (PA+SA), and a diet containing a PA-enriched supplement (≥ 80%) fed at ≤ 3% diet DM (PA). The effect of FA treatment was significant for all sources of milk fat ($P \leq 0.05$) and mean differences were separated using superscripts. Adapted from dos Santos Neto et al., (2021b).



CHAPTER THREE:
META-ANALYSIS EXAMINING THE EFFECT OF PALMITIC ACID SUPPLEMENTATION ON MOLAR CHANGES IN DE NOVO AND PREFORMED MILK FATTY ACIDS

Abstract

To determine the effect of supplemental palmitic acid (PA) on the molar yields of milk fat sources and individual fatty acids (FA), we conducted a meta-analysis of 1,215 individual cow observations from 16 studies conducted at Michigan State University. All studies included a control diet (CON) with no supplemental FA (n = 557) and a diet supplemented with a PA-enriched supplement containing 80-90% PA or a FA blend containing ≥ 80% PA (n = 658) fed at 1.5% diet DM (PA). On a DM basis, diets included (mean ± SD) 29.8% ± 2.1 NDF, 27.2% ± 2.2 starch, and 16.9% ± 0.51 CP. The production parameters across both treatments averaged 28.5 ± 3.4 kg DMI, 45.5 ± 4.5 kg/d milk, 1.70 ± 0.22 kg/d milk fat, and 1.45 ± 0.13 kg/d milk protein. The statistical model included the random effects of study, cow within study, and period or day of treatment within study. Sources of FA were classified as de novo (< 16 carbons), mixed (16-carbon), and preformed (> 16 carbons). Molar yields of individual FA were calculated using the FA mass yield (g/d) and the individual FA molecular weight. Supplementation of PA had no effect on de novo FA yield ($P = 0.22$) but increased C4:0 yield ($P < 0.01$) by 4% and decreased the molar yields of C8:0, C10:0, C12:0, and C14:0 ($P < 0.01$) by 5%, 9%, 10%, and 6%, respectively. Compared to CON, PA increased mixed FA yield ($P < 0.01$) by increasing the yields of C16:0 and *cis*-9 C16:1 ($P < 0.01$) by 17% and 8%, respectively. Supplementation of PA had no effect on preformed FA yield, but it decreased C18:0 ($P < 0.01$) by 3% and increased *cis*-9 C18:1 ($P < 0.01$) by 3%. The shift in FA profile is likely due to positional distribution of specific FA in triglycerides (TG) for maintenance of milk fat fluidity.

Introduction

Supplementing dairy rations with fatty acids (FA) is common practice in the United States to increase the energy density of the diet and increase the supply of FA to the mammary gland for milk fat synthesis (Rabiee et al, 2012). Most commercial FA supplements primarily contain combinations of palmitic acid (PA), stearic acid (SA), and oleic acid (OA). Effects of FA supplementation on milk fat yield are often dependent on the FA profile of the supplement and the level of milk production and stage of lactation of the dairy cow (de Souza et al., 2019; de Souza et al., 2021). Many meta-analyses evaluating the effect of FA supplementation on production performance have been reported, although most all analyses use treatment means from published studies. Meta-analyses using individual observations is considered the "gold standard" because it improves the consistency of the data analysis and allows for exploration of hypotheses using variables that may not be reported in a published study (Simmonds et al., 2005; Lean et al., 2009).

Recently, a meta-analysis using published studies observed supplementation of PA increased the yield of 16-carbon FA in milk, decreased the yield of FA <16 carbons, and had no effect on the yield of FA >16 carbons (dos Santos Neto et al., 2021b). Milk fat is comprised primarily of triglycerides (TG) that contain de novo FA (< 16 carbons) that are synthesized in the mammary gland using acetate and beta-hydroxybutyrate (BHBA) and preformed FA (≥ 16 carbons) that originate from dietary sources or adipose tissue. In this paper, I will refer to FA < 16 carbons as "de novo", FA of 16 carbons as "mixed", and FA >16 carbons as "preformed". Medium chain and long chain FA saturated FA (SFA) from C8:0 to C18:0 are commonly esterified at the *sn*-1 and *sn*-2 position of the TG whereas short chain FA from C4:0 to C6:0 and unsaturated FA (UFA) are commonly esterified at the *sn*-3 position during milk fat synthesis (Jensen, 2002).

Medium and long chain FA compete for positioning on the TG, and esterification of PA has been shown to initiate TG synthesis and dictate the abundance and positioning of other FA on TG (Moore and Christie, 1979; Tzompa-Sosa et al., 2014). Short chain FA are predominantly esterified at the *sn*-3 position of TG likely because they play a role in regulating milk fat synthesis to maintain milk fluidity (Barbano and Sherbon, 1980; Enjalbert, 1998).

Describing FA yields on a mass basis are traditionally used to evaluate changes in milk fat yield. However, describing FA yields on a molar basis may provide a more mechanistic approach for understanding changes in milk fat regulation and synthesis. During TG synthesis, only one FA can be esterified at any one position, regardless of its molecular weight. Molar yields allow for standardization of FA contribution to milk fat by adjusting for differences in FA molecular weights. Therefore, the objective of this meta-analysis was to evaluate the effect of supplemental PA on molar yields of individual FA and sources using individual cow observations to determine the effect of PA on molar yields of milk FA. We hypothesized that supplementation of PA would increase mixed FA yield with no effect on the yields of de novo FA or preformed FA, and PA would shift FA profile on a molar basis in favor of increased yields of short chain FA over medium chain FA and UFA over SFA due to competition during TG synthesis.

Materials and Methods

Data Set and Treatment Diets

Data from individual lactating cows(n=1215) from 16 studies conducted at Michigan State University from 2013 to 2020 were used in this meta-analysis. Studies included in the dataset are shown in Table 3.1. All studies contained a control diet (CON; n=557) with no supplemental FA and a treatment diet supplemented with a PA-enriched supplement containing 80-90% PA or a FA blend containing $\geq$ 80% PA (PA; n=658). The descriptive statistics for the FA profile of the PA-enriched supplements and FA blends are presented in Table 3.2. The PA-enriched supplements were included at ≤ 3% of diet DM for all studies and the average inclusion was 1.61% of diet DM. Descriptive statistics for diet nutrient composition are presented in Table 3.3 and intake and production performance are presented in Table 3.4.

Sample Analysis and Calculations

Sample collection and analysis were similar for all studies included in the dataset. Feed ingredients and orts samples were collected and dried for 72 h at 55ºC in a forced-air oven and ground in a Wiley mill (1 mm screen; Arthur H. Thomas, Philadelphia, PA) and analyzed for neutral detergent fiber (NDF), starch, crude protein (CP), and FA profile according to Boerman et al. (2017). Two milk samples were collected at each milking; one aliquot was collected in a tube with preservative and stored at 4 ºC for analysis of fat, true protein, and lactose (AOAC, 1990, method 972.160; CentralStar DHI, Grand Ledge, MI), and a second aliquot was collected and stored at -20ºC for milk FA analysis as described by Lock et al. (2013).

Intakes of NDF and FA were calculated using the nutrient composition of the diet and DMI for each individual observation. For Latin square designs, milk fat yield was determined using a composition of milk collected during the last 4 or 5 days of the treatment period. For block designs, the FA yield was determined using a composition of milk collected during 3 consecutive milkings every 7 d. Yields of FA in g/d were calculated using milk fat yield, individual FA concentrations, and molecular weight of each FA correcting for glycerol content and other milk lipids (Piantoni et al., 2013). Molar yields of individual FA were calculated by dividing the individual FA yield in g/d by their corresponding molecular weight presented in Table 3.5 Molar yields were calculated for all individual FA used in the analysis and then summed as follows: de novo (< 16 carbon), mixed (16-carbon), and preformed (> 16 carbon). The desaturation indices for C14, C16, and C18 were calculated by dividing the molar yields of the unsaturated products (*cis*-9 C14:1, *cis*-C16:1, and *cis* C18:1) by the sum of the unsaturated product and the saturated substrate (C14:0, C16:0, and C18:0, respectively).

Statistical Analysis: Meta-Analysis

A meta-analysis was performed to evaluate the overall effect of supplementing diets with PA on milk FA yield using individual observations across studies. All data were analyzed using SAS PROC MIXED (version 9.4, SAS Institute) with the linear model:

$$Y_{ijlm} = \mu + T_i + B_j + C_l + P_m + e_{ijlm},$$

where Y_{ijlm} = the dependent variable, μ = the overall mean, T_i = the fixed effect of treatment, B_j = the random effect of study, C_l = the random effect of cow within study, P_m = the random effect of period (Latin square design) or day of treatment (blocked design) within study, and e_{ijlm} = residual error. Statistical significance was declared at $P \leq 0.05$ and tendencies $P \leq 0.10$.

Statistical Analysis: Meta-Regression

A meta-regression was performed to evaluate the relationship between increasing dietary intake of PA on milk FA molar yields. Quadratic relationships between PA intake and FA molar yields were evaluated and determined to be insignificant, therefore, only linear relationships are reported. Data were analyzed using SAS PROC MIXED (version 9.4, SAS Institute) with the linear model:

$$Y_{ijklm} = A_i + D_j * X + B_k + C_l + P_m + e_{ijklm},$$

where Y_{ijklm} = the dependent variable, A_i = the intercept, D_j = the slope, X = PA intake, B_k = the random effect of study, C_l = the random effect of cow within study, P_m = the random effect of period (Latin square design) or day (blocked design) of treatment within study, and e_{ijklm} = the residual error. Study-adjusted values were incorporated into the regression figures as previously described by St. Pierre (2001). Statistical significance was declared at $P \leq 0.05$ and tendencies $P \leq 0.10$.

Results

Meta-Analysis

Results from the meta-analysis on a molar basis are presented in Table 3.5. Compared to CON, PA had no effect on de novo FA yield ($P = 0.22$), but PA increased C4:0 yield ($P < 0.01$) by 4%, had no effect on C6:0 yield ($P = 0.40$), and decreased the yields of C8:0, C10:0, C12:0, and C14:0 ($P < 0.01$) by 5, 9, 10, and 6% , respectively. Compared to CON, the PA treatment increased mixed FA molar yield ($P < 0.01$) by 17% mmol/d by increasing C16:0 ($P < 0.01$) and *cis*-9 C16:1 ($P < 0.01$) by 17 and 8%, respectively. Compared to CON, PA had no effect on preformed FA molar yield ($P = 0.69$), although it decreased C18:0 yield ($P < 0.01$) by 3% and increased *cis*-9 C18:1 yield ($P < 0.01$) by 3%.

Results from the meta-analysis on a mass basis followed a similar pattern as yield on a molar basis (Table 3.6). Compared to CON, PA decreased de novo FA yield ($P < 0.01$) by 5%, increased mixed FA yield ($P < 0.01$) by 17%, and had no effect on preformed FA yield ($P = 0.71$). Supplementation of PA increased C4:0 yield ($P < 0.01$) by 5% while decreasing the yields of C8:0, C10:0, C12:0, and C14:0 ($P < 0.01$) by 6, 9, 10, and 6% respectively. Compared to CON, PA increased the yields of C16:0 ($P < 0.01$) and *cis*-9 C16:1 ($P < 0.01$) by 17 and 10%, respectively. Compared to CON, PA decreased C18:0 ($P < 0.01$) and increased *cis*-9 C18:1 ($P < 0.01$) yield by 3%.

Meta-Regression: Molar Basis

Increasing dietary PA intake had no effect on the molar yields of de novo (Figure 3.3; $P = 0.95$) or preformed (Figure 3.5; $P = 0.84$) FA but linearly increased mixed FA yield (Figure 3.4;

$P < 0.01$). Increasing PA intake linearly increased C4:0 yield ($P < 0.01$) while linearly decreasing the yields of C8:0 ($P < 0.01$), C10:0 ($P < 0.01$), C12:0 ($P < 0.01$), and C14:0 ($P < 0.01$). Increasing dietary PA intake linearly increased the yields of C16:0 ($P < 0.01$) and *cis*-9 C16:1 ($P < 0.01$) and linearly decreased C18:0 yield ($P < 0.01$) and linearly increased *cis*-9 C18:1 yield ($P < 0.01$). Increasing dietary PA increased the C18 desaturation index ($P < 0.01$), decreased the C16 desaturation index ($P < 0.01$), and had no effect on the C14 desaturation index ($P = 0.98$).

Meta-Regression: Mass basis

Increasing dietary PA intake increased mixed FA yield (Figure 3.7; $P < 0.01$) and tended to linearly decrease de novo FA yield (Figure 3.6; $P = 0.06$) but had no effect on preformed FA yield (Figure 3.8; $P = 0.94$). Increasing dietary PA intake linearly increased C4:0 yield ($P < 0.01$) and linearly decreased the yields of C10:0 ($P < 0.01$), C12:0 ($P < 0.01$), and C14:0 ($P < 0.01$). Increasing PA intake tended to increase C6:0 yield ($P = 0.06$) and *cis*-9 C14:1 yield ($P = 0.08$) and tended to decrease C8:0 yield ($P = 0.10$). Lastly, increasing PA intake linearly increased C16:0 yield ($P < 0.01$) and *cis*-9 C16:1 yield ($P < 0.01$) and tended to increase *cis*-9 C18:1 yield ($P = 0.07$) and decrease C18:0 yield ($P = 0.08$).

Discussion

To our knowledge, this is the first report of the effect of PA supplementation on the incorporation of de novo FA and preformed FA into milk fat on a molar basis using individual cow observations. A recent meta-analysis using published study means evaluated the effect of dietary supplementation of PA on milk FA yield on a mass basis and observed supplementation of PA increased mixed FA yield, decreased de novo FA yield, and had no effect on preformed FA yield compared to a control diet with no FA supplementation (dos Santos Neto et al., 2021b). We observed similar results to that paper when describing FA yield on a mass basis. Supplementation of long chain FA increases the proportion of preformed FA in milk fat while decreasing the proportion of de novo FA.

Incorporating two or three short chain FA in milk TG is needed to balance the mass of one long chain FA whereas only one short chain FA is needed on a molar basis (Enjalbert et al., 1998). Additionally, only one molar unit of a FA can be esterified at each position of the TG, but the molecular weight of that FA can change the overall weight of the TG. Therefore, we hypothesized that the effect of PA supplementation on milk FA using molar yields would provide a more accurate representation of changes in milk FA regulation compared to changes on a mass basis. Performing meta-analyses using data from individual observations is more advantageous compared to published treatment means because these analyses limit study bias and allow for consistent statistical analysis compared to analyses performed using published studies (Simmonds et al., 2005; Lean et al., 2009). We recognize that the individual cow observations used in this paper were only from one population of dairy cows housed at one farm facility.

Supplementation of PA increased mixed FA yield but had no effect on the yields of de novo FA or preformed FA which was observed in the meta-analysis and meta-regression. However, it shifted de novo FA profile in favor of increased C4:0 yield and decreased yields of C8:0 to C14:0 and shifted preformed FA yield in favor of increased *cis*-9 C18:1 and decreased C18:0. Our meta-analysis evaluating the effect of PA on FA yields on a mass basis and the meta-analysis conducted by dos Santos Neto et al. (2021b) observed supplementation of PA decreased de novo FA yield. Increasing PA is decreasing the overall mass of de novo FA by substituting short chain FA for medium chain FA, whereas on a molar basis the incorporation of de novo FA is unchanged. Our results emphasize the importance of evaluating changes in de novo FA yield on a molar basis to understand how supplementation of FA effects incorporation of short and medium chain FA in milk fat.

Toral et al. (2013) evaluated the relationship between diet-induced changes in milk fat composition and estimated melting point in cows, ewes, and goats and determined that the variation of the estimated melting point of milk fat was narrow compared to the variation of the concentration of individual FA in milk. Additionally, the melting point of FA increases as the number of carbon units increase in SFA, whereas, increasing the double bonds in UFA decreases the melting point. Therefore, supplementation of long chain SFA increases the melting point when incorporated into TG and FA with lower melting points are likely needed to balance these changes. We observed yields of C4:0 and *cis*-9 C18:1 increased with PA supplementation likely due to an increase in demand for FA with lower melting points for maintenance of milk fat fluidity. In agreement, Hansen and Knudsen (1979b) observed infusing mammary epithelial cells with PA increased the synthesis C4:0 but had no effect on other de novo FA from C6:0 to C16:0.

Palmityl-CoA was the preferred substrate for the initial acyl transferase compared to myristyl-CoA, stearyl-CoA, and oleyl-CoA substrates in bovine mammary microsomes (Kinsella and Gross, 1973) suggesting PA may play a role in initiation of TG synthesis, which, in turn, might increase the amount of TG in milk fat Medium and long chain SFA are predominately esterified at the *sn*-1 and *sn*-2 position of TG during milk fat synthesis (Jensen, 2002). Increasing the incorporation of PA in TG increases the competition with other FA esterified at the same position (Tzompa-Sosa et al., 2014), and in the current study PA decreased the yields of C8:0 to C14:0. Likely, de novo synthesized PA also decreased, but this effect was masked by the increase in preformed PA supplied in the diet. In agreement, Enjalbert et al. (1998) observed infusing PA decreased the mean chain length of synthesized FA likely due to the increase in the dietary supply of PA decreasing the demand for de novo synthesized medium chain FA. Whereas, short chain FA and UFA are primarily esterified at the sn-3 position during TG synthesis suggesting they are important for milk fat regulation and fluidity (Barbano and Sherbon, 1980; Jensen, 2002). We hypothesize that the mammary gland utilized dietary supplied PA over synthesizing medium chain de novo FA potentially sparing acetate for use by other tissues although this theory warrants additional research.

Although there was no overall effect on preformed FA yield, supplementation of PA decreased C18:0 and increased *cis*-9 C18:1 yield. Long chain FA have different preferences towards positions on the TG depending on the unsaturation of the FA. C18:0 and *cis*-9 C18:1 have preference towards the *sn*-1 position although *cis*-9 C18:1 has higher secondary preference towards the *sn*-3 position (Jensen, 2002). Presumably, PA decreased C18:0 yield due to increased competition at the *sn*-1 position of the TG while also requiring more *cis*-9 C18:1 esterification at the *sn*-3 position for maintenance of milk fluidity or decreased competition. Additionally, we hypothesize that the increase in *cis*-9 C18:1 was due to desaturation of C18:0 in the mammary gland. Saturated or *trans*-C18:1 FA can be desaturated in the mammary gland by Stearyl-CoA desaturase (SCD), an enzyme that inserts a *cis*-double bond on carbon 9 of FA (Moore and Christie, 1979). The C14 desaturation index is often used as a proxy of SCD activity in the mammary gland because almost all of C14 FA are synthesized de novo, but the desaturation of C18:0 in the mammary gland is most important for milk fat synthesis and fluidity due to the high amounts of C18:0 leaving the rumen from to biohydrogenation (Bauman et al., 2011). Supplementation of PA had no effect on the C14 desaturation index, decreased the C16 desaturation index, and increased the C18 desaturation index; thus, SCD seemed to preferentially act upon C18 FA when PA supplementation increased. Presumably, the C16 desaturation index decreased because of the high demand of PA needed to make TG, whereas increasing the C18 desaturation index likely increased due to the demand of unsaturated FA needed to balance the increase in PA incorporation as the desaturation of C18:0 to *cis*-9 C18:1 (Palmquist, 2006). Although Bernard et al. (2013) suggests the action and regulation of SCD activity is at the gene expression level, it is unclear if dietary factors or metabolites are the main regulatory factors suggesting the effect of FA supplementation of SCD activity warrants further research.

Measuring FA yield on a molar basis is important for understanding changes in milk FA composition, especially when examining the effect of supplemental FA on de novo FA yield (Enjalbert et al., 1998). In our study, measuring changes in FA yield on a mass basis overestimated the effect of PA on de novo FA yield likely due to PA decreasing medium chain FA while increasing short-chain FA, thus decreasing overall de novo FA mass yield. Expressing these changes on a molar basis showed no overall difference in de novo FA yield between CON and PA because PA shifted de novo FA synthesis toward more C4:0 synthesis and less of all others. Measuring FA yield on a molar basis allows for a better understanding of the contribution of FA to milk fat because the molecular weight is accounted for, therefore the incorporation of individual FA in milk TG are standardized. When solely evaluating the CON diet, the relative concentration of de novo FA on a molar basis were 9.1 percentage units higher whereas mixed FA and preformed FA were 2.2 and 6.8 percentages lower, respectively, compared to on a mass basis. We observed that evaluating the relative concentration of FA using molar yields better reflects the contribution of de novo FA to milk fat compared to on a mass basis and highlights the important contribution of de novo FA to milk fat synthesis. Describing FA yields on a molar basis is important for understanding the regulation of milk fat synthesis and the effect of FA supplementation on de novo FA and preformed FA.

Conclusion

Supplementation of PA had no overall effect on the overall molar yields of de novo FA or preformed FA but shifted FA synthesis in favor of increased C4:0 and *cis*-9 C18:1 yield presumably to counter the increase in C16:0 FA and thus maintain milk fat fluidity. These changes were not as obvious when FA yields are expressed on a mass basis. Future research should evaluate the effect of altering the supply of de novo FA and preformed FA on the molar yields of milk FA to determine the effects on the incorporation and regulation of FA into milk fat.

Tables and Figures

Table 3.1. List of studies in the data set used in the analysis.

Authors	Year	Study design[1]
Unpublished	2014	LSD
Garver et al.	2015*	LSD
de Souza et al.	2016	LSD
de Souza et al.	2017	LSD
de Souza and Lock	2018	LSD
de Souza and Lock	2018	RCBD
de Souza et al.	2018	SP-LSD
de Souza and Lock	2019	RCBD
de Souza et al.	2019	T-LSD
Negreiro et al.	2019*	T-LSD
Burch et al.	2020*	SP-LSD
Negreiro et al.	2020*	CD
Western et al.	2020	T-LSD
Western et al.	2020	CD
de Souza et al.	2021	RCBD
Negreiro et al.	2021*	SP-LS

*Denotes studies published as ASDA Abstract

[1] LSD = Latin square design, RCBD = randomized complete block design, SP-LSD = split plot Latin square design, T-LSD = truncated Latin square design, and CD = crossover design.

Table 3.2. Descriptive statistics of palmitic acid-enriched supplements (PA)[1] in the dataset.

FA, g/100g FA	Mean	Min	Max	SD
C16:0	85.1	76.8	91.1	4.45
C18:0	2.01	0.31	5.05	1.33
cis-9 C18:1	8.82	4.90	13.6	2.50

[1] PA = diet supplemented with FA blend containing ≥ 80% PA or a PA-enriched FA supplement containing 80-90% PA (n=658)

Table 3.3. Descriptive statistics of the nutrient composition of the treatment diets in the dataset.

	CON[1]				PA[2]			
Item, % DM	Mean	Min	Max	SD	Mean	Min	Max	SD
DM								
NDF	30.6	25.3	37.0	2.87	29.9	24.3	36.1	2.71
Forage NDF	20.5	14.5	24.0	2.45	20.2	12.5	24.0	2.79
CP								
Starch	26.6	15.9	31.9	3.59	26.7	15.9	31.9	3.27
FA	3.01	1.68	4.85	0.68	4.36	3.19	5.10	0.52
C16:0	0.53	0.30	1.85	0.21	1.76	0.55	2.18	0.27
C18:0	0.09	0.05	0.21	0.03	0.16	0.05	0.45	0.09
cis-9 C18:1	0.56	0.42	1.17	0.12	0.66	0.51	1.17	0.09
cis-9,12 C18:2	1.55	1.20	2.40	0.28	1.50	1.21	1.85	0.17
cis-9,12,15 C18:3	0.39	0.10	1.81	0.56	0.17	0.09	0.22	0.04

[1] CON = diets containing no supplemental FA (n=556)

[2] PA = diet supplemented with FA blend containing ≥ 80% PA or a PA-enriched FA supplement containing 80-90% PA (n=658)

Table 3.4. Descriptive statistics of production performance and intake of individual cows in the dataset.

	CON[1]				PA[2]			
Item	Mean	Min	Max	SD	Mean	Min	Max	SD
Intake, kg/d								
DM	28.1	4.40	41.4	5.80	27.6	4.60	39.9	5.20
NDF	8.57	1.40	12.9	2.00	8.27	1.20	12.6	1.80
FA intake, g/d								
C16:0	146	20.6	529	54.5	488	69.4	734	108
C18:0	26.0	3.50	51.9	9.50	44.9	4.60	171	29.7
cis-9 C18:1	155	23.7	335	42.0	182	29.4	315	40.9
cis-9,12 C18:2	432	65.0	789	108	415	57.9	722	92.2
cis-9,12,15 C18:3	116	7.50	722	176	46.3	6.90	85.0	15.0
Yield, kg/d								
Milk	45.7	18.5	73.9	10.0	46.0	13.6	76.4	10.5
Fat	1.68	0.41	3.37	0.43	1.80	0.53	4.26	0.48
Protein	1.44	0.67	2.34	0.28	1.48	0.52	3.06	0.33
Lactose	2.21	0.78	3.62	0.52	2.21	0.53	3.85	0.55
3.5% FCM	47.1	19.1	77.1	10.1	49.0	14.5	92.6	11.3
ECM	47.1	19.6	75.4	9.69	48.9	15.1	84.0	11.0
Content, %								
Fat	3.72	0.92	7.46	0.79	3.96	1.73	7.81	0.77
Protein	3.19	1.36	4.54	0.37	3.25	2.13	4.62	0.37
Lactose	4.82	2.38	5.18	0.26	4.79	3.39	5.18	0.22

[1] CON = diets containing no supplemental FA (n=556)

[2] PA = diet supplemented with FA blend containing ≥ 80% PA or a PA-enriched FA supplement containing 80-90% PA (n=658)

Table 3.5. Molecular weight of 4-carbon to 18-carbon fatty acids (FA).

FA	Molecular weight, g/mol
C4	88.1
C6	116
C8	144
C10	172
C12	200
C14	228
C16	256
C18	285

Table 3.6. Milk fatty acid (FA) yield on a molar basis from cows fed treatment diets.

	Treatment[1]			*P*-value
	CON	PA	SEM	Trt
Source[2], mmol/d				
De novo	2203	2183	110	0.22
Mixed	2115	2469	90	<0.01
Preformed	2085	2080	140	0.69
Individual FA, mmol/d				
C4:0	520	543	30	<0.01
C6:0	262	260	10	0.40
C8:0	125	119	6	<0.01
C10:0	273	249	20	<0.01
C12:0	273	246	20	<0.01
C14:0	735	693	60	<0.01
cis-9 C14:1	52	50	2	<0.01
C16:0	2020	2370	90	<0.01
cis-9 C16:1	95	103	8	<0.01
C18:0	529	516	40	<0.01
cis-9 C18:1	1039	1072	90	<0.01
cis-11 C18:1	36	36	4	0.23
cis-12 C18:1	25	24	2	<0.01
cis-9,12 C18:2	126	125	6	0.66
cis-9,12,15 C18:3	18	17	1	<0.01

[1] CON = no supplemental FA (n=556), PA = diet supplemented with FA blend containing ≥ 80% PA or a PA-enriched FA supplement containing 80-90% PA (n=658)

[2] FA classified as: de novo < 16-carbons, mixed = 16-carbons, and preformed > 16-carbons

Table 3.7. Milk fatty acid (FA) yield on a weight basis from cows fed treatment diets.

	Treatment[1]			
	CON	PA	SEM	*P*-value
Source[2], g/d				
De novo	387	368	17.8	<0.01
Mixed	542	632	24.4	<0.01
Preformed	604	603	39.5	0.71
FA, g/d				
C4:0	45.7	47.9	3.10	<0.01
C6:0	30.4	30.1	1.90	0.24
C8:0	18.1	17.1	0.90	<0.01
C10:0	47.0	42.7	2.85	<0.01
C12:0	54.8	49.2	3.38	<0.01
C14:0	180	169	8.57	<0.01
cis-9 C14:1	11.6	11.3	0.56	<0.01
C16:0	518	607	23.3	<0.01
cis-9 C16:1	24.1	26.4	2.02	<0.01
C18:0	151	146	10.9	<0.01
cis-9 C18:1	293	302	26.3	<0.01
cis-11 C18:1	10.1	10.1	1.09	0.52
cis-12 C18:1	7.01	6.64	0.42	<0.01
cis-9,12 C18:2	35.3	35.1	1.72	0.37
cis-9,12,15 C18:3	5.10	4.69	0.40	<0.01

[1] CON = no supplemental FA, PA = diet supplemented with FA blend containing ≥ 80% PA or a ≤ 90% PA-enriched FA supplement

[2] FA classified as: de novo < 16-carbons, mixed = 16-carbons, and preformed > 16-carbons

Figure 3.1. Effect of supplementing palmitic acid (PA) on the relative concentration of milk fatty acid (FA) sources on a molar basis (mmol/100 mmol FA) and a mass basis (g/100g FA). Treatments diets were a control diet with no supplemental FA (CON) and a diet supplemental with a FA blend containing ≥ 80% PA or a PA-enriched FA supplement containing 80-90% PA. The relative concentration on a molar basis was 34.5% de novo FA, 33.0% mixed FA, and 32.5% preformed FA for CON and 32.1% de novo FA, 36.9% mixed FA, and 31.0% preformed FA for PA. The relative concentration on a mass basis was 25.4% de novo FA, 35.2% mixed FA, and 39.4% preformed FA for CON and 22.8% de novo FA, 39.5% mixed FA, and 37.7% preformed FA for PA.

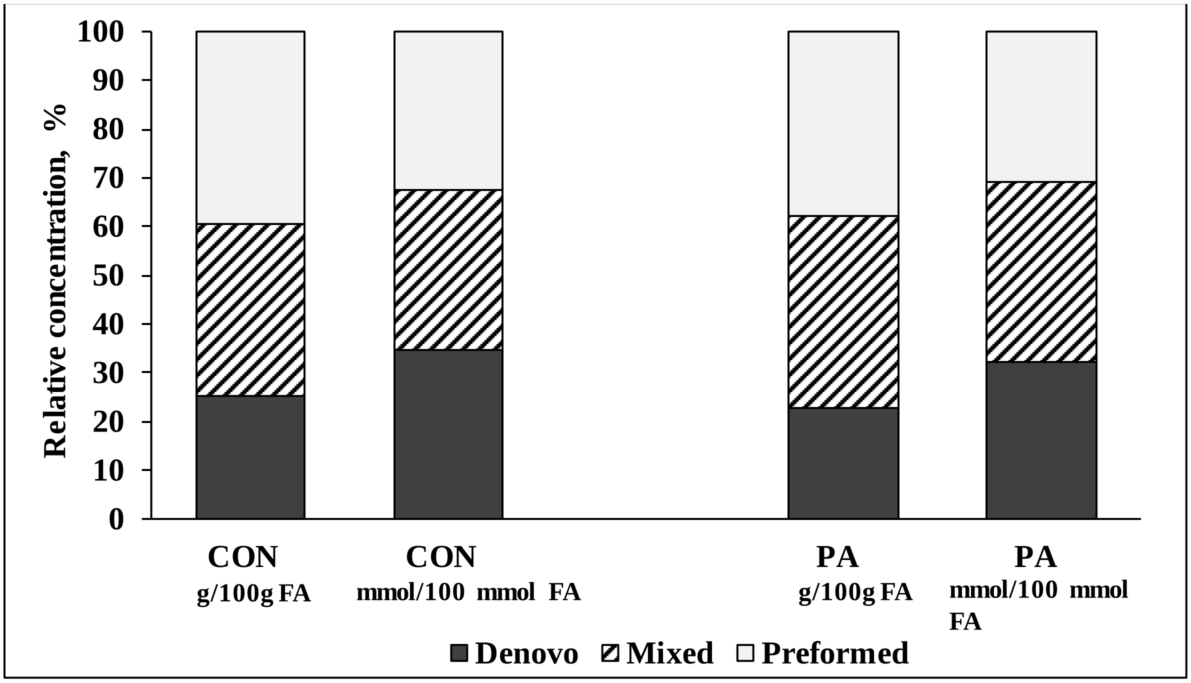


Figure 3.2. Difference of fatty acid (FA) yield between a diet containing palmitic acid (PA) compared to a control diet (CON) on a molar basis (mol/d) and mass basis (g/d) for all sources of FA (A) and de novo FA (B). Treatments diets were a control diet with no supplemental FA (CON) and a diet supplemented with a FA blend containing ≥ 80% PA or a PA-enriched FA supplement containing 80-90% PA. The difference between PA and CON on a molar basis was -0.91% de novo FA, 16.7% mixed FA, and -0.24% preformed FA. The difference between PA and CON on a mass basis was -4.91% de novo, 16.6% mixed FA, and -0.17% preformed FA.

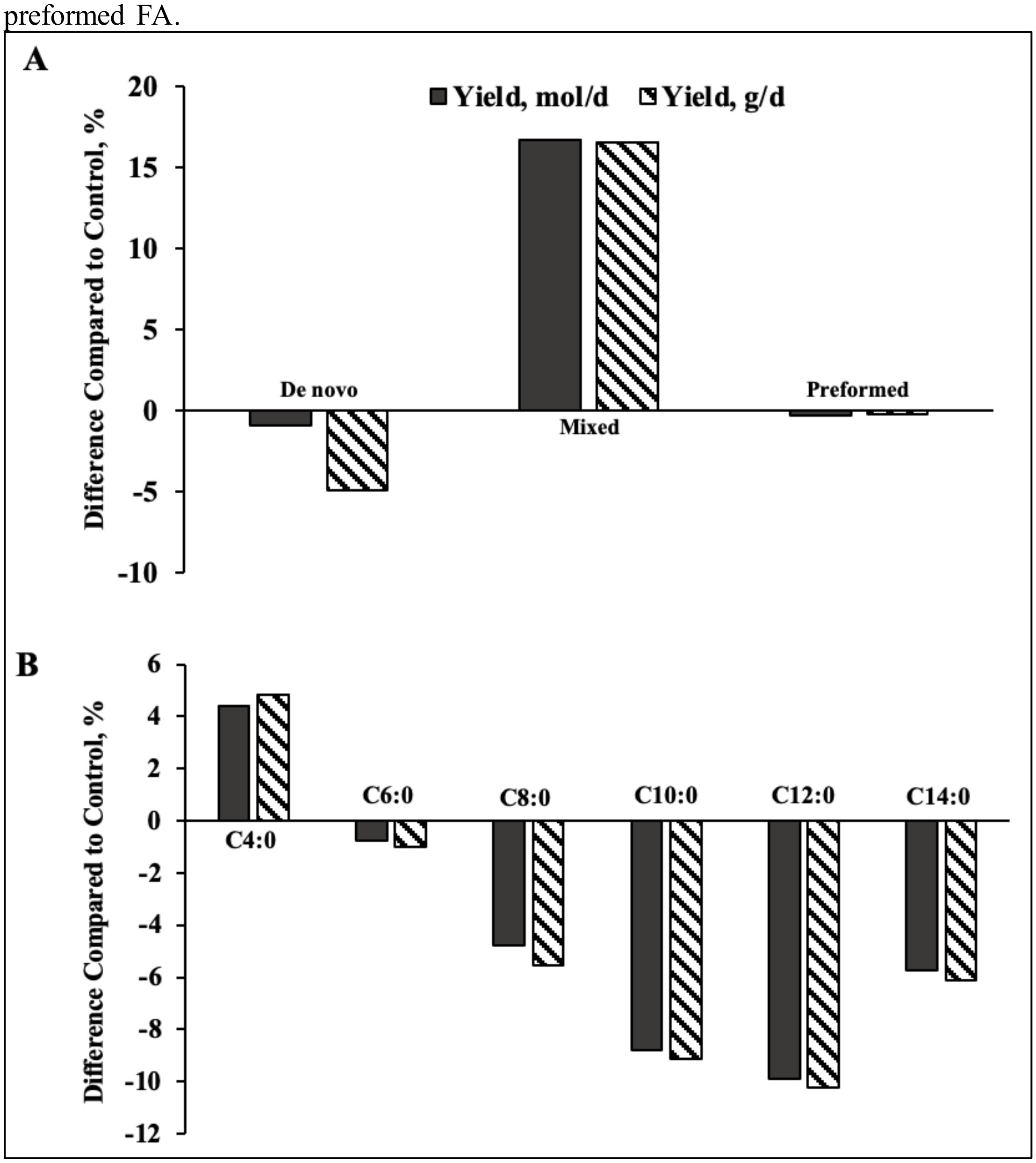


Figure 3.3. Meta-regression evaluating the effect of increasing dietary palmitic acid (PA) intake (g/d) on de novo fatty acid (FA) yield on a molar basis (A) and a mass basis (B). Treatments diets were a control diet with no supplemental FA (CON) and a diet supplemental with a FA blend containing ≥ 80% PA or a PA-enriched FA supplement containing 80-90% PA. Increasing dietary PA intake had no effect on de novo FA yield ($P = 0.95$) on a molar basis and tended to linearly decrease de novo FA yield ($P = 0.06$) on a mass basis.

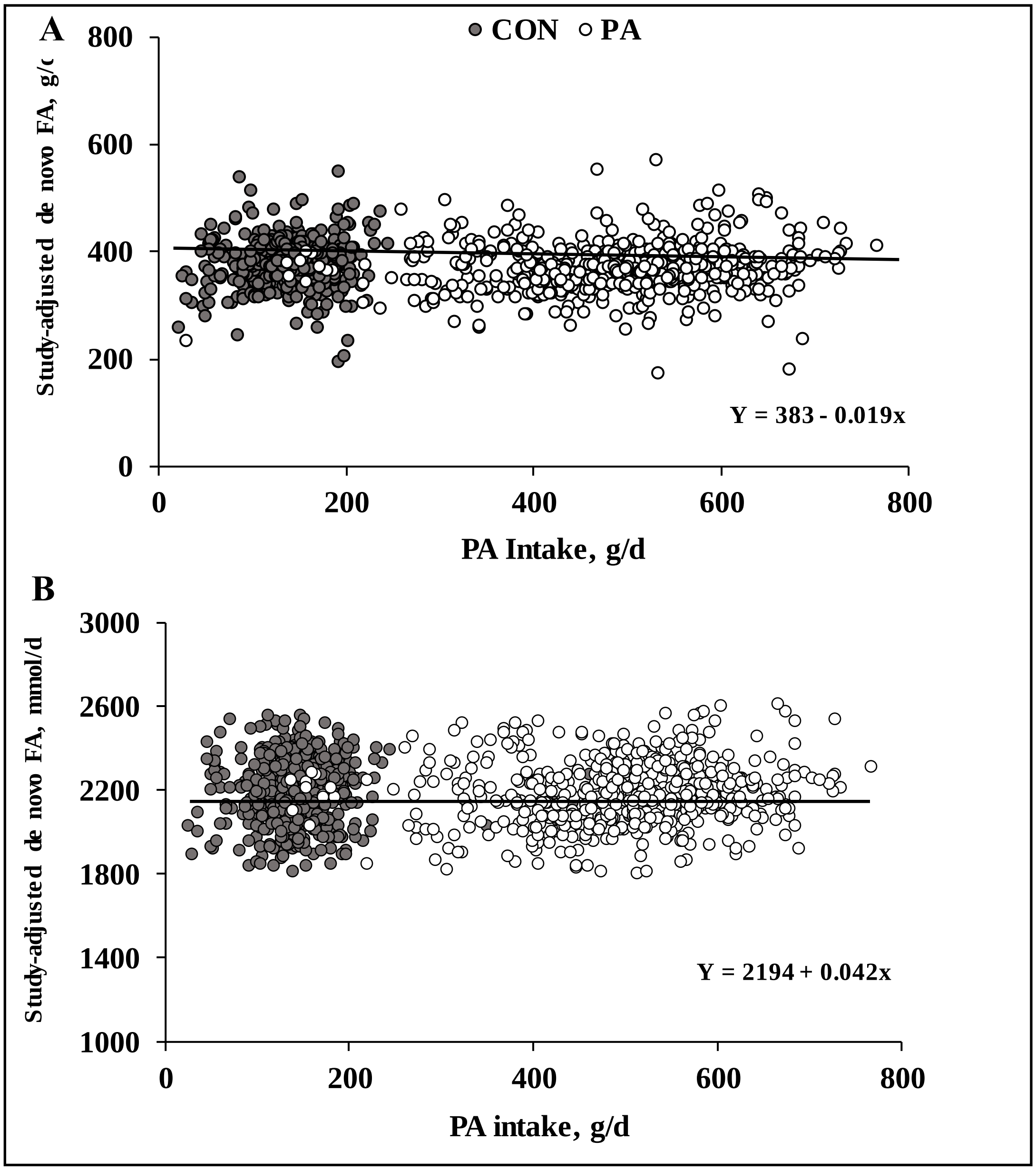


Figure 3.4. Meta-regression evaluating the effect of increasing dietary palmitic acid (PA) intake (g/d) on mixed fatty acid (FA) yield on a molar basis (A) and mass basis (B). Treatments diets were a control diet with no supplemental FA (CON) and a diet supplemental with a FA blend containing ≥ 80% PA or a PA-enriched FA supplement containing 80-90% PA. Increasing dietary PA intake linearly increased mixed FA yield ($P < 0.01$) on molar basis and mass basis.

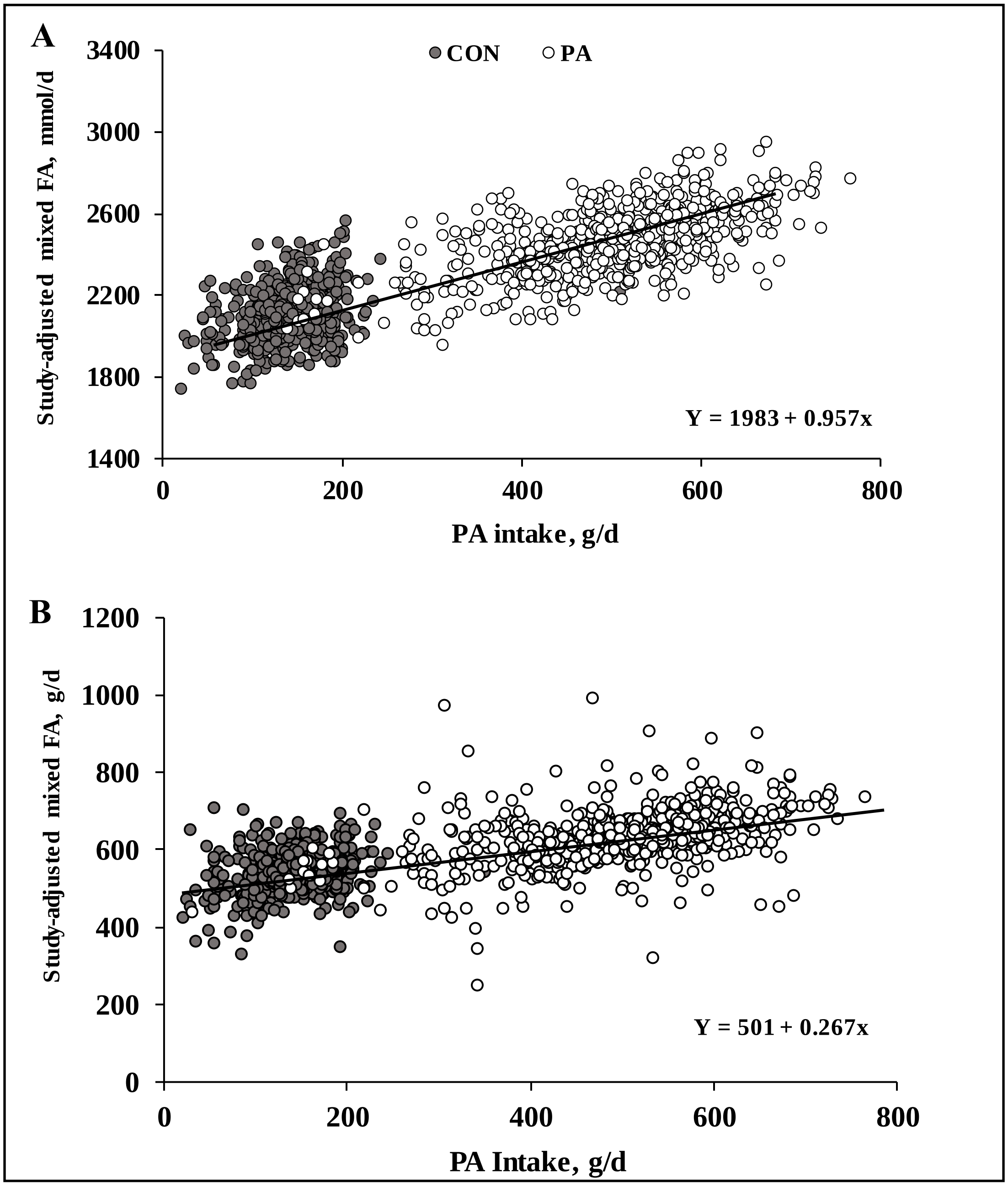


Figure 3.5. Meta-regression evaluating the effect of increasing dietary palmitic acid (PA) intake (g/d) on preformed fatty acid (FA) yield on a molar basis (A) and mass basis (B). Treatments diets were a control diet with no supplemental FA (CON) and a diet supplemental with a FA blend containing ≥ 80% PA or a PA-enriched FA supplement containing 80-90% PA. Increasing dietary PA intake had no effect on preformed FA yield on a molar basis (*P* = 0.84) or mass basis (*P* = 0.94).

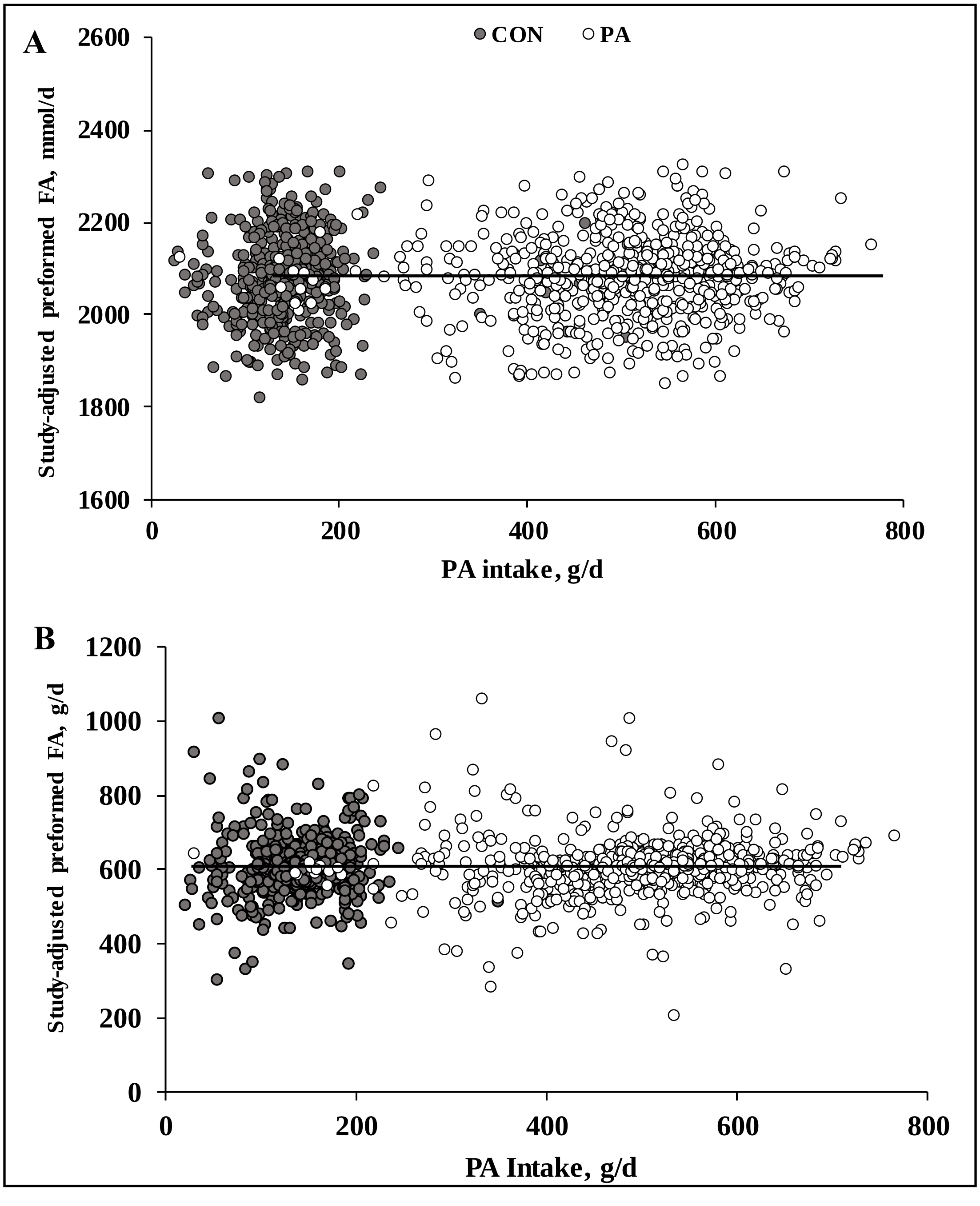
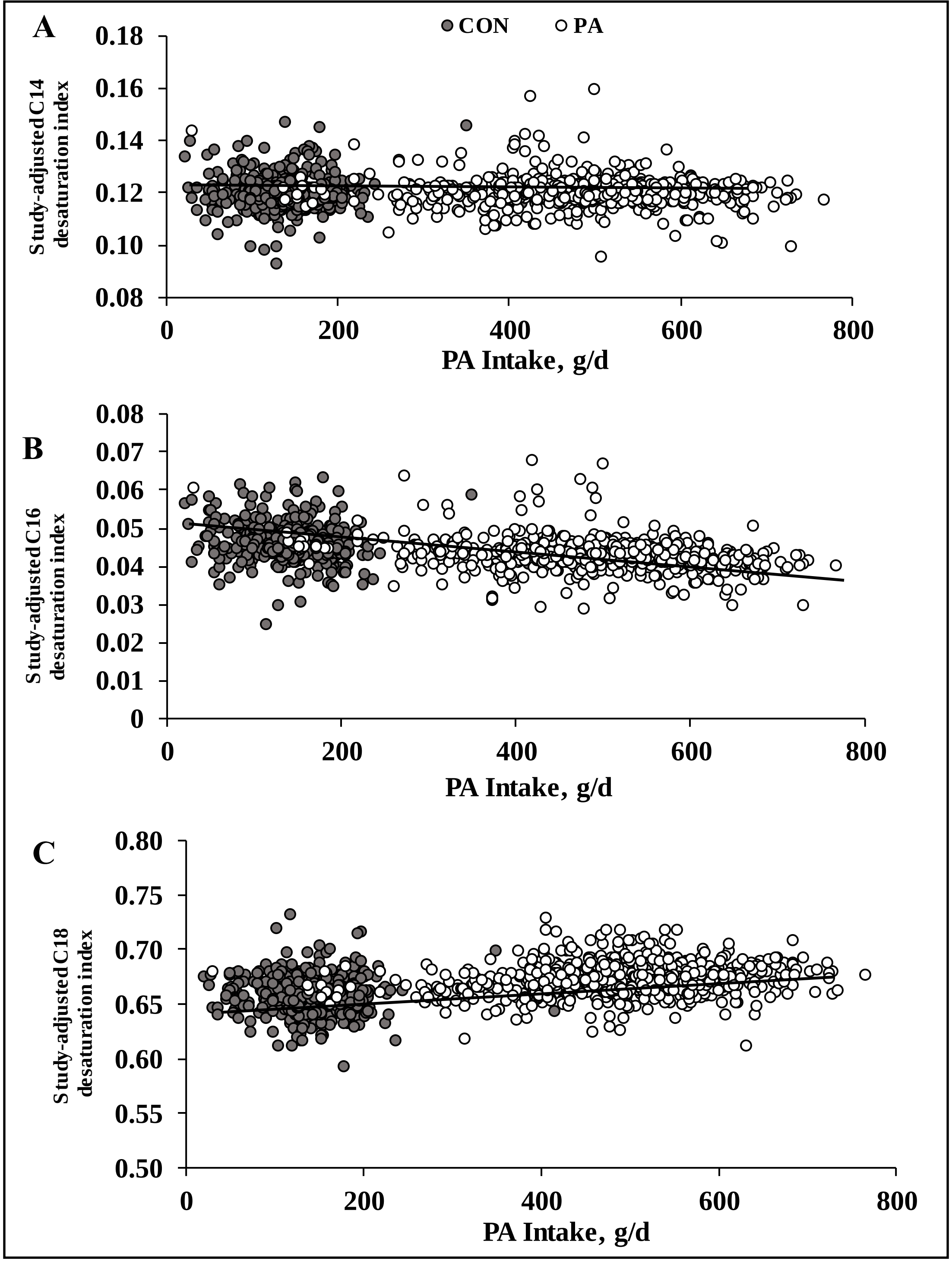


Figure 3.6. Meta-regression evaluating the effect of increasing dietary palmitic acid (PA) intake (g/d) on the C14 desaturation index (A), C16 desaturation index (B), and C18 desaturation index (C). Treatments diets were a control diet (CON) with no supplemental fatty acids (FA) and a diet supplemental with a FA blend containing ≥ 80% PA or a PA-enriched FA supplement containing 80-90% PA. Increasing dietary PA intake had no effect on the C14:0 desaturation index (*P* = 0.98) across all treatment diets, decreased C16 desaturation index (*P* < 0.01), and increased C18 desaturation index (*P* < 0.01).



CHAPTER FOUR:
MAMMARY GLAND RESPONSES TO ALTERING THE DIETARY SUPPLY OF DE NOVO AND PREFORMED FATTY ACIDS: EFFECT ON THE YIELDS OF MILK AND MILK FATTY ACIDS

Abstract

The objective of this study was to evaluate the effect of supplementing different combinations of sodium acetate (NaAc), palmitic acid (PA), and whole cottonseed (WCS) on the yields of milk and milk FA in high producing dairy cows. Thirty-two multiparous Holstein cows (133 ± 57 DIM, 50.5 ± 7.2 kg/d milk) were used in a 4 x 4 Latin Square split plot design with a 2 x 2 factorial arrangement of subplot treatments to determine the interaction among supplementing diets with acetate, PA, and long chain FA. Cows were blocked by milk yield and allocated to a main plot receiving a basal diet (n = 16) with no supplemental PA (Low PA) or a basal diet (n = 16) with 1.5% of a FA supplement containing 85% PA (High PA). In each Latin Square, the following arrangement of treatment diets were fed in 14 d periods: 1) a control diet (CON), 2) the control diet supplemented with 3.0% NaAc (AC), 3) the control diet supplemented with 12% WCS (CS), and 4) the control diet supplemented with 3.0% NaAc and 12% WCS (CS+AC). The PA supplement and NaAc replaced soyhulls, and WCS replaced cottonseed hulls and meal. All diets were balanced for 30.1% neutral detergent fiber (NDF), 23.0% forage NDF, 28.0% starch, and 16.9% crude protein (CP). The statistical model included the random effect of cow nested within basal diet treatment and fixed effect of basal diet, period, NaAc, WCS, and their interactions. Sources of FA were classified as de novo (< 16 carbons), mixed (16-carbon), and preformed (> 16 carbons). Three-way interactions between NaAc, basal diet, and WCS were observed for the yields of milk fat ($P < 0.01$) and FCM ($P = 0.08$) where AC and CS+AC increased the yields of milk fat and FCM in the Low PA diet but CS+AC increased the yields of milk fat and FCM compared to the other treatments in the High PA diet. Three-way interactions were observed for the molar yields of de novo FA ($P = 0.06$), mixed FA ($P = 0.04$), and preformed FA ($P = 0.05$). Compared with Low PA, High PA increased milk fat content ($P < 0.01$), mixed FA yield ($P < 0.01$), and tended to increase C4:0 yield ($P = 0.08$). Diets containing NaAc increased DMI ($P < 0.01$) and the yields of milk fat ($P < 0.01$), ECM ($P < 0.01$), FCM ($P < 0.01$) compared to diets without NaAc. Additionally, diets containing NaAc increased the yields of de novo FA ($P < 0.01$), mixed FA ($P < 0.01$), and preformed FA ($P < 0.01$) compared to diets without NaAc. Diets containing WCS increased milk yield ($P < 0.01$), tended to increase the yields of FCM ($P = 0.06$) and protein ($P = 0.07$), and decreased DMI ($P < 0.01$) compared to diets without WCS. Diets containing WCS increased preformed FA yield ($P < 0.01$) but decreased the yields of de novo FA ($P < 0.01$) and mixed FA ($P < 0.01$) compared to diets without WCS. The results from our study highlight the contribution of FA sources to milk fat synthesis, especially de novo FA. Additionally, our results emphasize the importance of balancing de novo FA and preformed FA supply to the mammary gland to maximize milk fat production in high producing dairy cows.

Introduction

Bovine milk fat is comprised of approximately 98% triglycerides (TG) with fatty acids (FA) originating from de novo or preformed sources (Jensen, 2002). De novo FA (≤ C14) are synthesized in the mammary gland using acetate and beta-hydroxybutyrate produced from ruminal fermentation of dietary carbohydrates, preformed FA (≥ C18) originate from dietary sources or adipose tissue mobilization, and mixed FA (C16) originate from both de novo and preformed sources (MacGibbon and Taylor, 2006). Dietary strategies to increase milk fat yield in dairy cows have primarily focused on increasing the yields of mixed FA and preformed FA supply to the mammary gland through supplemental FA containing palmitic acid (PA), stearic acid (SA), and oleic acid (OA), and oilseeds such as whole cottonseed (WCS) and soybean (Rico et al. 2015; de Souza et al., 2018). Additionally, a recent meta-analysis observed that supplementing diets with PA-enriched supplements increased milk fat yield by increasing mixed FA yield compared to control diets (dos Santos Neto et al., 2021b). Supplementation of PA is of great interest because C16 is the major FA in milk TG and may play a key role in initiation of milk FA synthesis in the mammary gland due to its high affinity for esterification at the *sn*-1 position (Moore and Christie, 1979).

Increasing milk fat yield through de novo FA synthesis can be complex with many dietary and management practices affecting VFA production and FA synthesis (Bauman and Griinari, 2003; Woolpert et al., 2017). Providing acetate as a substrate for FA synthesis increases yield of C4 to 16 FA and consequently milk fat yield (Urrutia and Harvatine, 2017; Urrutia et al., 2019). Interestingly, supplementation of acetate increased the synthesis of C16:0 over C4:0 to C14: (Matamoros et al., 2022) in agreement with Christie and Moore (1979) that suggested C16 is the favored product of de novo FA synthesis with optimal usage of reducing equivalents and substrates.

The regulation of milk fat synthesis occurs at the esterification of FA to the glycerol backbone (Moore and Christie, 1979; Palmquist, 2006), therefore a balance of de novo FA and preformed FA supply to the mammary gland is needed for maintenance of milk fat fluidity. Glasser et al. (2008a) observed that yields of C4 to C16 FA were positively related to C18 yield in milk fat and proposed an interdependent relationship between de novo FA and preformed FA where the two sources to a certain extent drive the synthesis of one another. In agreement, Gresti et al. (1993) quantified FA composition of TG from bovine milk fat and observed that TG containing a mix of short or medium chain FA and long chain FA were preferentially synthesized over TG containing only short, medium, or long chain FA. Our hypothesis was that NaAc, PA, and WCS would increase milk fat yield compared to a control diet, whereas, with the greatest increase in milk fat yield will be achieved by feeding a combination of NaAc, PA, and WCS that increases the supply and improves the balance of FA available in the mammary gland for TG synthesis. Therefore, the objective of our study was to evaluate the effects and interactions of supplementing different combinations of NaAc, PA, and WCS on production responses and milk FA synthesis.

Materials and Methods

Study Design and Treatment Diets

All experimental procedures were approved by the Institutional Animal and Care Use Committee at Michigan State University, East Lansing. Thirty-two mid lactation multiparous Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center were assigned randomly to a treatment sequence in a 4 x 4 Latin Square split plot design with a 2 x 2 factorial arrangement of subplot treatments. All cows received a common diet during a 14-d preliminary period followed by four 14-d treatment periods balanced for carryover effects. Cows were assigned to a main plot with 16 cows receiving a diet with no supplemental PA (Low PA) and 16 cows receiving a diet with 1.5% of a PA-enriched supplement containing 85% PA (High PA) replacing soyhulls in the Low PA diet. The average starting production was (mean ± SD) 50.9 ± 7.1 and 50.0 ± 6.8 kg/d milk, 1.64 ± 0.27 and 1.73 ± 0.23 kg/d milk fat, and 1.51 ± 0.20 and 1.53 ± 0.16 kg/d milk protein for Low PA and High PA, respectively. Within each basal diet, the treatments in the Latin square were: 1) a control diet (CON), 2) the control diet supplemented with 3.0% NaAc (AC)(Wausau Chemical Cooperation; Wausau, WI) to target approximately 10 moles of acetate per day, 3) the control diet supplemented with 12% WCS (CS) to increase dietary FA content by 2.2% , and 4) the control diet supplemented with 3.0%NaAc and 12% WCS (CS+AC). The NaAc replaced 3% soyhulls, and WCS replaced a mixture of ~8% cottonseed hulls and ~4% cottonseed meal to balance fiber and protein content. The inclusion of WCS was based on a dose response study conducted in our lab in which 8% and 16% WCS yielded more milk and milk fat than 0% and 24% WCS (Bales et al., unpublished). In that study, 8% and 16% yielded similar milk fat but more WCS shifted the FA profile toward more preformed FA. We postulated that 12% WCS would be optimal to supply preformed FA. The inclusion of NaAc was based on studies conducted by Urrutia and Harvatine (2017) and Urrutia et al. (2019) who observed infusing 10 mol/d acetate and feeding NaAc to target 10 mol/d acetate increased the yields of milk fat, and specifically those synthesized de novo (C4 to 16).

All experimental diets were formulated to meet the nutrient requirements of high producing dairy cows and balanced for (DM basis) 30.1% neutral detergent fiber (NDF), 23.0% forage NDF, 28.0% starch, and 16.9% crude protein (CP). The DM values of forages were determined twice weekly and used to adjust diets. A base diet containing forages, ground corn, soybean meal, protein mix, and vitamin and mineral mix was mixed in a wagon daily. Then, NaAc, the PA-enriched supplement, WCS, WCS meal, WCS hulls, and soyhulls were added to the base diet in a tumble mixer for each experimental diet. All cows were fed 115% of expected intake at 0800 h daily. Feed access was blocked from 0600 to 0800 h for orts collection and offering of new feed. Cows were housed in individual tie-stalls and water was available ab libitum.

Data and Sample Collection

The study was designed to have four 14-d treatment periods, and production data and samples were collected during the last 4 d of each treatment period. Diet ingredients (~0.5 kg) and orts samples (~12.5%) were collected daily and composited by period and cow for further analysis. Milk yield and intake were recorded daily. Two milk samples were collected at each milking; one aliquot was collected in a tube with preservative and stored at 4ºC for milk component analysis, and the second was collected and stored at -20ºC for milk FA analysis. Blood samples were collected every 8 h and centrifuged at 3,000 x *g* for 15 min at 4ºC for plasma extraction. Fecal samples were collected every 8 h and composited by cow and period. Body weight (BW) was measured twice during each collection period and body condition score (BCS) was recorded by 3 trained investigators according to Wildman et al. (1982).

Sample Analysis

Feed ingredients and orts samples were dried for 72 h at 55 ºC in a forced-air oven to determine DM. The samples were ground in a Wiley mill (1 mm screen; Arthur H. Thomas, Philadelphia, PA), and analyzed for NDF, 240-h indigestible NDF, starch, CP, and FA profile according to Boerman et al. (2017). Milk samples were analyzed for fat, true protein, lactose, and milk urea nitrogen (MUN) by mid-infrared spectroscopy (AOAC, 1990, method 972.160; Central Star DHI, Grand Ledge, MI). Yields of 3.5% FCM (FCM), energy-corrected milk (ECM), and milk components were calculated from each milking using milk yield and component concentration, summed for a total daily yield, and averaged for the treatment periods. Milk samples used for FA analysis were composited by period and cow based on milk fat yield. Then, milk lipids were extracted, and FA methyl esters were prepared and analyzed by gas chromatography as described by Lock et al. (2013). Individual FA yield on a mass basis (g/d) and molar basis (mol/d) were calculated using milk fat yield, FA concentration, and molecular weight of each FA correcting for glycerol content and other milk lipids (Piantoni et al., 2013; see Table 3.7).

Statistical Analysis

All data were analyzed using PROC GLIMMIX in SAS (version 9.4, SAS Institute, Cary, NC) using the following linear model:

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

where Y_{ijkl} = the dependent variable, μ = overall mean, $C(B)_{i(j)}$ = random effect of cow nested in basal diet (i = 1 to 16), P_k = fixed effect of period (k = 1 to 4), B_j = fixed effect of basal diet (j = 1 to 2), A_l = fixed effect of NaAc (l = 1 to 2), W_m = fixed effect of WCS (m = 1 to 2), $A_l \times W_m$ = the interaction of NaAc and WCS, $B_j \times A_l$ = interaction between basal diet and NaAc, $B_j \times W_m$ = interaction between basal diet and WCS, $B_j \times A_l \times W_m$ = the interaction of basal diet, NaAc, and WCS, and e_{ijklm} = the residual error. Main effects were declared significant at $P \leq 0.05$ and tendencies $P \leq 0.10$, and interactions were declared significant at $P \leq 0.10$ and tendencies at $P \leq 0.15$.

Results

Production Responses: Three-Way Interaction Among Basal Diet, NaAc, and WCS

Nutrient composition are reported in Table 4.1, and DMI and production responses are reported in Table 4.2. A three-way interaction was observed for the yields of milk fat ($P < 0.01$) and 3.5% fat-corrected milk ($P = 0.08$), milk fat content ($P = 0.04$), and a tendency for an interaction was observed for energy-corrected milk yield ($P = 0.14$) and MUN ($P = 0.12$). In the Low PA diets, AC and CS+AC increased the yields of milk fat and FCM compared to CON and CS, whereas, in the High PA diets, CS+AC increased the yields of milk fat and FCM compared to the other treatments and AC increased milk fat yield compared to CON and CS (Figure 4.2; 4.3). In the Low PA diets, AC increased milk fat content compared to the other treatments and

CS+AC increased milk fat content compared to CON and CS. In the High PA diets, AC and CS+AC increased milk fat content compared to CON and CS (Figure 4.1).

Production Responses: Two-Way Interaction Between NaAc and WCS

Variables with a two-way interaction between NaAc and WCS with no three-way interaction among basal diet, NaAc, and WCS are reported here. A two-way interaction was observed for DMI ($P = 0.03$) and a tendency for an interaction was observed for the yields of milk ($P = 0.11$), protein ($P = 0.14$), and lactose ($P = 0.13$). Compared to CON and AC, CS and CS+AC decreased DMI but CS+AC increased DMI compared to CS.

Production Responses: Main Effect of Basal Diet, NaAc, and WCS

Compared to the Low PA diet, High PA increased the content of milk fat ($P < 0.01$) and MUN ($P = 0.01$). Diets supplemented with NaAc increased DMI ($P < 0.01$) compared to diets without NaAc. Supplementation with NaAc increased the yields of milk fat ($P < 0.01$), ECM ($P < 0.01$), and FCM ($P < 0.01$) but had no effect on the yields of milk ($P = 0.53$), protein ($P = 0.87$) or lactose ($P = 0.44$) compared to diets without NaAc. Diets containing NaAc increased the contents of milk fat ($P < 0.01$) and lactose ($P < 0.01$), decreased MUN ($P < 0.01$), and had no effect on milk protein content ($P = 0.69$) compared to diets without NaAc. Diets containing WCS decreased DMI ($P < 0.01$) compared to diets without WCS. Diets containing WCS increased the yields of milk ($P < 0.01$) and lactose ($P = 0.02$) and tended to increase the yields of FCM ($P = 0.06$) and protein ($P = 0.07$) compared to diets without WCS. Diets containing WCS had no effect on the yields of milk fat ($P = 0.42$) or ECM ($P = 0.18$). Diets containing WCS had no effect on the contents of milk fat ($P = 0.24$), protein ($P = 0.11$), lactose ($P = 0.99$), or MUN ($P = 0.33$). There were no effects of treatments or interactions observed for BW, BW change, BCS, or BCS change ($P > 0.15$).

Milk FA Responses: Three-Way Interaction Among Basal Diet, NaAc, and WCS

Sources of FA were classified as de novo (< 16 carbons), mixed (16-carbon), and preformed (> 16 carbons). The following results are reported on a molar basis (mol/d) as presented in Table 4.3. The yield of individual FA and sources on a mass basis (g/d) are presented in Table 4.4. A three-way interaction among basal diet, NaAc, and WCS was observed for the yields of de novo FA ($P = 0.06$), mixed FA ($P = 0.04$), and preformed FA ($P = 0.05$). In the Low PA diets, AC increased de novo FA yield compared to the other treatments and CS decreased de novo FA yield compared to the other treatments. In the High PA, CS decreased de novo FA yield compared to the other treatments but AC and CS+AC had no effect compared to CON (Figure 4.4). In the Low PA diets, AC increased mixed FA yield compared to the other treatments, CS+AC increased mixed FA yield compared to CON and CS, and CS decreased mixed FA yield compared to the other treatments. In the High PA diets, AC and CS+AC increased mixed FA yield compared to CON and CS, whereas, CS decreased mixed FA yield compared to CON (Figure 4.5). In the Low PA diets, CS and CS+AC increased preformed FA yield compared to CON and AC, whereas, in the High PA diets CS+AC increased preformed FA yield compared to the other treatments and CS increased preformed FA yield compared to CON and AC (Figure 4.6). A three-way interaction was observed for the yields of C4:0 ($P = 0.04$), C6:0 ($P < 0.01$), C8:0 ($P < 0.01$), C16:0 ($P < 0.01$), *cis*-9 C16:1 ($P = 0.03$), and C18:0 ($P = 0.03$), and a tendency for an interaction for C14:0 ($P = 0.12$). The AC and CS+AC treatments increased C4:0 yield compared to CON and CS to a greater magnitude in the High PA diets compared to the Low PA diets. In the Low PA diets, AC increased C16:0 yield compared to the other treatments and CS+AC increased C16:0 yield compared to CON. In the High PA diets, AC and CS+AC increased C16:0 yield compared to CON and CS, whereas, CS decreased C16:0 yield compared to CON. In the Low PA diets, CS and CS+AC increased C18:0 yield compared to CON and AC and AC increased C18:0 yield compared to CON. In the High PA diets, CS+AC increased C18:0 yield compared to the other treatments, CS increased C18:0 yield compared to CON and AC, and AC increased C18:0 yield compared to CON.

Milk FA Responses: Two-Way Interaction Between NaAc and WCS

Variables with a two-way interaction between NaAc and WCS with no three-way interaction among basal diet, NaAc, and WCS are reported here. A two-way interaction between NaAc and WCS was observed for the yields of C10:0 ($P = 0.02$), C12:0 ($P = 0.08$), and *cis*-9 C14:1 ($P = 0.07$). The CS+AC treatment decreased the yields of C10:0 and C12:0 compared to CON and AC, and CS decreased the yields of C10:0 and C12:0 compared to the other treatments. Compared to CON and AC, CS and CS+AC decreased *cis*-9 C14:1 yield.

Milk FA Responses: Main Effect of Basal Diet, Acetate, and WCS

Compared to Low PA, High PA increased the yields of mixed FA ($P < 0.01$) and tended to increase C4:0 yield ($P = 0.08$). Supplementation of PA had no effect on the yields of de novo FA ($P = 0.89$) or preformed FA ($P = 0.90$). Diets containing NaAc increased the yields of de novo FA ($P < 0.01$), mixed FA ($P < 0.01$), and preformed FA ($P = 0.03$) compared to diets without NaAc. Diets containing NaAc increased the yields of FA from C4:0 to C16:0 ($P < 0.01$) and C18:0 ($P < 0.01$) and tended to increase *cis*-9 C18:1 yield ($P = 0.07$) compared to diets without NaAc. Diets containing WCS increased preformed FA yield ($P < 0.01$) but decreased the yields of de novo FA ($P < 0.01$) and mixed FA ($P < 0.01$) compared to diets without WCS. Diets containing WCS decreased the yields of FA C6:0 to C16:0 ($P < 0.01$) but increased the yields of all 18-carbon FA ($P < 0.01$) except *cis*-9,12 C18:2 and *cis*-9,12,15 C18:3 compared to diets without WCS.

Discussion

Supplementing diets with NaAc, PA, and WCS in dairy cows has been previously evaluated either independently or in combinations although interactions between all three have yet to be explored. Urrutia et al. (2019) and Matamoros et al. (2022) fed NaAc to mid-lactation dairy cows and observed that NaAc increased milk fat yield by increasing the yields of de novo and mixed FA. In addition, supplementing diets with PA consistently increases milk fat yield by increasing mixed FA yield while effects on the yields of de novo FA and preformed FA are more variable (de Souza and Lock, 2018; dos Santos Neto et al., 2021b). Rico et al. (2017) observed a tendency for an interaction between basal diet and PA dose for milk fat yield where increasing dietary inclusion of PA quadratically increased milk fat yield in diets containing soyhulls (3.30% diet DM FA), but cubically increased milk fat yield in diets containing WCS (4.58% diet DM FA) suggesting a balance of FA is important to enhance milk fat synthesis. To our knowledge, no studies in the literature have evaluated the interactions between supplementing NaAc, PA, and WCS on milk FA yields. Based on the proposed interdependent relationship between de novo FA and preformed FA (Glasser et al., 2008a), we hypothesized that diets that enhance de novo FA synthesis and increase preformed FA supply to the mammary gland would maximize milk fat yield by balancing the incorporation of short and long chain FA into milk TG. Improving the understanding of the relationship between de novo FA and preformed FA will allow for development of dietary strategies that increase milk fat yield beyond simply supplementing diets with FA or oilseeds.

We observed a three-way interaction between NaAc, PA, and WCS on the yields of milk fat, de novo FA, mixed FA, and preformed FA emphasizing the importance of balancing the supply of FA to the mammary gland. Additionally, we observed a three-way interaction for major milk FA for TG formation such as C4:0, C16:0, and C18:0 highlighting the relationship between dietary FA supplementation and milk FA profile, and the high affinity of FA to specific positions on milk TG. Supplementation of acetate provides carbon units and reducing equivalents for synthesis of de novo FA in the mammary gland (Moore and Christie, 1979) and supplemental preformed FA increases the supply of 16-carbon and 18-carbon FA to the mammary gland. In agreement with our hypothesis, diets containing only NaAc and diets containing only PA increased milk fat yield, whereas, diets containing NaAc, PA, and WCS to enhance the synthesis of de novo FA and preformed FA led to the greatest increase in milk fat yield. Importantly, diets containing NaAc and WCS increased the yields of milk fat by increasing all sources of FA compared to diets with only WCS. This supports the theory that de novo FA are likely the limiting factor during milk TG synthesis when the supply of long chain FA is increased (Barbano and Sherbon, 1980; Glasser et al. 2008a).

Milk fat synthesis is highly regulated with individual FA being preferentially located on specific positions on the TG. Long chain FA and saturated FA (SFA) are predominately located at the *sn*-1 and *sn*-2 position while short chain FA and unsaturated FA (UFA) are predominately located at the *sn*-3 position of the TG (Jensen, 2002). The esterification of short chain FA and UFA at the *sn*-3 position is important for maintenance of milk fat fluidity and may be a key regulatory step for milk TG synthesis (Barbano and Sherbon, 1980; Enjalbert et al., 1998). Moore and Christie (1979) proposed that C16 plays an important role in initiation of TG synthesis as palmitoyl-CoA is the preferred substrate for acylation of *sn*-glycerol-3-phosphate. The control diet in High PA yielded similar milk fat yield to AC and CS+AC in the Low PA diet highlighting the important contribution of C16 for milk fat synthesis. Additionally, in agreement with Matamoros et al. (2022), supplementation of NaAc increased mixed FA yield to a greater extent than de novo FA yield suggesting C16 is the preferred product of de novo FA synthesis when acetate supply is increased (Moore and Christie, 1979).

The relationship between the incorporation of de novo FA and preformed FA into TG is not well understood. Dorea and Armentano (2017) observed that supplementing diets with unsaturated FA decreased de novo FA synthesis at high levels, but these decreases were compensated by an increase in long chain FA supply to the mammary gland. These findings represent a substitution of preformed FA for de novo FA when the supply of preformed FA is increased or when the mammary gland is experiencing milk fat depression. On the contrary, Glasser at al. (2008a) proposed an interdependent relationship between de novo FA and preformed FA where a balance of both sources is needed to increase milk fat yield. In our current study, supplementation of NaAc increased de novo FA yield in the Low PA diets but had no effect in the High PA diets. This was presumably due to substitution of preformed C16 FA for de novo FA, or NaAc driving the synthesis of C16 FA over C4 to C14 as shown in Figure 4.5. Enjalbert et al. (1998) and Dorea and Armentano (2017) observed dietary PA accounted for approximately 20% of total C16 in milk fat when a control diet was fed, and the contribution of C16 increased to 60% in milk fat when the dietary supply of PA increased. Tzompa-Sosa et al. (2014) observed that increasing the molar content of C16 in TG mediated the positioning and amount of other FA in TG with increasing C16 negatively correlated with the amount of C6:0 to C10:0 at the *sn*-2 position and C14:0 at the *sn*-1 position of TG. Substitution of preformed FA for de novo FA and mixed FA likely occurred in diets containing WCS. Presumably, the mammary gland was using available long chain FA for TG formation rather than synthesizing de novo FA, whereas the addition of NaAc and PA in diets containing WCS improved the yields of all milk FA sources. Likely, substitution of FA occurs less when the balance of short and long chain FA is improved in the mammary gland because of the high demand of all available FA for increased TG synthesis, thus representing a more interdependent relationship between sources of milk fat. Although stereospecific distribution of FA in TG was not evaluated in the current study, we hypothesize that supplementation of PA increased the esterification of C16:0 at the *sn*-1 position increasing the presence of medium and long chain FA at the *sn*-2 position of TG. As previously mentioned, balancing the supply of de novo FA and preformed FA increased overall TG synthesis, therefore, decreasing the competition and substitution between FA on the *sn*-1 and *sn*-2 position of the TG. Additionally, our results highlight the importance of short chain FA for maintenance of milk fat fluidity as supplementation of PA increased C4:0 yield at the *sn*-3 position to complete TG synthesis (Jensen, 2002). In agreement, Hansen and Knudsen (1987b) observed that PA increased the synthesis of C4:0 in mammary epithelial cells.

Expressing the yields of FA on a molar basis provides a more mechanistic approach to understanding milk fat synthesis than does expressing them on a mass basis, which overestimates the contribution of preformed FA and underestimates the contribution of de novo FA to milk fat (Enjalbert et al., 1998). When evaluating the relative concentration of FA sources on a molar basis and mass basis, we observed similar results where NaAc increased the concentrations of de novo FA and mixed FA and WCS increased preformed FA, but the overall contribution of FA sources to milk fat differs between the two methods (Figure 4.7; 4.8). The concentration of de novo FA in milk fat from cows fed the control diet is 10 percentage units higher whereas mixed FA and preformed FA contribution is 5 percentage units lower when expressed on a molar basis compared to a mass basis. Thus, expressing FA on a molar basis helps demonstrate the key role for de novo FA synthesis in milk fat production and the incorporation of preformed FA into milk fat. I believe that dietary strategies to enhance de novo FA synthesis should receive more emphasis.

For our study, using a split plot was the most effective and practical design to evaluate the interaction between the treatment diets compared to using a factorial design that required more experimental units and treatment periods. Although diets containing supplemental PA were only fed to 16 cows, the positive effect of PA on the yields of milk fat and mixed FA has been consistently observed in the literature as summarized in our recent meta-analysis (dos Santo Neto et al., 2021b), therefore, we manipulated the supply of PA in the basal diets and supplemented NaAc and WCS within the Latin square. The WCS replaced a mixture of WCS hulls and meal to balance the content of fiber and protein allowing the supply of FA in the oilseed to be the primary difference between the diets.

Acetate was delivered in the form of NaAc to target 10 mol/d of acetate, but the actual intake of acetate was 12 and 11.5 mol/d for AC and CS+AC, respectively. The NaAc replaced soyhulls in the control diet due to the lack of an ideal sodium control supplement that balances both increases in DCAD and rumen buffering capacity (Matamoros et al., 2021), and effects of sodium chloride and sodium bicarbonate (NaHCO) on production performance are inconsistent in the literature. Milk fat depression occurs in dairy cows when diets cause decreases in rumen pH or contain high levels of polyunsaturated FA that increase the presence of alternative biohydrogenation intermediates, and dietary buffers are often supplemented to alleviate decreases in rumen pH (Bauman et al., 2011). Kalscheur et al. (1997) observed feeding NaHCO in low forage diets increased rumen pH and decreased *trans*-C18:1 FA in milk. Urrutia et al. (2019) and Matamoros et al. (2022) observed NaAc decreased *trans*-10 C18:1 FA content in milk, although the decrease in *trans*-10 C18:1 FA accounted for a 0.8-1.3% increase in milk fat content, therefore they attributed increases in the yields of milk fat and de novo FA to increased acetate supply. In the current study, acetate supplementation decreased *trans*-10 C18:1 FA yield, although low levels were detected in milk fat (0.35 g/100 g FA) indicating cows were not at risk for diet-induced milk fat depression, and likely the effects of NaAc on milk FA yields were due to increases in acetate supply.

Conclusion

To our knowledge, no studies in literature have evaluated the interactions between supplementing diets with NaAc, PA, and WCS to evaluate the relationship between enhancing all sources of milk fat. In agreement with our hypothesis, diets supplemented with NaAc, PA, and WCS increased milk fat yield to greater extent than diets supplemented to target one FA source emphasizing the importance of balancing the supply of de novo FA and preformed FA to the mammary gland for maximization of milk fat synthesis. Evaluating the contribution of FA to milk fat yield on a molar basis highlighted the importance of de novo FA and their role in TG synthesis. Dietary strategies that enhance de novo FA synthesis and preformed FA supply to the mammary gland are important for increasing TG synthesis and milk fat yield.

Tables and Figures

Table 4.1. Ingredient and nutrient composition of treatment diets[1].

	Low PA[2]				High PA[2]			
Ingredient, % DM	CON	AC	CS	CS+AC	CON	AC	CS	CS+AC
Corn Silage	37.3	37.3	37.3	37.3	37.3	37.3	37.3	37.3
Alfalfa Silage	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Corn Grain	18.9	18.9	18.9	18.9	18.9	18.9	18.9	18.9
Soybean Meal	8.50	8.50	8.50	8.50	8.50	8.50	8.50	8.50
Protein Mix[3]	6.33	6.33	6.33	6.33	6.33	6.33	6.33	6.33
Min/Vit Mix[4]	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08
Ion Mix[5]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Soyhulls	7.33	4.33	7.33	4.33	5.80	2.80	5.80	2.80
Sodium Acetate[6]	--	3.00	--	3.00	--	3.00	--	3.00
PA-enriched Supplement[7]	--	--	--	--	1.50	1.50	1.50	1.50
Whole Cottonseed	--	--	12.0	12.0	--	--	12.0	12.0
Cottonseed Hulls	7.80	7.80	--	--	7.80	7.80	--	--
Cottonseed Meal	4.20	4.20	--	--	4.20	4.20	--	--
Item, % DM								
CP	16.4	16.1	16.9	16.5	16.3	16.0	16.7	16.3
aNDFom	32.1	30.1	29.6	27.3	31.1	29.1	28.5	26.3
Starch	28.1	28.1	28.3	28.5	28.1	28.1	28.2	28.5
Total FA	2.04	2.01	3.49	3.40	3.35	3.32	4.89	4.72
C16:0	0.35	0.34	0.66	0.64	1.54	1.53	1.93	1.85
C18:0	0.07	0.06	0.10	0.10	0.13	0.12	0.16	0.16
C18:1	0.40	0.39	0.63	0.61	0.43	0.43	0.66	0.64
C18:2	1.07	1.06	1.92	1.87	1.07	1.06	1.92	1.86
C18:3	0.11	0.11	0.12	0.12	0.11	0.11	0.12	0.11

[1] CON= diet with no supplemental fatty acids (FA), AC = diet with 3% DM sodium acetate (NaAc), CS = diet with 12% DM whole cottonseed (WCS), and CS+AC = diet containing 3% DM NaAc and 12% DM WCS.

[2] Low PA = diet with no supplemental FA and High PA = diet with a FA supplement containing 85% palmitic acid (PA).

[3] Contains 41.3% Amino Plus, 11.9% calcium carbonate, 18.6% Caledonia Pass, 10.2% corn grain, 1.04% Smartamine M, 14.6% sodium sesquinate, and 2.45% urea.

[4] Contains 20.4% calcium carbonate, 12.5% calcium phosphate, 4.47% sodium sesquinate, 14.1% white salt, 15.7% Min AD, 0.03% vitamin A, 0.01% Vitamin 500, 0.34% vitamin E, 14.7% corn grain, 0.92% Energizer Tallow, 0.51% Micro 5, 2.36% CFE Selenium, 0.15% magnesium oxide, and 4.70% magnesium sulfate.

[5] Contains 56.03% potassium and 0.32% sodium.

[6] Wausau Chemical Cooperation; Wausau, WI

[7] Pro Palm 85, Perdue-Agribusiness, Salisbury, MD. Supplement contained approximately (g/100g FA) 87.1% C16:0, 1.0% C18:0, 6.4% *cis*-9 C18:1, and 1.5% *cis*-9,12 C18:2

Table 4.2. Milk production responses and dry matter intake (DMI) for cows fed treatment diets.

	Treatment[1]					Basal diet[2]			*P*-value[3]				
Item	CON	AC	CS	CS+AC	SEM	Low PA	High PA	SEM	Basal diet	NaAc[4]	WCS[5]	NaAc × WCS	Basal diet × NaAc × WCS
DMI, kg/d	32.5^{a}	32.7^{a}	30.3^{c}	31.5^{b}	0.59	32.1	31.4	0.78	0.57	<0.01	<0.01	0.03	0.59
Yield, kg/d													
Milk	47.7	47.4	48.1	48.9	1.30	48.8	47.2	1.79	0.52	0.53	<0.01	0.11	0.75
ECM	50.2^{c}	51.7^{b}	49.9^{c}	52.8^{a}	1.23	51.0	51.4	1.69	0.87	<0.01	0.18	0.03	0.14
3.5% FCM	50.0^{c}	51.8^{b}	50.0^{c}	53.1^{a}	1.26	50.7	51.8	1.73	0.64	<0.01	0.06	0.05	0.08
Fat	1.81	1.94	1.80	1.97	0.27	1.83	1.93	0.07	0.27	<0.01	0.42	0.19	<0.01
Protein	1.53	1.52	1.54	1.56	0.04	1.58	1.50	0.05	0.30	0.87	0.07	0.14	0.32
Lactose	2.34	2.33	2.36	2.40	0.07	2.40	2.32	0.10	0.58	0.44	0.02	0.13	0.66
Component, %													
Fat	3.83	4.13	3.84	4.04	0.08	3.80	4.20	0.10	<0.01	<0.01	0.24	0.12	0.04
Protein	3.24	3.23	3.22	3.21	0.05	3.23	3.22	0.06	0.88	0.69	0.11	0.87	0.59
Lactose	4.88	4.92	4.89	4.92	0.02	4.89	4.92	0.03	0.47	<0.01	0.99	0.46	0.35
MUN, mg/dL	11.74	10.95	11.77	11.12	0.19	10.94	11.85	0.24	0.01	<0.01	0.33	0.51	0.12
Milk/DMI	1.48	1.46	1.58	1.57	0.03	1.54	1.51	0.05	0.59	0.14	<0.01	0.58	0.28
ECM/DMI	1.54	1.57	1.65	1.70	0.02	1.59	1.64	0.03	0.33	<0.01	<0.01	0.32	0.82
3.5% FCM/DMI	1.54	1.58	1.65	1.71	0.03	1.59	1.65	0.03	0.16	<0.01	<0.01	0.45	0.25
BW, kg	735	733	731	735	11.3	727	740	15.8	0.56	0.63	0.60	0.21	0.94
BW change[6]	12.4	8.08	9.01	7.00	2.21	8.98	9.26	1.56	0.90	0.15	0.31	0.60	0.68
BCS	3.07	3.06	3.05	3.08	0.05	3.00	3.12	0.07	0.23	0.57	0.99	0.19	0.87
BCS change[6]	0.03	-0.03	0.01	0.02	0.02	-0.01	0.02	0.01	0.16	0.22	0.47	0.16	0.26

$^{a-d}$Means in a row with different superscripts differ ($P < 0.05$). Conducted only if interaction $P < 0.10$.

[1] CON= diet with no supplemental fatty acids (FA), AC = diet with 3% DM sodium acetate (NaAc), CS = diet with 12% DM whole cottonseed (WCS), and CS+AC = diet containing 3% DM NaAc and 12% DM WCS.

[2] Low PA = diet with no supplemental FA and High PA = diet with a FA supplement containing 85% palmitic acid (PA).

[3] *P*-values refer to results from ANOVA for main effects of basal diet, NaAc, and WCS, the interaction between NaAc and WCS, and the interaction between basal diet, NaAc, and WCS.

[4] NaAc = diets containing 3% NaAc compared to diets with no NaAc.

[5] WCS = diets containing 12% WCS compared to diets with no WCS

[6] Change per treatment period.

Table 4.3. Summation by source and individual fatty acid (FA) yield measured in moles per day for cows fed treatment diets.

	Treatment[1]					Basal diet[2]			*P*-value[3]				
Item	CON	AC	CS	CS+AC	SEM	Low PA	High PA	SEM	Basal diet	NaAc[4]	WCS[5]	NaAc × WCS	Basal diet × NaAc × WCS
Source[6], mmol/d													
De Novo	2657^{b}	2784^{a}	2389^{c}	2635^{b}	75	2607	2626	100	0.89	<0.01	<0.01	0.03	0.06
Mixed	2645^{c}	2921^{a}	2441^{d}	2812^{b}	80	2483	2926	107	<0.01	<0.01	<0.01	0.10	0.04
Preformed	1983	2045	2313	2392	56	2190	2179	73	0.90	<0.01	<0.01	0.72	0.05
FA, mmol/d													
C4:0	547	615	547	620	18	550	614	24	0.07	<0.01	0.76	0.78	0.04
C6:0	297	325	278	310	9	294	311	12	0.35	<0.01	<0.01	0.52	<0.01
C8:0	147	156	133	145	5	145	146	6	0.89	<0.01	<0.01	0.57	<0.01
C10:0	335^{a}	336^{a}	287^{c}	306^{b}	11	323	309	14	0.50	0.02	<0.01	0.02	0.19
C12:0	348^{a}	349^{a}	288^{c}	304^{b}	11	331	314	15	0.42	0.06	<0.01	0.08	0.37
C14:0	897^{b}	933^{a}	811^{c}	892^{b}	26	904	863	35	0.41	<0.01	<0.01	0.01	0.12
cis-9 C14:1	74^{a}	73^{a}	57^{b}	59^{b}	3	69	63	4	0.28	0.54	<0.01	0.07	0.85
C16:0	2518^{b}	2767^{a}	2370^{c}	2700^{a}	75	2378	2799	102	<0.01	<0.01	<0.01	0.10	<0.01
cis-9 C16:1	127	129	106	111	5	114	122	7	0.41	0.01	<0.01	0.32	0.03
C18:0	460	516	637	688	20	561	589	25	0.44	<0.01	<0.01	0.79	0.03
trans-9 C18:1	11	11	15	15	0.40	13	12	0.5	0.25	0.23	<0.01	0.38	0.29
trans-10 C18:1	26	22	31	27	0.18	29	24	0.2	0.18	<0.01	<0.01	0.26	0.52
trans-11 C18:1	41^{c}	49^{b}	57^{a}	59^{a}	0.24	56	48	0.3	0.08	<0.01	<0.01	0.07	0.16
trans-12 C18:1	21	21	31	31	1.0	29	24	1	0.03	0.21	<0.01	0.15	0.34
cis-9 C18:1	913	924	1015	1048	27	961	988	34	0.59	0.07	<0.01	0.34	0.27
cis-11 C18:1	44	43	46	47	2	48	42	2	0.06	0.77	<0.01	0.35	0.21
cis-12 C18:1	30	29	42	40	2	38	33	2	0.05	0.09	<0.01	0.33	0.66
cis-13 C18:1	5	5	6	6	0.22	6	5	0.3	0.32	0.07	<0.01	0.28	0.19
cis-9,12 C18:2	170	171	162	165	4.1	167	167	5	0.93	0.16	<0.01	0.55	0.27
cis-9,12,15 C18:3	18^{a}	18^{a}	15^{b}	15^{b}	0.43	17	16	0.5	0.22	0.88	<0.01	0.07	0.15

$^{a-d}$Means in a row with different superscripts differ ($P < 0.05$). Conducted only if interaction $P < 0.10$.

[1] CON= diet with no supplemental fatty acids (FA), AC = diet with 3% DM sodium acetate (NaAc), CS = diet with 12% DM whole cottonseed (WCS), and CS+AC = diet containing 3% DM NaAc and 12% DM WCS.

[2] Low PA = diet with no supplemental FA and High PA = diet with a FA supplement containing 85% palmitic acid (PA).

[3] *P*-values refer to results from ANOVA for main effects of basal diet, NaAc, and WCS, the interaction between NaAc and WCS, and the interaction between basal diet, NaAc, and WCS.

[4] NaAc= diets containing 3% NaAc compared to diets with no NaAc.

[5] WCS = diets containing 12% WCS compared to diets with no WCS

[6] FA sources determined as de novo < 16 carbons, mixed = 16-carbon, and preformed = > 16 carbons.

Table 4.4. Summation by source and individual fatty acid (FA) yield measured in grams per day for cows fed treatment diets.

	Treatment[1]					Basal diet[2]			*P*-value[3]				
Item	CON	AC	CS	CS+AC	SEM	Low PA	High PA	SEM	Basal diet	NaAc[4]	WCS[5]	NaAc × WCS	Basal Diet × NaAc × WCS
Source, g/d[6]													
De Novo	455^{b}	471^{a}	405^{c}	443^{b}	13.1	447	440	17.6	0.76	<0.01	<0.01	0.02	0.06
Mixed	678^{c}	743^{a}	635^{d}	721^{b}	20.3	639	749	27.6	<0.01	<0.01	<0.01	0.10	<0.01
Preformed	565	577	667	682	15.4	623	623	20.4	0.99	0.03	<0.01	0.85	0.30
FA, g/d													
C4:0	48.2	54.6	48.2	54.6	1.61	48.7	54.1	2.10	0.08	<0.01	0.99	0.96	0.01
C6:0	34.5	37.7	32.3	36.0	1.08	34.2	36.1	1.43	0.35	<0.01	<0.01	0.52	<0.01
C8:0	21.5	22.6	19.3	20.9	0.67	20.9	21.2	0.88	0.77	<0.01	<0.01	0.33	0.04
C10:0	57.4^{a}	58.2^{a}	49.4^{c}	52.7^{b}	1.90	55.6	53.2	2.53	0.51	<0.01	<0.01	0.09	0.07
C12:0	69.7^{a}	69.9^{a}	57.7^{c}	61.0^{b}	2.29	66.3	62.8	3.03	0.42	0.06	<0.01	0.08	0.37
C14:0	205^{b}	213^{a}	185^{c}	204^{b}	5.89	206	197	7.94	0.41	<0.01	<0.01	0.01	0.12
cis-9 C14:1	16.8^{a}	16.6^{a}	12.9^{b}	13.4^{b}	0.69	15.6	14.2	0.94	0.28	<0.01	<0.01	0.07	0.85
C16:0	646^{b}	710^{a}	608^{c}	692^{a}	19.3	610	718	26.2	<0.01	<0.01	<0.01	0.10	<0.01
cis-9 C16:1	32.4	33.1	27.0	28.2	1.25	29.2	31.3	1.71	0.44	<0.01	<0.01	0.51	0.02
C18:0	131	147	181	196	5.70	160	168	7.25	0.44	<0.01	<0.01	0.79	0.03
trans-9 C18:1	3.06	3.16	4.17	4.19	0.11	3.77	3.53	0.15	0.25	0.23	<0.01	0.38	0.29
trans-10 C18:1	7.44^{b}	5.95^{c}	8.52^{a}	7.62^{b}	0.48	8.17	6.59	0.64	0.09	<0.01	<0.01	0.10	0.99
trans-11 C18:1	11.7^{c}	13.8^{b}	16.1^{a}	16.8^{a}	0.69	15.7	13.5	0.85	0.08	<0.01	<0.01	0.07	0.16
trans-12 C18:1	6.02	6.04	9.02	8.73	0.28	8.07	6.83	0.38	0.03	0.21	<0.01	0.15	0.34
cis-9 C18:1	258	261	287	296	7.51	272	279	9.72	0.59	0.07	<0.01	0.34	0.27
cis-11 C18:1	12.3	12.0	13.1	13.2	0.46	13.5	11.8	0.59	0.06	0.77	<0.01	0.35	0.21
cis-12 C18:1	8.45	8.30	11.9	11.4	0.44	10.8	9.18	0.57	0.05	0.09	<0.01	0.33	0.66
cis-13 C18:1	1.46	1.44	1.65	1.58	0.06	1.59	1.47	0.09	0.32	0.07	<0.01	0.28	0.19
cis-9,12 C18:2	47.6	48.1	45.3	46.4	1.15	46.9	46.8	1.49	0.93	0.16	<0.01	0.55	0.27
cis-9,12,15 C18:3	5.03^{a}	4.88^{a}	4.10^{b}	4.23^{b}	0.12	4.70	4.41	0.15	0.17	0.87	<0.01	0.01	0.04

[a-d] Means in a row with different superscripts differ ($P < 0.05$). Conducted only if interaction $P < 0.10$.

[1] CON= diet with no supplemental fatty acids (FA), AC = diet with 3% DM sodium acetate (NaAc), CS = diet with 12% DM whole cottonseed (WCS), and CS+AC = diet containing 3% DM NaAc and 12% DM WCS.

[2] Low PA = diet with no supplemental FA and High PA = diet with a FA supplement containing 85% palmitic acid (PA).

[3] *P*-values refer to results from ANOVA for main effects of basal diet, NaAc, and WCS, the interaction between NaAc and WCS, and the interaction between basal diet, NaAc, and WCS.

[4] NaAc = diets containing 3% NaAc compared to diets with no NaAc.

[5] WCS = diets containing 12% WCS compared to diets with no WCS

[6] FA sources determined as de novo < 16 carbons, mixed = 16-carbon, and preformed = > 16 carbons.

Figure 4.1. Interaction among basal diet, NaAc, and whole cottonseed (WCS) on milk fat content. An interaction between basal diet, NaAc, and WCS was observed for milk fat content ($P = 0.04$). Basal diets included a Low PA diet with no supplemental fatty acids (FA) and a High PA diet containing a FA supplement with 85% palmitic acid (PA). Treatment diets were: CON = diet with no supplemental FA, AC = diet with 3% DM sodium acetate (NaAc), CS = diet with 12% DM WCS, and CS+AC = diet containing 3% DM NaAc and 12% DM WCS. Means within basal diet differ with different letters ($P < 0.05$).

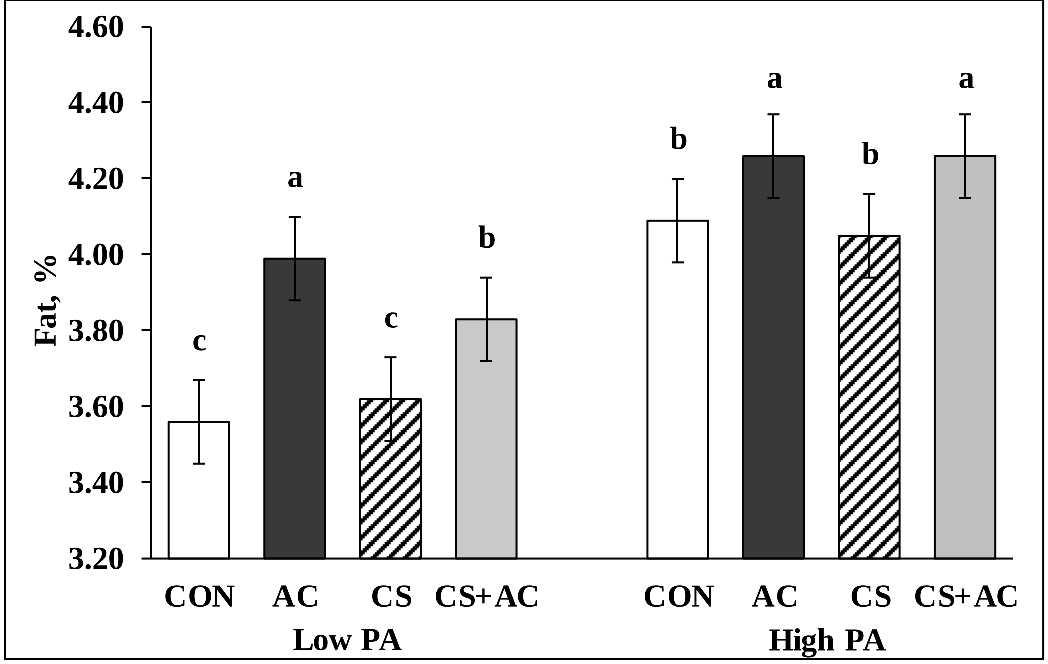


Figure 4.2. Interaction among basal diet, NaAc, and whole cottonseed (WCS) on milk fat yield. An interaction between basal diet, NaAc, and WCS was observed for milk fat yield ($P < 0.01$). Basal diets included a Low PA diet with no supplemental FA and a High PA diet containing a FA supplement with 85% palmitic acid (PA). Treatment diets were: CON = diet with no supplemental FA, AC = diet with 3% DM sodium acetate (NaAc), CS = diet with 12% DM WCS, and CS+AC = diet containing 3% DM NaAc and 12% DM WCS. Means within basal diet differ with different letters ($P < 0.05$).

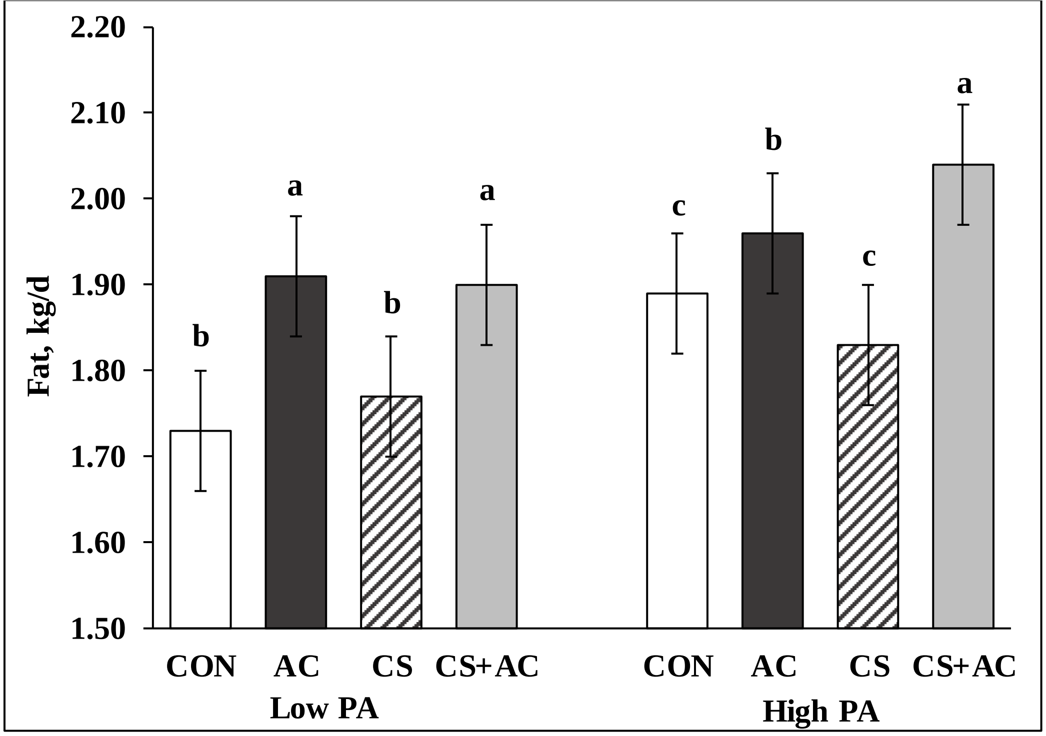


Figure 4.3. Interaction among basal diet, NaAc, and whole cottonseed (WCS) on 3.5% FCM (FCM) yield. An interaction between basal diet, NaAc, and WCS was observed for FCM yield (*P* = 0.08). Basal diets included a Low PA diet with no supplemental fatty acids (FA) and a High PA diet containing a FA supplement with 85% palmitic acid (PA). Treatment diets were: CON = diet with no supplemental FA, AC = diet with 3% DM sodium acetate (NaAc), CS = diet with 12% DM WCS, and CS+AC = diet containing 3% DM NaAc and 12% DM WCS. Means within basal diet differ with different letters (*P* < 0.05).

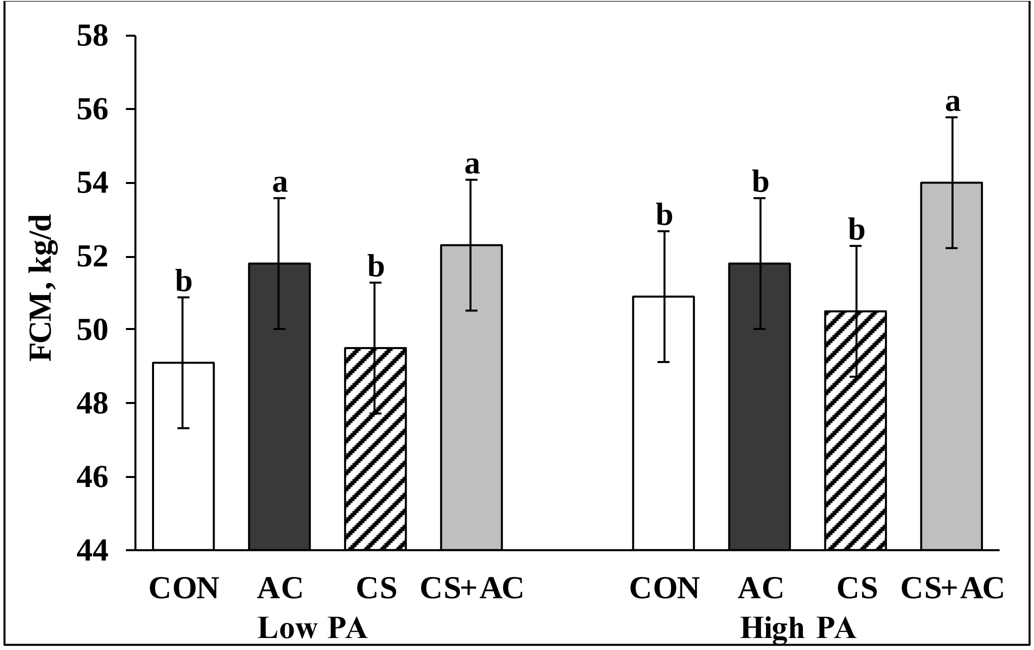


Figure 4.4. Interaction among basal diet, NaAc, and whole cottonseed (WCS) on de novo fatty acid (FA) yield. An interaction between basal diet, NaAc, and WCS was observed for de novo FA yield (*P* = 0.06). Basal diets included a Low PA diet with no supplemental fatty acids (FA) and a High PA diet containing a FA supplement with 85% palmitic acid (PA). Treatment diets were: CON = diet with no supplemental FA, AC = diet with 3% DM sodium acetate (NaAc), CS = diet with 12% DM WCS, and CS+AC = diet containing 3% DM NaAc and 12% DM WCS. Means within basal diet differ with different letters (*P* < 0.05).

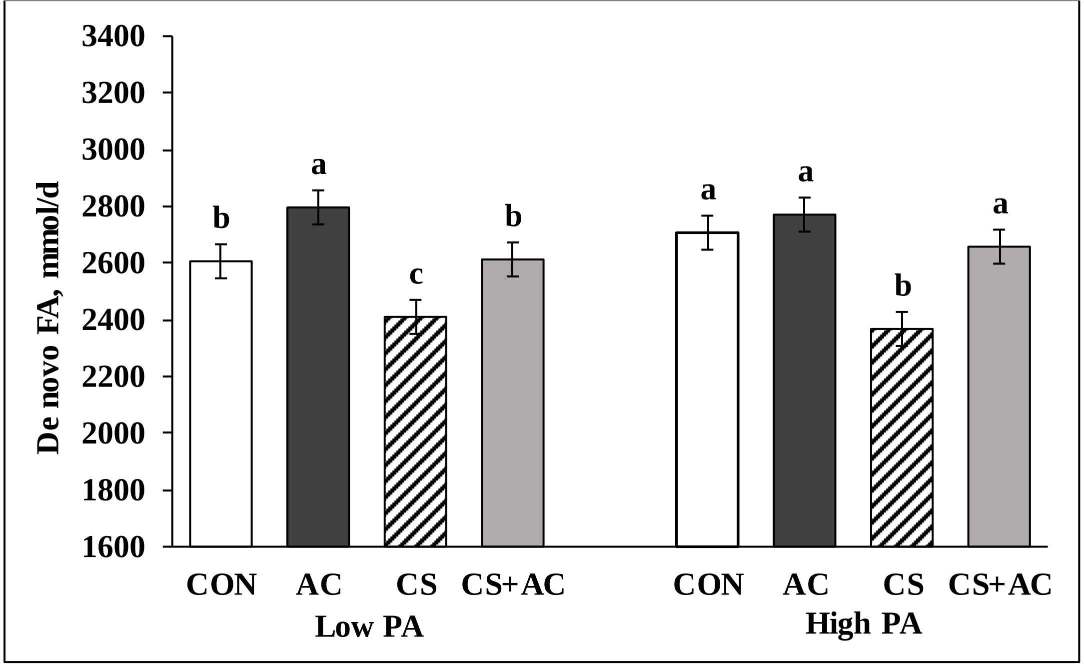


Figure 4.5. Interaction among basal diet, NaAc, and whole cottonseed (WCS) on mixed fatty acid (FA) yield. An interaction between basal diet, NaAc, and WCS was observed for mixed FA yield (*P* = 0.04). Basal diets included a Low PA diet with no supplemental fatty acids (FA) and a High PA diet containing a FA supplement with 85% palmitic acid (PA). Treatment diets were: CON = diet with no supplemental FA, AC = diet with 3% DM sodium acetate (NaAc), CS = diet with 12% DM WCS, and CS+AC = diet containing 3% DM NaAc and 12% DM WCS. Means within basal diet differ with different letters (*P* < 0.05).

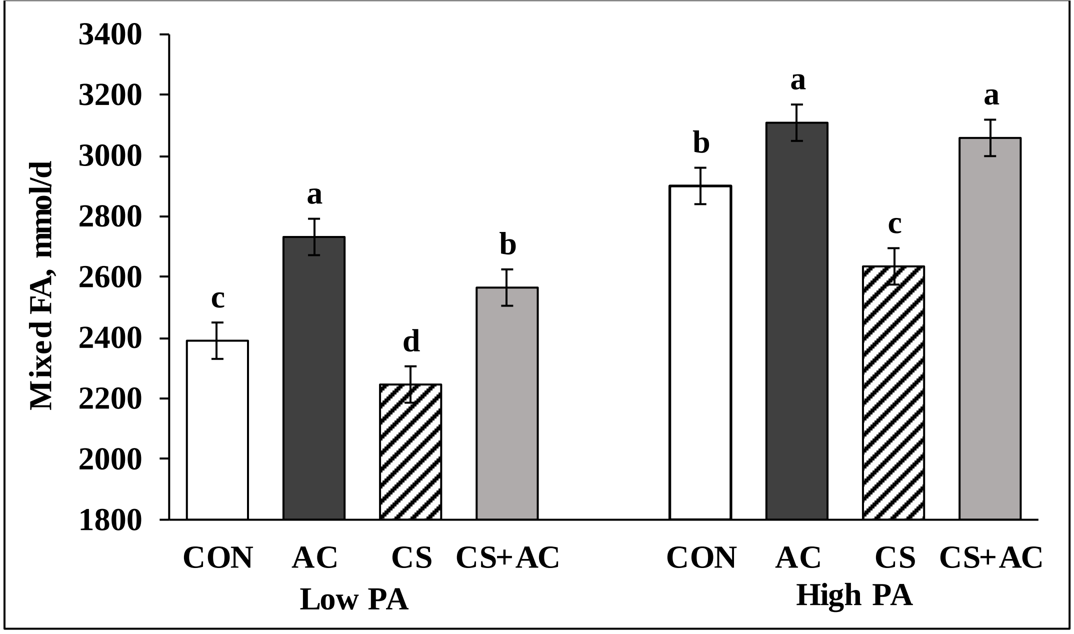


Figure 4.6. Interaction among basal diet, NaAc, and whole cottonseed (WCS) on preformed fatty acid (FA) yield. An interaction between basal diet, NaAc, and WCS was observed for preformed FA yield (*P* = 0.05). Basal diets included a Low PA diet with no supplemental fatty acids (FA) and a High PA diet containing a FA supplement with 85% palmitic acid (PA). Treatment diets were: CON = diet with no supplemental FA, AC = diet with 3% DM sodium acetate (NaAc), CS = diet with 12% DM WCS, and CS+AC = diet containing 3% DM NaAc and 12% DM WCS. Means within basal diet differ with different letters (*P* < 0.05).

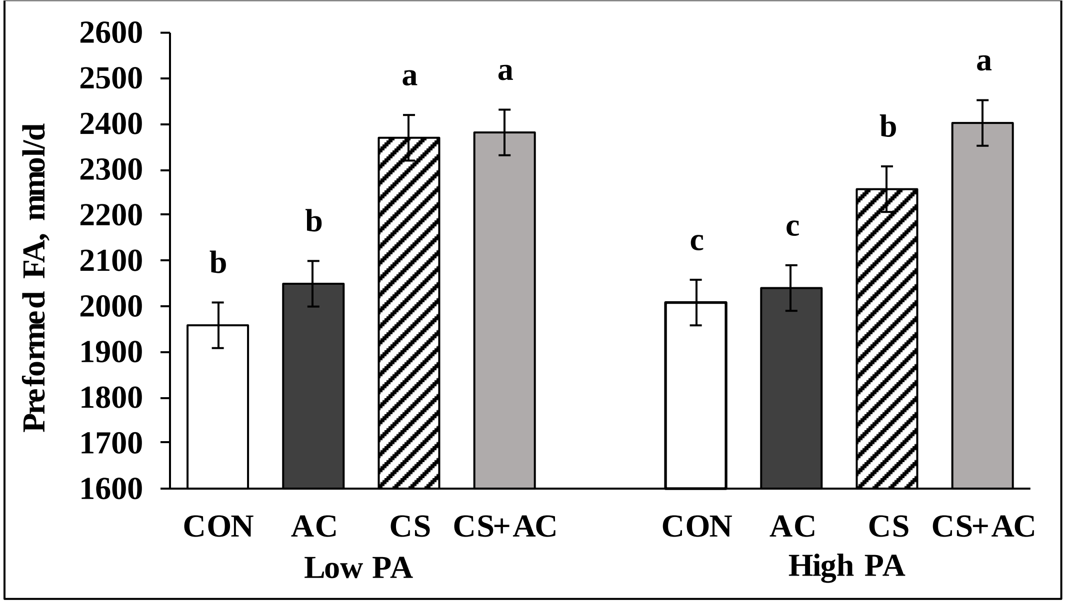
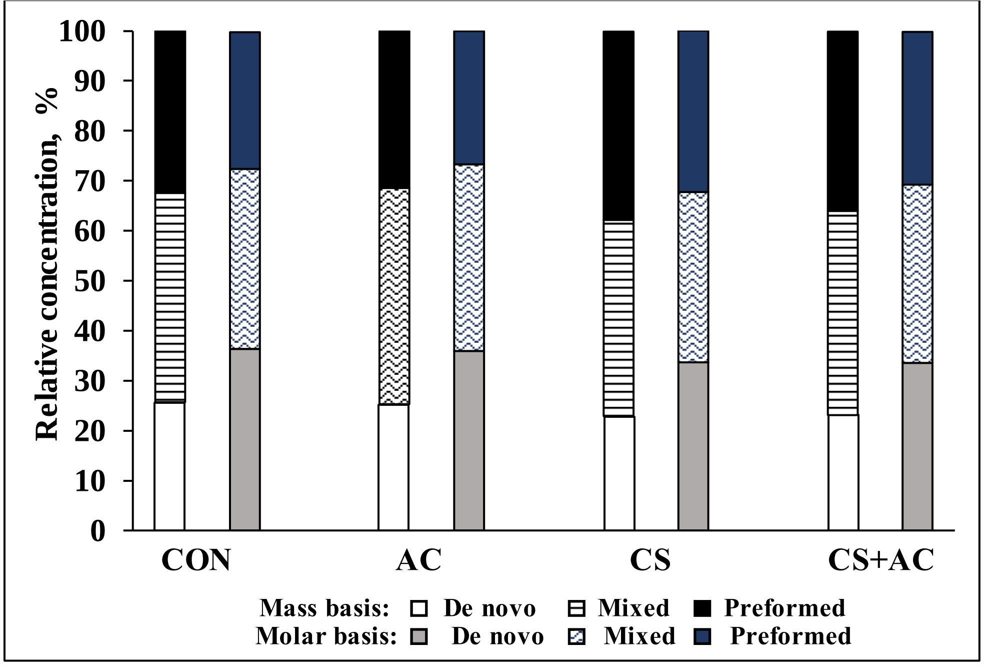


Figure 4.7. The relative concentration of fatty acid (FA) sources on a mass basis (g/100g FA) and molar basis (mmol/100mmol FA) for treatment diets across both basal diets. Basal diets included a Low PA diet with no supplemental FA and a High PA diet containing a FA supplement with 85% palmitic acid (PA). Treatment diets were: CON = diet with no supplemental FA, AC = diet with 3% DM sodium acetate (NaAc), CS = diet with 12% DM WCS, and CS+AC = diet containing 3% DM NaAc and 12% DM WCS. In the order of de novo FA, mixed FA, and preformed FA, the relative concentration on a weight basis is 25.7%, 41.9%, and 36.1% for CON, 22.9%, 39.3%, and 37.7% for CS, 25.3%, 43.4%, and 31.4% for AC, and 23.1%, 40.9%, and 35.9% for CS+AC, respectively. In the order of de novo FA, mixed FA, and preformed FA, the relative concentration on a molar basis is 36.4%, 36.1%, and 27.4% for CON, 33.7%, 34.1%, and 32.3% for CS, 36.0%, 37.4%, and 26.7% for AC, and 33.6%, 35.7%, and 30.6% for CS+AC, respectively.



CHAPTER FIVE:

OVERALL CONCLUSIONS

Milk fat of dairy cows is comprised of de novo FA that are synthesized in the mammary gland and preformed FA that originate from the diet. Improving our understanding of the relationship between these and how dietary changes affected these FA sources is important for improving our understanding of milk fat synthesis. Using molar yields of FA allows us to improve the understanding of diet-induced changes in milk FA composition and the incorporation of FA into milk fat. To our knowledge, no studies in the literature have evaluated the effect of supplementing diets with substrates or FA that target all sources of milk fat nor evaluated these changes on a molar basis. Therefore, the objective of our studies was to determine the effect of supplementing diets with PA on the molar yields of FA sources and determine the effect of supplementing diets with NaAc, PA, and WCS to balance the supply of FA sources to the mammary gland. In combination, these studies evaluated mammary gland responses to different supplementations to improve our understanding of milk fat synthesis in lactating dairy cows.

In Chapter Three, I determined that supplementation of PA increased mixed FA molar yield but had no effect on the yields of de novo FA or preformed FA. On a molar basis, supplementation of PA shifted FA in favor of increased yields of C4:0 and *cis*-9 C18:1 and decreased yields of C8:0 to C14:0 and C18:0. Some of these effects were not obvious if FA yields were expressed on a mass basis.

In Chapter Four, I observed three-way interactions for the effects of NaAc, PA, and WCS for milk fat yield on the molar yields of de novo FA, mixed FA, and preformed FA. Additionally, we observed a three-way interaction for the molar yields of C4:0, C16:0, and C18:0. Our results emphasize the importance of balancing the supply of de novo FA and preformed FA to the mammary gland to increase milk fat synthesis. We observed diets containing NaAc and PA independently increased milk fat yield, whereas diets containing NaAc, PA, and WCS led to the greatest increase in milk fat yield. In addition, diets containing NaAc and WCS increased the yields of milk fat by increasing all sources FA compared to diets with only WCS. In agreement with Chapter Three, the contribution of FA to milk fat was more easily observed when expressed on a molar basis compared to a mass basis and highlighted the importance of enhancing de novo FA synthesis to increase milk fat yield.

Overall, the results from our studies emphasize the importance of targeting increases in yields of both de novo and preformed FA to enhance milk fat synthesis. Secondly, our results highlighted the importance of PA for increasing milk fat synthesis. In Chapter Three, supplementation of PA linearly increased mixed FA yield compared to the control diet, and in Chapter Four the High PA control diet had similar milk fat yield as the Low PA diets containing NaAc and WCS. Additionally, the results from our studies highlight the contribution of de novo FA to milk fat yield and the importance of evaluating diet-induced changes in milk FA composition using molar yields. Enhancing de novo FA synthesis may play a key role in increasing milk fat synthesis and the incorporation of preformed FA into milk fat. We observed diets containing NaAc and WCS increased the yields of milk fat by increasing both de novo and preformed FA compared to diets with only WCS. In agreement with Glasser et al. (2008a), we observed an interdependent relationship between de novo FA and preformed FA where diets containing NaAc, PA, and WCS increased milk fat yield to a greater extent than diets containing only one source. Our results add to previously published literature and will help to improve our understanding of milk fat regulation and synthesis. More importantly, the results from our studies will aid in developing dietary strategies to be used by dairy nutritionists to increase milk fat yield thus increasing milk gross income for the producers.

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