EFFECTS OF FAT SUPPLEMENTATION ON MILK PRODUCTION OF MID-LACTATION DAIRY COWS DURING WARM WEATHER

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ABSTRACT

Heat stressed dairy cows typically have reduced dry matter intake (**DMI**) and milk production making them less profitable. Fatty acid (**FA**) supplements are sometimes used to mitigate heat stress. We hypothesized that feeding a FA supplement of 59% palmitic and 29% oleic acids would improve milk production during summer months. We conducted 2 studies to test this hypothesis. Study 1 was conducted in the summer of 2022 using 40 Holstein cows (95±38 DIM; mean ± standard deviation; 47±10 kg milk/d; and 646±88 kg BW, 12 were primiparous), and Study 2 was conducted in the summer of 2023 using 41 Holstein cows (93±38 DIM; 46±11 kg milk/d; 694±114 kg BW; 17 were primiparous). Cows were fed a common diet for a 6 wk preliminary period, blocked by parity, days in milk, and energy corrected milk/metabolic body weight, and randomly assigned to a control (CON) or fat supplemented (FAT) diet. In both studies, CON and FAT diets were corn silage based and contained ~20% forage NDF and 17% CP. The FAT diet contained an additional 1.23% PA and 0.61% OA as a Ca-salt. The FAT diet increased yields of milk fat 0.07 kg/d, energy-corrected milk 1.6 kg/d, and tended to increase milk yield 1.2 kg/d compared to CON. The FAT diet did not affect DMI, yields of milk protein or lactose, or body weight. The FAT diet increased yields of milk FA of >16 carbon and 16 carbon while decreasing milk FA <16 carbons as well as increasing plasma NEFA concentration. In conclusion, feeding a supplemental FA containing 59% palmitic and 29% oleic FA at 2.09% of diet DM to dairy cows improved milk production 2.9% during warm weather.

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LIST OF ABBREVIATIONS

BCS = Body condition score

BW = Body weight

CON = Control treatment

CP = Crude protein

DIM = Days in milk

DM = Dry matter

DMI = Dry matter intake

ECM = Energy corrected milk

FA = Fatty acid

FAT = Fat supplemented treatment

FCM = Fat corrected milk

HSP = Heat shock protein

ME = Metabolizable energy

MP = Metabolizable protein

NDF = Neutral detergent fiber

NEFA = Non-esterified fatty acid

NEL = Net energy of lactation

OA = Oleic acid

PA = Palmitic acid

PFTN = Pair fed thermoneutral

PPARα = Peroxisome proliferator-activated receptor alpha

RUP = Rumen undegradable protein

SD = Standard deviation

THI = Temperature humidity index

TMR = Total mixed ration

CHAPTER 1

INTRODUCTION

During warm weather, dairy cows are susceptible to heat stress which has numerous detrimental effects on the cows. The severity of heat stress can be categorized by the temperature humidity index (**THI**) value, which combines the ambient temperature and relative humidity of an environment. Heat stress can cause decreased milk and component production as well as lower feed intake when compared to thermoneutrality (Knapp & Grummer, 1991). Fat supplementation often has positive effects on dairy cows, including increased yields of milk and components (dos Santos Neto, de Souza, Lock 2021). Fat supplements are commonly used in the dairy industry to improve production responses of heat stressed cows.

The effects of fat supplementation on milk production during heat stress have been studied for many years (Roskopf et al., 2023; Wang et al., 2010; Moody et al., 1967). Fat supplementation should benefit heat stressed cows theoretically by increasing the energy density of the diet and by decreasing metabolic heat production due to the lower heat increment associated with metabolizing fat (Weiss et al., 2009; Wang et al., 2010; Roskopf et al., 2023). Milk and component production, particularly yields of fat and fat corrected milk, are increased and rectal temperatures are decreased when fat supplements are fed during heat stress (Roskopf et al., 2023; Wang et al., 2010). While fat supplementation does increase the energy density of a diet, it does not guarantee that digestible energy intake will increase (Weiss et al., 2009), and a recent study has shown that when fed a fat supplement, high producing cows had higher milk fat content than medium producing cows during heat stress (Akhlaghi et al., 2019).

We suspect that the effects of fat supplementation during warm weather may be related to the type of fatty acids (FA) in the supplement. Recently, a study feeding supplements containing

different ratios of C16:0 and *cis*-9 C18:1 FA found that supplements higher in *cis*-9 C18:1 increased milk and milk fat yield more than supplements lower in *cis*-9 C18:1, and that a 60:30 blend of these FA was most effective (de Souza, St-Pierre & Lock, 2019). That study was not focused on how the supplements affect heat stressed cows. Our goal was to determine how a fat supplement containing 60% C16:0 and 30% *cis*-9 C18:1 affects milk production of mid-lactation dairy cattle during warm weather and potential heat stress.

CHAPTER 2

LITERATURE REVIEW

Heat Stres

Biology of Heat Stress

Heat stress results from a variety of environmental and biological factors. Cows release heat from the body through evaporative heat loss from their skin and increased respiration. They also manage higher temperatures through non-evaporative cooling, which depends on the temperature gradient between the animal and its environment and includes convective heat loss (Berman et al., 2003). The effectiveness of conductive and evaporative heat loss decreases as ambient temperature rises above 10° C until it becomes ineffective at the animal's body temperature, 39° C. The effectiveness of evaporative cooling also depends on the humidity level of the environment (Collier et al., 2019; Berman et al., 2003). Heat stress often leads to increased respiration rates and body temperature in cows which causes daily maintenance costs to go up (Sammad et al., 2020). Long term exposure to high temperature and humidity can result in acclimatization and acclimation. Acclimation refers to a singular environmental stressor causing a phenotypic response in an animal while acclimatization is a response to multiple stressors occurring simultaneously. Acclimatization often fades when the thermal stress lessens; however, if the stress is present for years the acclimations can become permanent, and the animal is considered "adapted" to its environment. Cows in temperate regions have a harder time managing heat stress due to the inconsistent heat pattern making it difficult to adapt physiologically (Beede & Collier, 1986). High producing cows create more metabolic heat which increases their susceptibility to high thermal loads and heat stress (Collier et al., 2019; Berman et al., 2003). The ability of a cow to effectively cool itself is limited as hot environmental

conditions become more extreme, making it clear that research on how to help cows handle hot weather is needed.

Heat Stress Management

Heat stress costs the US dairy industry $1.5 billion annually, so effective management strategies to alleviate heat stress are important (St-Pierre et al., 2003). The use of shading structures, particularly on open lot dairy farms, is an effective way to reduce heat stress by minimizing radiation heat transfer (Ji et al., 2020). The use of sprinklers and fans have been studied with mixed results (Levit et al., 2021; Perano et al., 2015). A benefit of sprinkler systems is the large size of the water droplet allows it to contact the skin directly for evaporative cooling (Ji et al., 2020). Perano et al (2015) found that when there is high humidity, sprinklers alone are unable to alleviate heat stress, but the conductive cooling effect from water beds circulating cooled water decreased rectal temperature and respiration rate in heat stressed cows. A study by Levit et al (2021) utilized temperature sensors in the reticulorumen to determine the cooling needs of cows instead of relying on cooling measures implemented at regularly timed intervals. When using sensor data to determine how frequently cows were exposed to a sprinkler and fan cooling system authors reported increased milk protein concentrations as well as higher ECM and FCM yields (Levit et al., 2021). Cuellar et al (2023) reported that there are genes regulating milk yield during heat stress that are independent of genes controlling body temperature. Thus to select for more thermotolerant cows we should select for cows that have less milk yield depression during heat stress rather than focusing on breeding for cows that maintain a lower body temperature (Cuellar et al., 2023).

Temperature Humidity Index

Temperature humidity index (THI) is a number that combines the ambient temperature and relative humidity of an environment and is often used to determine if an animal is heat stressed. THI is a value based on an equation from the 1971 NRC

THI = (1.8*Temperature C^{o}+32) – (0.55-0.0055*Relative Humidity) * (1.8*Temperature C^{o}-26)

The THI threshold for considering a lactating cow as heat stressed varies depending on the region and stage of lactation. Using the daily mean THI is the most comprehensive value because it includes daytime and nighttime values (Chang-Fung-Martel et al., 2021). In the United States Midwest region, high producing lactating cows have a heat stress THI threshold of 68, with some variation depending on the production response being studied (North et al., 2023; Mbuthia et al., 2022; Kaufman et al., 2018). Previous studies have not agreed on the number of hours above the threshold required for a cow to be heat stressed, reporting anywhere from 10-17 hours per day before heat stress signs are observed (Kaufman et al., 2019; Zimbelman et al., 2009). By identifying the threshold at which animals begin experiencing symptoms of heat stress it is possible to know when to start implementing measures to mitigate the thermal load. The uncertainty surrounding the amount of time animals must be exposed to high THI before heat stress effects are felt provides an opportunity for additional research to be done to better understand when and which additional cooling measures must be taken.

Heat Stress and Dry Matter Intake

Maximizing diet dry matter intake (**DMI**) is critical to maximize milk production. A reduction in DMI is often seen when cows are experiencing heat stress because reducing DMI can decrease the thermal load from digestion and metabolism (Beede & Collier, 1986). A

negative correlation has been found between THI and DMI, with intake decreasing to a greater extent as THI increases. When determining the extent of DMI decline, there is often a lag in observable effects. When comparing the DMI decrease two days after a high THI incidence there is a greater drop in intake than the decrease observed during the high THI day (West et al., 2003). Long term heat stress can decrease DMI more severely when compared to shorter durations of heat stress. While some variables are affected by the duration of heat stress, DMI remains relatively stable once a threshold is reached (Hou et al., 2021). It is clear that DMI is negatively affected by higher THI and heat stress.

Diet composition during heat stress also impacts the ability of cows to maintain high DMI during heat stress. Feeding higher fiber forages produces additional heat during fermentation, and the decrease in DMI is greater when cows are fed diets with more forage (Beede & Collier, 1986). Another study found that cows fed diets higher in fat had lower respiration and rectal temperatures than when they were fed diets higher in concentrates. The authors attributed this to the decreased DMI when cows were fed a higher fat diet, resulting in lower heat from fermentation (Drackley et al., 2003). This suggests that fat supplementation is one way to mitigate the effects of heat stress.

Production level and stage of lactation are major factors impacting heat stress effects on DMI. High producing cows often experience a greater thermal load, and they often have an accelerated and larger decline in intake to reduce heat production (Collier et al., 2019). Compared to mid-lactation cows, early lactation animals are the most susceptible to DMI decreases during high THI conditions. There are no response differences between multiparous and primiparous animals (Chang-Fung-Martel et al., 2021). Further research to understand the

interaction between production, THI, and DMI can identify ways to manipulate environmental and nutritional factors to mitigate heat stress.

Heat Stress and Production

Heat stress consistently results in milk yield and components declining based on a variety of factors. Milk yield decreases are reported to range from 1.2 to 9.1 kg/d based on production level and location of the study (Mbuthia et al., 2022; Baumgard et al., 2011). When comparing heat stressed cows and PFTN cows, milk yield declined in both groups but declined to a greater extent in the PFTN cows despite the same decreases in DMI (Wheelock et al., 2010). THI 68 is generally the threshold for when milk yield losses begin. (Kaufman et al., 2018; Zimbelman et al., 2009). Cows with longer exposure to heat stress have a greater reduction in milk yield compared to animals that experienced shorter periods of heat stress (Hou et al., 2021). Additionally, studies have found that milk yield declines linearly as THI increases at a rate of 0.69 - 1.2 kg per unit of THI. However, a lag time exists for the effect of heat stress on milk yield with the loss of milk yield per unit of THI being greater two days after a heat stress event, perhaps because it takes about 2 days for animals to digest and metabolize feed (Mbuthia et al., 2022; West et al., 2003). Milk yield declines are related to the severity and duration of high THI events, but the extent of the loss may not be immediately evident.

Milk components are also affected by warm weather and heat stress. Fat yield losses are common during heat stress periods. Fat yield does not have a clear heat stress threshold which makes it difficult to accurately assess the extent of the decline. If THI 68 is considered the heat stress threshold to assess production losses, fat yield declines at 0.02 - 0.1 kg/d (Mbuthia et al., 2022; M'Hamdi et al., 2021). Numerous studies have reported reduced milk protein yield and content (M'Hamdi et al., 2021; Sammad et al., 2020). As the time spent at THI above the heat

stress threshold increases, there is a greater loss of milk protein (Mbuthis et al., 2022). Following a heat stress event, protein content may have a lag time where the greatest reduction occurs in the 5 d thermoneutral recovery period (Ominski et al., 2002). Milk lactose also decreases when cows are heat stressed (Hou et al., 2021; Itoh et al., 1998). A study comparing the effects of long-term heat stress to short term heat stress found lactose yield was impacted more severely when the heat stress period was longer (Hou et al., 2021).

Heat stressed cows consistently have decreased milk fat, protein, and lactose yields. The interactions between environment, production level, and time under stress need to be considered when considering nutritional and management strategies to improve milk and component yields during heat stress.

Adipocytes and Heat Shock Proteins

Heat stress affects bovine adipocytes through alterations in gene expression and signaling pathways related to adipogenesis and lipolysis (Kim et al., 2024; Faylon et al., 2015). Kim et al. (2024) reported heat stressed bovine preadipocytes had an increase *PPARγ* expression, a gene involved in lipid storage in adipocytes as well as the transcription factor C/EBPα involved in adipogenesis. These findings demonstrate how heat stress directly impacts adipose tissue through transcriptional regulation (Kim et al., 2024).

Heat stress also affects the gene expression and concentration of heat shock proteins (**HSP**) (Archana et al., 2017; Hu et al., 2016). Heat stress proteins chaperone denatured proteins to ensure correct unfolding and refolding and prevent aggregation of denatured proteins (Archana et al., 2017). Kim et al. (2024) reported HSP70 was expressed as early as 3 h after adipocytes were exposed to heat stress conditions but returned to thermoneutral levels by 24 h after exposure to heat stress, suggesting it could be an early indicator of heat stress in cattle. Hu

et al (2016) looked at HSP70 in mammary epithelial cells and found HSP70 elevated up to 24 h after exposure to heat stress, indicating a difference between adipocytes and mammary cells for HSP70 expression. Hu et al (2016) also found casein synthesis was decreased in mammary epithelial cells during heat stress when HSP70 was increased, and authors suggested this effort to protect mammary epithelial cells was part of the reason milk protein synthesis is lower in heat stressed cows. Authors did not study alphalactalbumin synthesis in the Hu et al (2016) study, and it is possible a decrease in alphalactalbumin synthesis is partially responsible for the drop in milk production.

Metabolic Changes to Heat Stress

In studies comparing heat stressed cows to pair fed thermoneutral cows (**PFTN**), the pair fed cows have their feed intake reduced to match the decreased intake observed in heat stressed cows. When comparing PFTN to heat stressed cows, lactose yield decreases similarly in both groups. This suggests the decrease is due to lower feed intake and is not entirely temperature dependent (Rhoads et al., 2009). Later studies, however, found that heat stressed cows, compared to PFTN, had lower lactose yields despite similar rate of glucose appearance. This suggests a mechanistic difference between glucose supply and lactose production in heat stressed and PFTN cows (Baumgard et al., 2011). The potential for heat stress to cause metabolic changes in heat stressed cows warrants additional research into the mechanistic causes of decreasing milk components. This research could focus on how heat stress alters plasma glucose and how that leads to decreased yields of milk and lactose.

Dry Matter Intake

Studies comparing PFTN cows to heat stressed cows demonstrate that metabolic changes during heat stress are partially responsible for reduced production responses. Using PFTN cows

allows researchers to determine if production effects are because of decreased intake or metabolic changes specific to heat stress. When comparing the reduction in DMI to the expected decrease in milk yield based on lower DMI, heat stressed cows have significantly greater milk yield losses compared to the PFTN group. This demonstrates that there are mechanisms beyond reduced intake affecting milk yield and milk components (Baumgard et al., 2011; Rhoads et al., 2009). Despite similar decreases in DMI, heat stressed cows had greater nutrient digestibility compared to PFTN cows (Gao et al., 2017). This research into metabolic changes in heat stressed cows shows that strategies other than trying to increase intake in heat stressed cows are required to completely recover milk production.

Insulin

Insulin plays a role in several of these metabolic changes during heat stress. Compared to thermoneutral cows, heat stressed cows have higher insulin secretion (Itoh et al., 1998). Compared to PFTN cows, heat stressed cows do not lose the same amount of body weight, indicating they are not using adipose breakdown as a glucose sparing mechanism when feed intake is reduced. This is supported by increased insulin response to an epinephrine challenge comparing PFTN and heat stressed cows, which suggests this additional insulin is used as an antilipolytic signal (Baumgard et al., 2011). Heat stressed cows have greater levels of circulating blood insulin compared to PFTN cows. Based on glucose tolerance test data, this is because of increased pancreatic insulin release as opposed to reduced insulin removal, which supports the hypothesis that heat stressed cows try to minimize non-esterified fatty acid release when intake is lower (Baumgard et al., 2011; Wheelock et al., 2010).

Glucose

The decrease in plasma glucose is not proportional with the decline in DMI in heat stressed cows, suggesting sources other than propionate from feed digestion are used for gluconeogenesis (Gao et al., 2017; Baumgard et al., 2011). By reducing adipose tissue mobilization for energy, coupled with higher amounts of glucose leaving the circulating blood pool, glucose appears to be the primary energy source for heat stressed cows (Wheelock et al., 2010). This increase in whole body glucose utilization may redirect glucose away from the mammary gland for other body functions. This is supported by the lower lactose yield in heat stressed cows compared to PFTN cows, suggesting extra mammary tissues are prioritized for glucose utilization (Baumgard et al., 2011). Additionally, heat stressed cows have increased milk urea nitrogen and blood urea nitrogen, and decreased plasma amino acids and NEFA concentrations. This suggests higher amino acid deamination and extra mammary utilization of amino acids as gluconeogenic precursors (Gao et al., 2017). Feeding to enhance glucose production or redirect glucose towards to the mammary gland could be a way to enhance yields of milk and lactose in heat stressed cows.

Non-esterified Fatty Acid

Despite the similar reduction in DMI, PFTN cows had greater plasma NEFA concentrations than heat stressed cows. Baumgard et al. (2011) reported PFTN cows lost more body weight compared to heat stressed cows while Wheelock et al. (2010) observed PFTN and heat stressed cows lost a similar amount of body weight. PFTN cows losing more body weight than heat stressed cows while having greater plasma NEFA concentrations, suggests that heat stressed cows try to reduce body tissue mobilization when compensating for reduced intake (Baumgard et al., 2011; Rhoads et al., 2009). In an epinephrine challenge, PFTN cows had a

larger NEFA response than heat stressed cows, suggesting that adipose tissue in heat stressed cows becomes resistant to lipolytic stimuli (Baumgard et al., 2011). Additional studies could help understand how to manage production effects from this blunted adipose tissue mobilization response using nutrition.

Adipose Tissue, Insulin and Glucose

During exposure to long term heat stress, one study proposed that heat stressed cows had greater lipolysis inhibition and increased skeletal muscle proteolysis, which indicates continued reliance on carbon sources other than NEFA as heat stress continues (Hou et al., 2021). While insulin is increased in heat stressed cows, the mammary gland utilizes insulin independent mechanisms for glucose uptake (De Koster & Opsomer, 2013). This suggests additional factors overriding the mammary gland as the prioritized glucose endpoint in heat stressed cows. Cows in negative energy balance typically rely on mobilizing body tissue for milk FA. Due to reduced DMI, many heat stressed cows are also in a negative energy balance but rely more on other energy sources than mobilized body tissue and NEFA. The inhibiting effect of insulin on lipolysis does play a role in milk fat production. It has been observed that the increase in insulin is related to magnitude of milk fat decrease (Bauman et al., 2003).

These results indicate that blood metabolites and adipose tissue respond differently during heat stress events compared to thermoneutral cows with decreased DMI. Improving milk and component yields depends on understanding and balancing the interactions between reduced milk production, blood metabolites and nutrition of heat stressed cows.

Fat Supplements

Rumen Digestion of Fat Supplements

Unsaturated free fatty acids are toxic to rumen microbes, and rumen microbes biohydrogenate these unsaturated fatty acids to protect themselves (Jenkins, 1993). The amount of lipid added to a diet can alter fermentation and ruminal digestion of nonlipid feed components (Jenkins, 1993). This can affect the benefits of fat supplementation based on the type and composition of supplement fed. Unsaturated fat supplements can increase biohydrogenation intermediates compared to saturated fat supplements, as well as change the concentration of short, medium, and long chain fatty acids. Unsaturated fat supplements, such as oils, can also reduce digestion of fiber and saturated triglycerides (Harvatine & Allen, 2006; Allen, 2000).

Calcium salts of fatty acids have different effects on intake, production, and digestibility when compared to other types of fat supplement. Compared to a palmitic enriched triglyceride supplement, calcium salts of palmitic FA increased energy allocated to body reserves and increased BW and BCS change, but had lower yields of milk, milk fat, and milk energy output. Calcium salts also increase yields of preformed milk FA compared to palmitic enriched triglyceride supplements (de Souza & Lock, 2018). When compared to control diets, calcium salts of palm FA have been shown to decrease DMI (de Souza, Batistel & Lock, 2017) or have no effect on DMI (de Souza & Lock, 2018). Calcium salts can also increase yields of milk, milk fat, and ECM (de Souza & Lock, 2018; de Souza, Batistel & Lock, 2017).

Based on the research regarding types of fat supplements, there are many differences to consider when choosing which supplement to feed. Calcium salts have the benefit of being highly digestible and effective for protecting long chain unsaturated fatty acids from

biohydrogenation. That makes them an ideal method for feeding long chain unsaturated fatty acid supplements.

Dry Matter Intake

Supplemental fat has varying effects on dry matter intake (**DMI**). Studies have found that feeding a fat supplement, compared to a control diet, results in decreased DMI (dos Santos Neto et al., 2021; Lock, 2013), increased DMI (de Souza & Lock, 2018) or had no effect on DMI (Bales, Cinzori & Lock, 2024; Burch et al., 2021). The effect of fat supplementation on DMI is impacted by the fatty acid composition of the supplement. When comparing diets with increasing levels of C16:0 to each other, DMI increases linearly with the increase in C16:0 (Bales, Cinzori & Lock, 2024). When comparing supplements containing *cis*-9 C18:1, increasing the amount of *cis*-9 C18:1 in the supplement had no effect on DMI (Hu et al., 2024; de Souza, St-Pierre & Lock, 2019). Fat supplement composition interacts with production level to alter DMI in some studies. For example, low producing cows had greater DMI when fed supplements high in C16:0 while high producing cows had greater DMI when fed supplements containing more *cis*-9 C18:1 (de Souza et al., 2019). A study by Western, de Souza & Lock (2020), however, did not observe an interaction between DMI and the ratio of C16:0 and *cis*-9 C18:1, possibly due to different levels of unsaturated fatty acids in the supplements.

Milk Composition

Feeding a fat supplement consistently affects milk yield and components. When comparing a diet with a fat supplement to a control diet with no fat supplement, the supplemented diet usually increases milk yield and often increases milk energy output (Bales, Cinzori & Lock, 2024; Prom & Lock, 2021). Fat supplementation can increase milk yield to a

greater extent in multiparous than in primiparous cows and in high producing cows than low producers (Burch, Pineda & Lock, 2021; de Souza & Lock, 2018). Effects vary with the composition of the supplement and the amount of supplement included in the diet. Calcium salts of C16:0 increase milk yield as the amount of supplement included increases, and they also partition more energy to milk (dos Santos Neto, de Souza & Lock, 2018). When feeding fat supplements containing 16:0 and *cis*-9 C18:1, milk yield increases when the supplement contains greater amounts of *cis*-9 C18:1. The milk yield response, however, is often greater in high producing cows than in low producers (Hu et al., 2024, de Souza, St-Pierre & Lock, 2019).

Milk fat is often affected by adding a fat supplement. Fat supplements consistently increase yields of fat and fat corrected milk (**FCM**), and often increase milk fat content (Prom & Lock, 2021; Lock et al., 2013). As for milk yield, yields of milk fat and FCM typically increase more in multiparous cows than in primiparous cows and in high producing cows than in low producers (Burch, Pineda & Lock, 2021; de Souza & Lock, 2018). Variations in the content of *cis*-9 C18:1 in the supplement have inconsistent effects on milk fat. As the amount of *cis*-9 C18:1 increases in a supplement, milk fat content often decreases. Yields of milk fat and FCM often remain unchanged when the amount of *cis*-9 C18:1 is increased in a supplement compared to supplements lower in *cis*-9 C18:1. When looking at milk fat composition, however, de novo and mixed-source FA decrease while preformed milk FA increased (Hu et al., 2024; Prom & Lock, 2021). When comparing the interaction between production level and increasing amounts of *cis*-9 C18:1 in a supplement, high producing cows see an increase in fat yield and FCM yield while low producing cows have a decline in yields of milk fat and FCM (de Souza, St-Pierre & Lock, 2019).

Milk protein is sometimes impacted by fat supplementation. Like other milk components, there are inconsistent effects of fat supplementation on protein yield and content. Several studies reported no difference in protein yield or content between cows fed a fat supplement and cows on a control diet (Burch, Pineda & Lock, 2021; dos Santos Neto, de Souza & Lock, 2021; de Souza & Lock, 2018). In other studies, however, fat supplementation increased protein yield compared to cows on a control diet (de Souza & Lock, 2018). Increasing the amount of *cis*-9 C18:1 in a fat supplement increases protein yield, particularly in high producing cows compared to supplements lower in *cis*-9 C18:1 (de Souza, St-Pierre & Lock, 2019). Similarly, Hu et al. (2024) saw an increase in protein yield with increasing *cis*-9 C18:1, however authors did not include production level in their analysis. It is common, however, that fat supplementation will decrease protein content compared to a control diet (Bales, Cinzori & Lock, 2024; Burch, Pineda & Lock, 2021, dos Santos Neto, de Souza & Lock, 2021). Adding greater amounts of a supplement to a diet can decrease milk protein content, but does not affect protein yield (dos Santos Neto, de Souza & Lock, 2021). When feeding supplements with increasing amounts of *cis*-9 C18:1, protein content may decrease (Prom & Lock, 2020).

Milk lactose is also affected by fat supplementation. Milk lactose yield is often increased when cows are fed fat supplemented diets compared to a control diet, however this effect is seen more in high producing cows (Burch, Pineda & Lock, 2021). This increase is not consistently reported, however, and lactose yield remains unchanged when diets are supplemented with fat in some studies (dos Santos Neto, de Souza & Lock, 2021; Lock et al., 2013). Lactose content is consistently unchanged when cows are fed a fat supplemented diet compared to a control diet. When comparing lactose content and yield in cows fed fat supplements with varying amounts of

cis-9 C18:1, both lactose content and yield increase as the amount of *cis*-9 C18:1 increases in the fat supplement fed (Hu et al., 2024; de Souza, St-Pierre & Lock, 2019)

Fat supplementation consistently increases milk fat and FCM while other components like milk protein and lactose have a more varied response. It is clear that fat supplementation plays a beneficial role in dairy cow diets for increasing yield and content of milk components. Cow production level and parity interact with the fatty acid profile of a supplement. This interaction can alter the effectiveness of fat supplementation based on production goals and should be considered when choosing a supplement.

Milk Fatty Acids

De novo milk fatty acids (**FA**) are <17 carbon in length and synthesized in the mammary gland. De novo FA are often affected by fat supplementation. De novo FA yield decreases when cows are fed a fat supplement compared to a control diet (Bales, Cinzori & Lock, 2024; Burch, Pineda & Lock, 2021). Some studies, however, reported no change in de novo FA yield when a fat supplement was fed compared to control cows (Prom & Lock, 2021; Lock et al., 2013). Compared to cows fed a control diet, fat supplemented cows consistently decrease de novo FA content (Bales, Cinzori & Lock, 2024; dos Santos Neto, de Souza & Lock, 2021). When cows are fed fat supplements with varying amount of *cis*-9 C18:1, low producing cows decrease de novo FA yield while high producing cows either increase or have no change for de novo FA yield (Western, de Souza & Lock, 2020; de Souza, St-Pierre & Lock, 2019).

Preformed milk FA are >15 carbons in length and are extracted from plasma. Compared to cows on a control diet, fat supplemented cows increase both yield and content of preformed FA (Bales, Cinzori & Lock, 2024; Prom & Lock, 2021). Yields of preformed FA increase in cows

fed a fat supplement compared to control cows, but the increase is seen to a greater extent in higher producing cows (Burch, Pineda & Lock, 2021). Increasing the amount of *cis*-9 C18:1 in fat supplements found higher amounts of *cis*-9 C18:1 increased yields of preformed FA. Western, de Souza & Lock (2020) saw an increase in preformed FA yield in low and high producing cows through greater yield of all 18 carbon FA. De Souza, St-Pierre & Lock (2019) saw increased preformed FA yield only in medium and high producing cows by increasing the yield of *cis*-9 C18:1 while reducing yield of C16:0.

Mixed-source milk FA are 16 carbon in length and are extracted from plasma and synthesized de novo in the mammary gland. Mixed source FA yield and content had varied responses based on the type of fat supplement being fed. Feeding fat supplements higher in C16:0 often increases yield and content due to increases in C16:0 and C16:1 in milk fat (Bales, Cinzori & Lock, 2024; de Souza & Lock, 2018). As the amount of C16:0 supplementation increased in the diet, mixed FA yield and content increased to a greater extent (dos Santos Neto, de Souza & Lock, 2021). Cows fed fat supplements higher in *cis*-9 C18:1 can decrease the yield and content of mixed FA, partially due to increased yield of *cis*-9 C18:1 in milk fat and reduced C16:0, but the effect is primarily seen in low and medium producing cows (Western, de Souza & Lock, 2020; de Souza, St-Pierre & Lock, 2019).

Milk fat composition is affected by fat supplementation, however the results are varied based on the type of fat supplemented as well as cow production level and parity. To increase mixed FA yield, feeding a supplement higher in C16:0 is beneficial, but if the goal is to produce more preformed FA then feeding supplements higher in *cis*-9 C18:1 is more effective. Knowing the desired milk FA profile is important before choosing a fat supplement.

Body Weight and Body Condition Score

Body weight change and body condition score were not affected by fat supplementation compared to a control diet according to some studies (Burch, Pineda & Lock, 2021; dos Santos Neto, de Souza & Lock, 2021). De Souza & Lock (2018) reported that adding a fat supplement increased body weight change per day in fat supplemented cows compared to control diet, and that the effect was greater in primiparous cows than multiparous cows. This suggests that primiparous cows are putting more energy into body tissue reserves than multiparous cows.

Heat Stress and Fat Supplementation

Heat stressed cows often reduce dry matter and energy intake, and adding a fat supplement will increase the energy density of the diet, therefore increasing energy intake. This is not always an effective strategy because it does not mean dry matter or digestible energy intake will increase (Weiss et al., 2009; Harvatine and Allen, 2006). Fat supplementation has inconsistent effects on dry matter intake in heat stressed cows. Drackley (2003) reported cows on a high fat diet containing grease high in *cis*-9 C18:1 have decreased intake while Wang et al. (2010) observed no change in intake when fed a saturated fatty acid supplement containing mostly C16:0. The effects of feeding a fat supplement to heat stressed cows to improve health and production responses have been studied extensively with varying results for respiration rate, body temperature, DMI, or BW (Roskopf et al., 2023; Williams et al., 2021; Moody et al., 1967). Fat yield, fat content, and fat corrected milkare often increased when heat stressed cows are fed a fat supplement containing both C16:0 and C18:0 (Roskopf et al., 2023; Knapp & Grummer, 1991). Feeding high levels of fat can cause milk fat depression, when trans isomers of fatty acids are formed during biohydrogenation of polyunsaturated fatty acids leads to reduced milk fat synthesis in the mammary gland (Bauman et al., 2003). When considering the efficacy of fat

supplementation as a heat stress mitigation strategy, Akhlaghi et al. (2019) found that high producing cows use calcium salts of C16:0 and *cis*-9 C18:1 as well as C16:0 enriched fat supplements more efficiently than medium producing cows under heat stress conditions.

Feeding fat supplements to heat stressed cows also has effects on body temperature and body weight. Feeding a fat supplement theoretically could benefit heat stressed cows because digested fat is associated with less heat increment than all other digested nutrients. Several studies used rectal temperature to test for metabolic heat differences. Roskopf et al. (2023) observed no change in rectal temperature while other studies reported decreased rectal temperature in fat supplemented cows (Wang et al., 2010; Drackley et al., 2003). Researchers feeding free fatty acid supplements containing C16:0, C18:0, or a grease supplement high in *cis*-9 C18:1 observed no impacts on body weight when cows are experiencing heat stress (Afarani et al., 2023; Drackley et al., 2003). Roskopf et al. (2023) fed a microencapsulated protected fat supplement containing C16:0 and C18:0 and reported that body weight and body condition score of heat stressed cows were not changed when fed a fat supplement, but there was an increase in 4% FCM and ECM. This suggests that the additional energy in the diet is being partitioned to milk instead of body reserves. Several studies also report an apparent increase in efficiency when fat supplemented heat stressed cows produced more milk per unit of DMI than control cows while other studies report increased nitrogen use efficiency (Roskopf et al., 2023; Drackley et al., 2003). Wang et al. (2010) found that heat stressed cows fed a supplement containing primarily C16:0 did not lose body weight or BCS and had lower NEFA values than control cows. This may be because cows on the fat supplemented diet had a positive energy balance due to the energy density of the diet after adding a fat supplement.

Much of the previous research done has focused on fat supplements containing C16:0, C18:0, and *cis*-9 C18:1 in the form of free fatty acids, rumen protected fat supplements, and calcium salts. Feeding these fat supplements to heat stressed cows sometimes plays a beneficial role in production, body temperature, and body weight changes. While the effects on energy intake are unclear, there are sometimes increases in milk fat production as well as resilience against body tissue loss. These benefits indicate fat supplementation is a viable strategy for mitigating heat stress in dairy cows.

Conclusion of Literature Review and Objective of The Thesis

Milk production, intake, blood metabolites, and body weight are all influenced by environmental and dietary factors. Heat stress affects most cows at some point during the year and has negative effects on milk and milk composition in cows at all production levels to some extent. Fat supplementation has been shown to consistently improve milk yield and composition as well as body condition. Providing a fat supplement to heat stressed cows has been effective in some studies, but inconsistent results indicate that further research should be done looking at different environmental factors, cow production level, and supplement composition.

Our objective was to investigate the effects of feeding a fat supplement containing 60% C16:0 and 30% *cis*-9 C18:1 to heat stressed dairy cows on milk production and composition, blood metabolites, and body condition. Previous research has reported that supplements containing this composition of fatty acids are beneficial to cows at a varying levels of production and parity, which suggests it could have a positive effect on heat stressed cows. This research will increase our understanding of how dosage and composition of fat supplements impact heat stressed cows to promote optimal production and farm income.

CHAPTER 3

MATERIALS AND METHODS

Design and Treatments

Two studies were conducted at Michigan State University Dairy Cattle Teaching and Research Center using the same treatment design and experimental fat supplement. The first study took place in the summer of 2022 (Year 1), and the second study (Year 2) was conducted in the summer of 2023. We used 40 cows (12 were primiparous) in Year 1 and 41 cows, (17 were primiparous) in Year 2. Mean DIM, milk yield, and BW at the start of these studies were (mean ±SD) 95±38, 47±10 kg/d, and 646±88 kg for Year 1 and 93±38, 46±11 kg/d, and 694±114 kg for Year 2, respectively. We assigned cows to treatments with a randomized complete block design. Blocks were created based on parity (primiparous or multiparous), DIM (less than or greater than 110 DIM, the midpoint of both studies), and ECM/$BW^{0.75}$ to account for milk production differences due to body size,allowing two cows in each block. Dietary treatments were randomly assigned within those blocks. Michigan State's Institutional Animal Care and Use Committee approved all treatments and procedures.

Diets were corn silage-based and formulated to meet or exceed NASEM nutrient requirements of the average cow in the group (NASEM, 2021). All cows were fed a control (CON) diet during a 11-d preliminary period for Year 1 and an 8-d preliminary period for Year 2. Control cows continued to receive the CON diet for the next 6 wk. The high fat diet (FAT) was similar to the CON diet but with addition of a calcium salt of palmitic (C16:0) and oleic (*cis*-9 18:1) fatty acids (FA) at a 59:29 ratio (59% C16:0 and 29% *cis*-9 C18:1 (Spectrum Distinct, Perdue Agribusiness). The remaining FA in the supplement included primarily 18 Carbon FA and less than 2% C12:0 and C14:0. The fat supplement was included at 2.35% dry matter of the

FAT diet and was mixed into the TMR; thus, the FAT diet was supplemented with 1.23% palmitic and 0.61% oleic acids and soyhulls were removed. With the addition of the fat supplement, we also adjusted the protein content of the diet to keep the metabolizable protein per unit of ME similar and the two diets differed in soyhulls and protein supplements. In Year 2 we adjust the alfalfa content to keep the effective NDF similar in both diets. TMR composition and nutrient information for both studies are based off of book values and forage analysis before the studies began- listed in Table 3.1.

For Year 1 the preliminary period began on July 21, 2022, and treatment diets were fed from August 2 to September 12. Maximum and minimum daily temperatures averaged 27.5°C and 18.3°C, respectively and relative humidity averaged 69%. THI was an average of 70.6 reaching a maximum of 87.0 (Figure A.1). For Year 2, we fed preliminary diets starting June 22, 2023, and treatment diets were fed from July 1 to August 13. Maximum and minimum daily temperatures averaged 27.2°C and 18.9°C, respectively and relative humidity averaged 68%. THI was an average of 71.9 reaching a maximum of 86.9 (Figure A.2) Temperature and humidity data were collected using Hoboware temperature and humidity data loggers placed at cow height evenly spaced throughout the barn,and THI was calculated using the following equation from the NRC (1971):

$$THI = (1.8*AT_{avg} + 32) – [(0.55 - 0.0055 * RH) * (1.8 * AT – 26)],$$

AT is the ambient temperature °C and RH is the relative humidity %.

Cows were housed in a tie stall barn with each cow's access limited to their own feed. Access to feed was ad libitum for approximately 21 hours per day, except during milking, and restricted from 0900 h to 1000 h each day to collect refusals and provide new feed at 110% of

their expected intake. Cows were milked three times per day (0530, 1400, and 2100 h). All cows had ad libitum water available, even during milking. Stalls were bedded with fresh sawdust daily and were cleaned three times per day.

Table 3.1 Ingredient and nutrient composition of treatments

	Year 1 % of DM		Year 2 % of DM	
Ingredient	CON	HiFA	CON	HiFA
Corn Silage BMR	39.42	39.42	39.75	39.77
Alfalfa Haylage	11.67	11.67	11.86	12.76
Ground Corn	20.67	20.67	19.31	19.10
Cottonseed, Whole	5.00	5.00	7.01	6.99
Soybean Meal	9.17	10.67	9.84	9.91
Soybean Hulls	4.67	0.00	3.65	0.00
Base Vitamin/Mineral Mix[1,2]	2.08	2.08	2.09	2.14
High Cow Supplement Mix[3,4]	6.92	7.73	5.59	0.00
D&D Ion Plus	0.42	0.42	0.89	0.86
Amino Acid Mix[5]	0.00	0.00	0.00	6.13
Ca-salt of Palm FA[6]	0.00	2.35	0.00	2.35
Nutrient Composition				
Dry Matter[7]	48.7	48.9	51.7	51.5
Neutral Detergent Fiber (NDF)	29.4	26.5	28.5	26.2
Forage NDF	20.4	20.4	19.4	19.8
Effective NDF[8]	24.0	23.1	22.6	22.4
Metabolizable Protein (MP)	11.8	12.0	9.5	9.7
Starch	29.3	29.3	31.0	30.9
Crude Protein (CP)	16.8	17.4	16.8	17.2
Rumen Undegraded Protein[9]	42.6	42.9	42.6	43.0

[1] Vitamin and mineral mix (Study1) contained 14.7% fine ground corn grain, 0.4% calcium carbonate, 15.7% MIN-AD(MIN-AD Inc., Winnemucca, NV), 20.4% calcium carbonate, 14.1% white salt, 12.5% calcium phosphate di, 9.1% Magnesium ox, 4.7% Magnesium sulfate, 4.5% sodium sesquinate, 2.3% selenium, 0.5% Micro 5, 0.3% Vitamin E, 0.03% Vitamin A, <0.01 %Energizer Tallow, <0.01% Vitamin D3 500.

[2] Vitamin and mineral mix (Study 2) contained 22% ground corn, 21% MIN A (Min Ad Inc.), 20% Calcium carbonate, 19% calcium phosphate, 10% white salt, 5% sodium sesquinate, 2% selenium, and <1% of each of the following: tallow, Micro 5 (Alltech), Vitamin A, Vitamin E, Vitamin D.

[3]High supplement mix (Study 1) contained 41.3% Amino plus, 18.6% Caledonia Pass (Caledonia Farmers Elevator, Caledonia, MI), 14.6% sodium sesquinate, 11.9% calcium carbonate, 10.2% ground corn, 2.4% urea, 1% Spartamine M (Adisseo, Alpharetta, GA).

[4]High supplement mix (Study 2) contained 40.8% Amino plus, 17.7% ground corn, 14.6% sodium sesquinate, 11.9% spectrum AgriBlue, 11.9% calcium carbonate, 2.4% Urea, <1% Smartamine M (Adisseo, Alpharetta, GA).

5Amino Acid Mix contained 30.7% Amino plus, 24.3% Spectrum AgriBlue, 16.1% ground corn, 14.7% sodium sesquinate, 9.6% calcium carbonate, 2.4% urea, 0.9% Smartamine M (Adisseo, Alpharetta, GA), 0.9% ion plus.

[6]Ca-salt containing 8%Ca and 89%FA with a FA blend of approximately 59% C16:0 + 29% C18:1 cis-9.

[7]Expressed as percent of as fed.

Table 3.1 (cont'd)
[8]NDF of forages is 100% effective. NDF of cottonseeds is 50% effective and NDF of other feeds is 25% effective. Effective NDF not only stimulates rumination and buffering but also replaces starch so less buffering is needed (Spartan Dairy)
[9]Expressed as percent of CP

Data Collection and Analysis

Body condition score was measured by 3 trained researchers blind to treatment during the first and last week of both studies on a 5-point scale using 0.25 units (Wildman et al., 1982). Body weights were measured three times weekly after the afternoon milking. Milk samples were collected at 6 consecutive milkings each week starting 5 days before treatments in Year 1 and 3 days before treatments start for Year 2, with the week before treatment used as a covariate. Samples were stored at 4° C in a tube containing a preservative (Bronolab W-II liquid, Advanced Instruments, Norwood, MA). Samples were sent to CentralStar Cooperative Inc (Grand Ledge, MI) for analysis of fat, protein, lactose, somatic cell count, and milk urea nitrogen concentrations. Yields of ECM and milk components were calculated using the sum of component concentrations and milk yield from individual milkings. Milk energy (MilkE) was calculated using 9.29 x kg fat + 5.85 x kg true protein + 3.95 x kg lactose (NASEM 2021). Energy corrected milk (ECM) was calculated using [0.327 x kg milk) + (12.95 x kg milk fat) + (7.2 x kg milk protein)] which corrects milk to a 0.68Mcal/kg energy basis (Tyrrell and Reid, 1965). We also measured feed efficiency by calculating energy-corrected milk/dry matter intake as well as milk energy/feed net energy.

Additional milk samples were collected in tubes without a preservative for analysis of FA composition during wk 6 of both studies. Those milk samples were composited for each cow based on milk weight and fat concentration. Milk lipids were extracted, and fatty acid methyl esters were prepared and analyzed by gas chromatography as described previously (Lock et al., 2013; Bales, Cinzori & Lock, 2024). Yields of individual FA (g/d) in milk fat were calculated

using the measured FA concentration, the milk fat yield for the day of collection, and the molecular weight of each FA while correcting for glycerol content and other milk lipid classes (Piantoni et al., 2013).

Blood was sampled during wk 6 of Study 1 at -1 and +4 hours relative to feed gates opening at 1000 h into EDTA and NaF vacutainer tubes. For Year 2 we collected blood on a day 4°C below the average temperature for that month and a day 4°C above the average monthly temperature into EDTA and NaF tubes. We centrifuged blood samples at 2,000 X *g* for 15 min at 4°C to collect plasma then stored the plasma at -20°C until analysis. Plasma was analyzed for non-esterified fatty acids (NEFAs), glucose, and insulin concentrations at the Michigan State University Veterinary Diagnostic Laboratory using a Beckman Coulter AU series analyzer for chemistry analysis (Lansing, MI).

Statistical Analysis

Data were analyzed using the GLIMMIX model in SAS (version 9.4, SAS Institute Inc.) using the following model:

$$Y_{ijklmn} = \mu + D_i + P_j + W_k + Y_l + C_m(P_j) + W_n(Y_l) + D_i \times P_j + D_i \times W_k + P_j \times W_k + Di \times P_j \times W_k + pDM(P_j) + DIM(P_j) + e_{ijklmn.}$$

Where μ = overall mean, Di = fixed effect of treatment diet (CON and FAT), Pj = fixed effect of Parity, Wk = fixed effect of week, Y_l = fixed effect of year, $C_m(P_j)$ = random effect of cow nested within parity, $W_n(Y_l)$ = random effect of week nested into year, $D_i \times P_j$ = fixed effect of the interaction between treatment diet and Parity, $D_i \times W_k$ = fixed effect of the interaction between treatment diet and Week, $P_j \times W_k$ = fixed effect of the interaction between Parity and Week, $D_i \times P_j \times W_k$ = fixed effect of the three-way interaction treatment diet × parity × week, $pDM(P_j)$ = pre-diet measurement nested within parity used as a covariate, $DIM(P_j)$ = days in milk nested within parity used as a covariate, and e_{ijkl} = residual error. Data from pre-treatment

measurements were used as covariates nested within parity to avoid masking measurements between primiparous and multiparous cows. We used DIM nested within parity as covariates due to the different lactation curve patterns between primiparous and multiparous cows. We used first-order regressive as the covariance structure for repeated analysis. The interactions between treatment diet × year, parity × year, and the three-way interaction treatment diet × year × parity were initially included in the model but removed from the final model. We tested normality using box plots, normal probability, and homogeneity of variances. Significant differences were declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$ for main effects and interactions. We used the Kenward-Roger method to adjust denominator degrees of freedom. All data were expressed as least square means and SEM unless otherwise specified.

CHAPTER 4

RESULTS

Initially, we tested the interactions between treatment × year, parity × year, and the three-way interaction treatment × year × parity. Overall, there were no interactions ($P \geq 0.16$), except for a few variables (Appendix Table A.1). We observed a tendency for a three-way interaction between treatment × year × parity for DMI ($P = 0.15$). However, this is not relevant to our study because CON and FAT did not differ within primiparous or multiparous cows in either year 1 or year 2 ($P \geq 0.21$). Similarly, the tendency for interaction between parity and year for lactose content ($P = 0.14$) and the interaction between parity and year for BW ($P = 0.01$) are not relevant either. Lactose content did not differ between year 1 and year 2 in primiparous cows ($P = 0.97$), while in multiparous cows, it was slightly higher in year 1 than in year 2 (4.89 vs. 4.83 ± 0.02 g/100g [mean ± SEM], $P = 0.02$). Compared with year 1, BW was higher in year 2 in both multiparous (764 vs. 698 ± 5.93 kg [mean ± SEM], $P < 0.01$) and primiparous cows (591 vs. 562 ± 7.9 kg [mean ± SEM], $P = 0.01$). As these interactions do not change our interpretation and to avoid overfitting the model, we removed them from the final analysis. However, the least squares means, SEM, and *P*-values obtained considering these interactions can be seen in Appendix Table A.1. Our study evaluated the differences between a control diet and a fat supplemented diet during warm weather on milk production, milk fatty acid profile, and blood metabolites. The FAT diet increased the yields of milk fat (+1.1%), ECM (+1.0%) and 3.5% FCM (+1.0%) compared to the CON diet (all $P < 0.05$; Table 4.1). Compared to cows fed CON, cows fed FAT had decreased milk protein and lactose concentrations (all $P < 0.05$; Table 4.1) The FAT diet increased ECM per unit DMI compared to CON. The FAT diet tended to increase

overall milk yield 2.9% compared to CON ($P = 0.08$). We saw no differences between FAT and CON for BW, BW change, BCS, or BCS change (all $P > 0.6$; Table 4.1).

Milk fatty acids (**FA**) are derived from only de novo FA (<16 carbon FA) synthesized in the mammary gland or only preformed FA (>16 carbon FA) extracted from plasma. Mixed-source FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis or extraction from plasma. FAT decreased de novo milk FA yield and increased yields of mixed and preformed milk FA (all $P < 0.05$; Table 4.2) compared to CON. The increased yields of mixed and preformed FA came primarily from increases in C16:0 and *cis*-9 C18:1 (both $P < 0.05$). FAT decreased de novo FA concentration and increased mixed FA concentration (all $P < 0.05$) but did not affect concentration of preformed FA in milk.

FAT increased plasma nonesterified fatty acid (**NEFA**) concentration ($P <0.001$) compared to CON with no effects on the concentration of plasma glucose or insulin (both $P > 0.5$; table 4.5). Compared to cows fed CON, cows fed FAT had greater NEFA concentration 1 h before and 4 h after feeding (all $P < 0.05$) with no difference at 7 h after feeding. Compared to a cool day, on a warm day NEFA concentration was greater at 7 h post feeding ($P = 0.01$). A three-way interaction between parity, day, and time was observed for plasma NEFA levels ($P = 0.05$). Primiparous cows fed FAT had greater NEFA concentration in plasma 1 h before feeding compared to those fed CON ($P < 0.001$) but not after feeding. Compared to primiparous cows fed FAT, multiparous cows fed FAT had less plasma NEFA before feeding but greater plasma NEFA concentration at 4 and 7 h after feeding (all $P < 0.05$).

Plasma glucose concentration was greater in all cows on a cool day compared to a warm day ($P < 0.001$). We observed an interaction between day and time ($P < 0.05$) because of high

glucose concentration on cool days at 4 and 7 h after feeding compared to warm days. There was a three-way interaction between treatment, parity, and time ($P < 0.05$). At 7 h after feeding primiparous cows fed FAT had higher glucose concentration than multiparous cows fed FAT ($P < 0.05$).

We observed an interaction between treatment, parity, and time for plasma insulin concentration ($P < 0.05$). At 4 h after feeding, multiparous cows fed CON and primiparous cows fed FAT had greater insulin concentration compared to multiparous cows fed FAT (all $P < 0.05$). At 7 h after feeding multiparous cows fed FAT had greater plasma insulin concentration than primiparous cows fed FAT or CON (all $P <0.05$).

Discussion

As we hypothesized, there was an increase in milk fat yield and a tendency for increased milk yield on the FAT treatment. This is consistent with previous work looking at the effects of fat supplementation on milk yield. Adding fat supplements containing PA+OA have had varied responses based on factors such as the ratio of PA:OA and cow production level (Prom & Lock, 2021; de Souza et al., 2019). Hu et al (2024) reported a linear increase in milk yield and a tendency to increase milk fat yield when OA content was increased in PA+OA supplements which supports the increases we saw in yields of milk and milk fat. Hu et al (2024) used cows averaging 47.9 kg/d for starting milk production which is similar to the production level of our cows, 46 kg/d. A study by de Souza et al. (2019) found higher producing cows have greater production responses to supplements higher in OA while lower producing cows have a greater response to supplements with more PA. Cows in our study averaged 46 kg/d of milk which corresponds with the low producing group in the study by de Souza et al. (2019). This potentially explains why we only observed a tendency for increased milk yield for the FAT treatment

compared to CON. Future work could evaluate varying ratios of PA:OA during the warm months to compare with our results.

In our study, treatment had no effect on body weight (**BW**) or BW change. There are inconsistent reports on fat supplementation affecting BW in cows. Cows supplemented with PA often have no change in BW and it is hypothesized that the additional energy from the fat supplement is being partitioned to milk instead of tissue reserves (de Souza et al., 2018; Mathews et al., 2016). When comparing supplements containing PA+OA to a control diet, supplements containing OA can lead to increased energy partitioning to body tissue reserves, which is determined by increased BW and BCS change, with a fatty acid profile similar to the supplement used in our study (de Souza et al., 2019; de Souza et al., 2018). This could explain why compared to CON, our FAT cows had no differences in overall BW or BW change but tended to increase milk yield. Regardless of treatment, the primiparous cows had greater BW change per day compared to multiparous cows, which is supported by similar findings by de Souza & Lock (2018). This is not surprising as primiparous animals are still growing and often partition nutrients towards body tissue during the first lactation (Tucker, 2000).

We observed that compared to CON, FAT increased yields of C16:0 and *cis*-9 C18:1 milk FA (both $P < 0.001$) but decreased yields of C4 to 14 FA ($P < 0.01$) resulting in an overall increase in milk fat yield. Given that we included the fat supplement at 2.35% of diet DM, the FAT diet provided an additional 302 g C16:0 and 198 g C18 FA (20 g C18:0, 149 g C18:1, and 18 g C18:2)` to the ration of the average cow based on the DMI of cows on the CON and FAT diets and the percent of individual FA in the supplement. Compared to CON, the milk FA from cows fed the FAT diet had an additional 67 g of C16 FA and an additional 43 g of C18 FA. Thus, 22% of the C16 and 22% of the C18 FA from the supplement were captured in milk in the FAT

treatment group. Based on the similar increase in C16 and C18 milk FA relative to their consumption and that decrease in C4 to 14 FA yield, we suggest that the increase in C16 is mostly from preformed sources or that the FAT diet greatly reduced the activity of thiolase in the de novo FA synthesis pathway. The increased C16 and C18 FA yields of cows fed FAT may also have been caused by changes in FA digestibility. Previous research has found that increasing the amount of OA in a PA+OA supplement increased the digestibility of 16C, 18C, and total FA, which may also have contributed to the greater yield of C16 and C18 FA in milk of cows fed FAT in our study (de Souza et al., 2021; Western, de Souza & Lock, 2020). Further research could investigate how the digestibility and absorption of a 60:30 PA:OA supplement affects milk FA composition compared to a CON diet.

Another explanation for cows on the FAT diet having lower de novo and greater preformed milk FA (both $P<0.01$; Table 4.2) than CON cows is mild milk fat depression from the fat supplement. The fat supplement fed in our studies contained *cis*-9 C18:1, and feeding fat supplements containing unsaturated fatty acids has been linked to lower milk fat compared to cows on a control diet or diets with saturated fat supplements (Bauman et al., 2003; Griinari et al., 1998). We observed greater yields of *trans* C18:1 milk FA, including *trans*-10 C18:1(P =0.03) and *trans*-11C18:1 (P =0.10; table 4.2) in cows on the FAT diet than the CON diet. Previous research has found that cows experiencing milk fat depression have increased yields of *trans*-10 C18:1 and *trans*-11 C18:1 compared to control cows from intermediates formed during biohydrogenation of unsaturated dietary FA (Bauman et al., 2003; Griinari et al., 1998). A potential for decreased de novo FA synthesis due to milk fat depression is the biohydrogenation itnermediates inhibiting milk fat synthesis in the mammary gland by reducing activity of acetyl-CoA carboxylase and fatty acid synthase (Buaman et al., 2003; Piperova et al., 2000).

Previous studies feeding a fat supplement to cows at a similar production level to cows used on our studies reported mixed results. de Souza et al (2019) observed a reduction in overall milk fat through lower yields of de novo and mixed milk FA for low producing cows. Western, de Souza & Lock (2020) found low producing cows had greater milk fat yield when fed a supplement containing PA compared to a supplement of PA+OA. In that study, the milk fat in cows fed PA contained 20 g more de novo FA and 2.7 g more PA, although there was 22 g less preformed FA.

Some of the milk fatty acid changes we observed may be attributed to body weight or BCS changes observed between our CON and FAT cows. We observed similar concentration of plasma insulin ($P = 0.94$) between CON and FAT treatments while FAT had greater plasma NEFA concentration ($P < 0.01$) compared to CON. A study by Palmquist (2006) reported when plasma NEFA concentration is high the mammary gland takes up more plasma NEFA for long chain milk FA production while Dorea, French & Armentano (2017) found that plasma NEFA levels are positively correlated with the amount of C18:1 in milk fat. We saw that cows on the FAT diet had greater plasma NEFA and yields of C18:1, including *cis*-9 C18:1, in milk fat compared to cows on the CON diet. This suggests that cows on the FAT diet were partitioning some of the additional plasma NEFA to milk fat which may have contributed to the greater milk fat yield compared to cows fed CON.

The increased NEFA concentration we observed in FAT cows is consistent with previous research into fat supplementation. FAT cows did have a greater intake of fatty acids which could partially explain this increase in plasma NEFA concentration. Previous studies have reported varying results increasing the amount of OA in fat supplements on plasma NEFA concentration using cows with similar production level to ours. Prom & Lock (2021) observed the greatest

NEFA increase in supplements containing 30% OA, while Hu et al (2024) reported a linear decrease in NEFA as OA content increased. However, when comparing a supplement containing 60% PA 30% OA to a PA supplement, cows on the PA supplement had lower NEFA concentration (Western, de Souza & Lock, 2020). Hu et al (2024) reported reduced BW loss as NEFA concentration decreased with greater OA content which could suggest NEFA are being partitioned to adipose tissue. Cows in our study did not have significant BW change from treatment, explaining the increased NEFA concentration.

The effects of increasing OA ratio in a fat supplement on plasma glucose have been varied in previous research. As the ratio of OA increased, Hu et al (2024) saw an increase in plasma glucose concentration while Abou-Rjeileh et al (2023) found a decrease in plasma glucose, and Akhlaghi et al (2019) reported no change in plasma glucose concentration. Our overall treatment effect on plasma glucose was not significant and fits best with Akhlaghi et al (2019). Hu et al (2024) reported increased NDF digestibility when feeding a supplement higher in OA that could result in increased production of propionate, a glucose precursor. This could explain why we saw increased plasma glucose concentration in FAT cows after feeding compared to glucose concentration before feeding, although it does not explain the interaction with parity.

The effects of FAT on plasma insulin concentration follow the pattern of plasma glucose concentrations we observed. At 4 h after feeding, when multiparous cows fed FAT had greater glucose concentrations than primiparous cows fed FAT, the opposite was seen for plasma insulin concentration, so primiparous cows fed FAT had greater plasma insulin concentration than multiparous cows fed FAT at 4 h after feeding. Similarly, at 7 h after feeding, when FAT primiparous cows had greater plasma glucose than FAT multiparous cows, insulin concentration was greater in FAT multiparous cows than FAT primiparous cows. A potential reason that cows

fed CON did not have the same response as cows fed FAT, regardless of parity, is heat stress is often linked with decreased plasma glucose when compared to PFTN cows (Gao et al., 2017; Baumgard et al., 2011). It is interesting that FAT multiparous cows had plasma glucose and insulin concentrations opposite to primiparous cows. A possible explanation for FAT multiparous cows, compared to FAT primiparous cows, having greater plasma glucose at four hours after feeding and lower glucose at seven hours after feeding is the greater intake of feed ($P < 0.01$). The greater intake may have caused greater propionate production and increased glucose synthesis. This glucose could have contributed to the observed increased production of lactose ($P < 0.01$) in multiparous cows compared to primiparous cows as the mammary gland uses most of the plasma glucose in lactating cows (De Koster & Opsomer, 2013). Further research could investigate parity specific effects on plasma insulin, glucose, and NEFA concentration.

Table 4.1 Effects of fat supplementation on milk yield, milk composition, BW, BCS, and efficiency

	Treatments[1]		SEM	P Value
	CON	FAT		Trt
DMI (kg)	24.8	24.5	0.33	0.45
Yields (kg/d)				
Milk	40.5	41.7	0.88	0.08
Fat	1.44	1.52	0.03	0.03
Protein	1.21	1.22	0.02	0.47
Lactose	1.99	2.03	0.03	0.24
ECM	40.5	42.1	0.76	0.04
FCM	40.9	42.8	0.81	0.02
Milk Composition (%)				
Fat	3.66	3.73	0.07	0.32
Protein	2.99	2.94	0.02	0.02
Lactose	4.92	4.88	0.02	0.06
Efficiency Values				
Feed Efficiency (ECM/DMI)	1.63	1.71	0.02	<0.01
Milk Energy (Mcal/kg)	0.66	0.65	0.01	0.90
BW (kg)	652	653	7.07	0.81
BW Change (kg/d)	0.42	0.40	0.05	0.76
BCS	3.12	3.12	0.03	0.97
BCS Change (Unit/6wk)	-0.10	-0.08	0.03	0.63

[1]Treatments were a control diet or a diet containing a 60% palmitic 30% oleic fat supplement at 2.35% of diet DM.

Table 4.2 Yield of milk FA for dietary treatment[1]

Summations of milk FA[3,4]	Treatments[2]		SEM	P Value
	CON	FAT		Trt
FA yield (g/d)				
De novo	383	341	10.9	<0.01
Mixed	551	624	14.4	<0.01
Preformed	503	544	12.0	0.01
Selected individual FA (g/d)				
C4:0	40.7	43.6	1.45	0.16
C6:0	29.3	28.0	1.99	0.37
C8:0	17.7	15.6	0.58	0.01
C10:0	48.5	39.4	1.68	<0.01
C12:0	57.7	46.0	2.01	<0.01
C14:0	178	157	4.75	<0.01
C16:0	532	597	15.0	<0.01
Cis-9 C16:1	17.7	20.0	0.59	<0.01
C18:0	136	136	4.82	0.97
trans-6-8 C18:1	3.32	4.62	0.11	<0.01
trans-9 C18:1	2.48	3.46	0.08	<0.01
trans-10 C18:1	6.17	8.87	0.88	0.03
trans-11 C18:1	10.5	11.7	0.49	0.10
cis-9 C18:1	231	266	6.04	<0.01
cis-9, *cis*-12 C18:2	29.6	31.3	0.91	0.19
cis9, cis-12, *cis*-15 C18:3	3.48	3.36	0.11	0.41

[1](From table title) Samples for milk FA were collected during wk 6 of each study.

[2]Treatments were a control diet or a diet containing a 60% palmitic 30% oleic fat supplement at 2.35% of diet DM.

[3]De novo = Milk FA <16 carbons in length; mixed = milk FA 16 carbons in length; preformed = milk FA > 16 carbons in length

[4]Additional FA measured were not included in this list so selected individual FA values may not add up to summation values

Table 4.3 Concentration of milk FA for dietary treatment[1]

	Treatments[2]		SEM	P Value
Summations of milk FA[3,4]	CON	FAT		Trt
FA Concentration (g/100g)				
De novo	26.6	22.4	0.28	<0.001
Mixed	38.2	41.5	0.41	<0.001
Preformed	35.2	36.1	0.45	0.18
Selected individual FA (g/100g)				
C4:0	2.83	2.86	0.08	0.74
C6:0	2.03	1.85	0.05	<0.01
C8:0	1.23	1.03	0.01	<0.01
C10:0	3.36	2.58	0.06	<0.01
C12:0	3.99	3.02	0.08	<0.01
C14:0	12.3	10.3	0.17	<0.01
C16:0	36.9	40.0	0.40	<0.01
cis-9 C16:1	1.26	1.36	0.03	0.04
C18:0	9.58	9.03	0.22	0.08
trans-6-8 C18:1	0.22	0.29	<0.1	<0.01
trans-9 C18:1	0.17	0.22	<0.1	<0.01
trans-10 C18:1	0.36	0.44	0.02	<0.01
trans-11 C18:1	0.70	0.76	0.02	0.08
cis-9 C18:1	16.2	17.7	0.25	<0.01
cis-9, *cis*-12 C18:2	2.06	2.05	0.04	0.94
cis-9, cis-12, *cis*-15 C18:3	0.24	0.22	<0.1	<0.01

[1](From table title) Samples for milk FA were collected during wk 6 of each study.

[2]Treatments were a control diet or a diet containing a 60% palmitic 30% oleic fat supplement at 2.35% of diet DM.

[3]De novo = Milk FA <16 carbons in length; mixed = milk FA 16 carbons in length; preformed = milk FA > 16 carbons in length

[4]Additional FA measured were not included in this list so selected individual FA values may not add up to summation values

Table 4.4 Comparing blood metabolite response to fat supplementation broken out by time relative to feeding[1] and dietary treatment[2] by day

	CON				FAT				P Value	
Variable	-1 Hr	+4 Hr	+7 Hr	SEM	-1 Hr	+4 Hr	+7 Hr	SEM	Trt	Trt* Day
Cool Day[3]										
Insulin (µg/L)	5.37	13.6	10.5	0.73	5.30	13.4	10.7	0.72	0.94	0.82
Glucose (mg/dL)	57.5	49.3	56.4	1.02	57.8	48.2	56.0	0.99	0.59	0.72
NEFA (mEq/L)	0.104	0.084	0.084	0.006	0.174	0.092	0.089	0.006	<0.01	0.70
Warm Day[4]										
Insulin (µg/L)	6.24	12.7	9.92	0.73	5.55	12.3	11.4	0.71	0.94	0.82
Glucose (mg/dL)	57.9	46.2	51.9	0.99	55.1	47.4	51.6	0.99	0.59	0.72
NEFA (mEq/L)	0.113	0.073	0.092	0.006	0.172	0.094	0.106	0.006	<0.01	0.70

[1](From table title) Cows were fed at 1000 h. Blood was drawn at 0900 h, 1400 h, and 1700 h.
[2](From table title) Treatments were a control diet or a diet containing a 60% palmitic 30% oleic fat supplement at 2.35% of diet DM.
[3]Cool day refers to 7/18/2023 when the temperature was 23.8° C and the average yearly temperature for 7/18 is 27.7° C.
[4]Warm day refers to 8/4/2023 when the temperature was 27.7° C and the yearly average for 8/4 is 23.8°C

CHAPTER 5

OVERALL CONCLUSIONS

Fat supplements are commonly fed in the dairy industry to increase milk production as well as change the milk fatty acid profile. Because fats are energy dense and their digestion and metabolism produces less heat than dietary carbohydrates, theoretically fats should be especially beneficial for cows that are heat stressed. Based on studies demonstrating that heat stress occurs once THI is 68 or higher, the cows in our study were experiencing heat stress for most of the treatment period. Several studies have researched the impact of fat supplementation on heat stressed dairy cows, but results are not conclusive. We believe one reason that results have been inconsistent is that different fat supplements have different FA profiles. Recent work at Michigan State University demonstrated that a calcium salt containing 60% C16:0 30% *cis*-9 C18:1 is effective at increasing milk production in mid-lactation (de Souza, St-Pierre & Lock, 2019). The objective of my study was to determine if feeding a calcium salt fat supplement containing 60% C16:0 30% *cis*-9 C18:1 would improve milk production of cows during heat stress, and thus serve as a means to reduce the effects of heat stress. We also measured blood glucose, insulin, and NEFA, in an effort to understand the mechanisms responsible for observed responses.

We found that fat supplementation significantly increased the yields of milk fat and ECM and tended to increase milk yield. There was no change observed for other milk component yields, BW, or BCS. Increased yields of milk and milk fat are likely a result of increased energy density of the diet and the greater intake of fatty acids from the supplement. We did not observe a body weight change effect from fat supplementation, which suggests the additional FA were being partitioned to milk. Fat supplementation increased plasma NEFA concentration, likely from the additional fatty acid intake.

In conclusion, our study showed that feeding a calcium salt fat supplement containing 59% C16:0 and 29% *cis*-9 C18:1 at 2.35% of diet DM increased milk production in heat stressed dairy cows. Although the cows in this study were heat-stressed, based on THI averaging 71.2 and reaching a maximum of 87.0, the THI did not reach levels experienced by heat stressed cows in warmer climates. Future research should focus on production effects of this supplement on cows during periods with a higher THI. In addition, studies comparing effects of this fat supplement in both heat-stressed and cooled cows at the same time would help determine if the fat supplement specifically reduces heat stress.

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APPENDIX

Table A.1 Comparison of production between treatments[1] by parity showing that interactions with year were not significant

	Primi		Multi			P-value		
Item	CON	FAT	CON	FAT	SEM	Trt × year	Parity × year	Trt × Parity x year
DMI (kg)	22.2	21.9	27.6	27.3	0.47	0.58	0.23	0.15
Yields (kg/d)								
Milk	34.0	35.0	47.1	48.5	1.03	0.74	0.90	0.65
Fat	1.34	1.39	1.57	1.66	0.05	0.16	0.24	0.35
Protein	1.04	1.05	1.38	1.39	0.04	0.65	0.40	0.63
Lactose	1.70	1.72	2.29	2.35	0.05	0.95	0.74	0.61
ECM	35.8	36.8	45.5	47.4	1.12	0.24	0.42	0.38
FCM	36.3	37.5	45.8	48.1	1.10	0.17	0.42	0.39
Milk Composition (%)								
Fat	3.95	4.03	3.40	3.45	0.08	0.24	0.22	0.42
Protein	3.06	3.01	2.95	2.88	0.04	0.42	0.19	0.67
Lactose	4.96	4.93	4.88	4.85	0.02	0.24	0.14	0.99
Feed Efficiency (ECM/DMI)	1.61	1.69	1.66	1.74	0.02	0.56	0.66	0.25
BW (kg)	574	579	734	729	6.88	0.79	0.01	0.32
BW Change (kg/d)	0.46	.050	0.35	0.30	0.06	0.22	0.20	0.16
BCS	3.11	3.14	3.16	3.12	0.03	0.84	0.63	0.43
BCS Change (Unit/6wk)	-0.09	-0.06	-0.11	-0.11	0.04	0.34	0.48	0.28

[1]Treatments were a control diet or a diet containing a 60% palmitic 30% oleic fat supplement at 2.35% of diet DM.

Table A.2 Comparison of production and plasma metabolite concentrations between treatments[1] by parity

	Primi		SEM	Multi		SEM	
	CON	FAT		CON	FAT		Trt*parity
Milk yield and composition							
DMI (kg)	22.1	21.8	0.53	27.5	27.2	0.39	0.98
Milk (kg/d)	33.9	35.0	1.14	47.0	48.5	0.87	0.82
ECM[2] (kg/d)	35.6	36.8	1.23	45.5	47.4	0.92	0.68
Fat (%)	3.93	4.03	0.12	3.40	3.44	0.08	0.71
Fat (kg/d)	1.32	1.39	0.06	1.57	1.66	0.04	0.78
Protein (%)	3.05	3.05	0.03	2.94	2.88	0.03	0.77
Protein (kg/d)	1.03	1.05	0.03	1.37	1.39	0.02	0.96
Lactose (%)	4.96	4.92	0.03	4.87	4.84	0.02	0.92
Lactose (kg/d)	1.69	1.72	0.05	2.29	2.34	0.05	0.72
BW and BCS							
BW (kg)	570	578	11.3	733	729	8.47	0.40
BW change (kg/d)	0.47	0.51	0.09	0.36	0.29	0.07	0.37
BCS	3.10	3.14	0.05	3.15	3.11	0.04	0.30
BCS change	-0.09	-0.05	0.06	-0.10	-0.11	0.04	0.54
Plasma Metabolites							
Glucose (mg/dL)	53.3	52.7	1.37	53.1	52.7	1.16	0.94
NEFA (μM)	0.09	0.13	0.01	0.09	0.11	0.01	0.07
Insulin (μg/L)	9.59	9.65	0.95	9.87	9.90	0.80	0.97

[1]Treatments were a control diet or a diet containing a 60% palmitic 30% oleic fat supplement at 2.35% of diet DM.

Table A.3 Comparison of production between treatments[1] by parity for Year 1

	Primi		SEM	Multi		SEM	P Value	
	CON	FAT		CON	FAT		Trt	Trt *parity
Milk yield and composition								
DMI (kg)	22.7	21.6	0.87	27.1	27.2	0.5	0.32	0.31
Milk (kg/d)	34.9	34.3	2.14	47.1	48.9	1.41	0.64	0.36
ECM[2] (kg/d)	37.0	35.6	2.25	45.0	46.7	1.44	0.91	0.25
Fat (%)	4.01	3.91	0.15	3.28	3.33	0.10	0.81	0.44
Fat (kg/d)	1.39	1.34	0.09	1.54	1.61	0.06	0.89	0.28
Protein (%)	3.01	2.97	0.05	2.87	2.81	0.03	0.11	0.87
Protein (kg/d)	1.05	1.02	0.06	1.35	1.37	0.03	0.85	0.42
Lactose (%)	4.94	4.94	0.02	4.89	4.88	0.01	0.69	0.83
Lactose (kg/d)	1.73	1.70	0.10	2.30	2.38	0.06	0.75	0.38
BW and BCS								
BW (kg)	564	560	21.3	698	697	13.8	0.85	0.91
BW change (kg/d)	0.27	0.40	0.23	0.48	0.34	0.15	0.96	0.33
BCS	3.20	3.17	0.16	3.15	3.11	0.10	0.71	0.91
BCS change	<0.01	-0.05	0.10	-0.02	-0.03	0.06	0.65	0.68

[1]Treatments were a control diet or a diet containing a 60% palmitic 30% oleic fat supplement at 2.35% of diet DM.

Table A.4 Comparison of production between treatments[1] by parity for Year 2

	Primi		SEM	Multi		SEM	P Value	
	CON	FAT		CON	FAT		Trt	Trt*Parity
Milk yield and composition								
DMI (kg)	21.7	22.0	0.65	27.9	27.2	0.51	0.60	0.29
Milk (kg/d)	33.9	34.7	0.99	47.0	47.9	0.83	0.18	0.95
ECM[2] (kg/d)	35.4	37.4	1.35	46.0	47.8	1.10	0.03	0.92
Fat (%)	3.88	4.17	0.16	3.50	3.54	0.13	0.13	0.24
Fat (kg/d)	1.30	1.43	0.07	1.59	1.71	0.06	0.01	0.87
Protein (%)	3.12	3.04	0.04	3.02	2.95	0.03	0.01	0.98
Protein (kg/d)	1.06	1.06	0.03	1.41	1.41	0.02	0.99	0.83
Lactose (%)	4.96	4.91	0.05	4.86	4.80	0.04	0.12	0.85
Lactose (kg/d)	1.68	1.70	0.05	2.27	2.30	0.04	0.43	0.85
BW and BCS								
BW (kg)	585	594	5.59	769	760	4.67	0.90	0.02
BW change (kg/d)	2.78	3.46	0.81	1.04	1.46	0.65	0.30	0.80
BCS	3.10	3.10	0.06	3.15	3.11	0.04	0.60	0.62
BCS change	-0.12	-0.12	0.06	-0.16	-0.20	0.04	0.60	0.62

[1]Treatments were a control diet or a diet containing a 60% palmitic 30% oleic fat supplement at 2.35% of diet DM.

Table A.5 Comparison of milk FA yield between treatments[1] by parity[2]

	Primi		SEM	Multi		SEM	P Value
Summations of milk FA[3]	CON	FAT		CON	FAT		Trt*Parity
FA yield (g/d)							
De novo	343	299	17.3	423	383	12.8	0.89
Mixed	523	563	23.0	580	685	17.2	0.10
Preformed	462	485	18.9	544	603	14.7	0.29
Selected individual FA (g/d)							
C4:0	36.7	38.2	3.30	44.7	49.0	2.47	0.52
C6:0	26.8	25.0	2.25	31.8	31.0	1.68	0.70
C8:0	16.2	13.9	1.32	19.2	17.3	0.98	0.83
C10:0	43.9	34.9	3.79	53.1	43.9	2.83	0.96
C12:0	52.1	40.7	4.56	63.4	51.3	3.41	0.88
C14:0	158	138	10.8	198	178	8.06	0.94
C16:0	493	545	35.3	570	650	26.6	0.51
Cis-9 C16:1	16.4	17.4	1.32	19.0	22.7	1.01	0.10
C18:0	130	129	10.9	144	145	8.19	0.82
trans-6-8 C18:1	2.83	3.83	0.25	3.81	5.41	0.19	0.06
trans-9 C18:1	2.24	3.00	13.7	2.72	3.93	10.2	0.04
trans-10 C18:1	5.17	6.34	1.99	7.17	11.4	1.49	0.22
trans-11 C18:1	9.49	10.1	1.12	11.6	13.2	0.84	0.44
cis-9 C18:1	215	240	13.7	248	294	10.2	0.21
cis-9, *cis*-12 C18:2	25.2	25.6	2.05	33.9	36.9	1.53	0.32
cis9, cis-12, *cis*-15 C18:3	3.06	2.81	0.25	3.91	3.90	0.18	0.43

[1](From table title) Samples for milk FA were collected during wk 6 of each study.
[2]Treatments were a control diet or a diet containing a 60% palmitic 30% oleic fat supplement at 2.35% of diet DM.
[3]De novo = Milk FA <16 carbons in length; mixed = milk FA 16 carbons in length; preformed = milk FA > 16 carbons in length

Table A.6 Comparison of milk FA yield between treatments[1] by parity for Year 1

	Treatments[2]		SEM	P Value
Summations of milk FA[4]	CON	FAT		Trt
FA yield (g/d)				
De novo	385	319	25.7	0.01
Mixed	590	623	36.0	0.36
Preformed	507	536	25.8	0.26
Selected individual FA (g/d)				
C4:0	41.3	41.6	3.61	0.95
C6:0	29.4	25.9	2.48	0.17
C8:0	17.5	14.1	1.42	0.02
C10:0	47.4	34.6	3.83	<0.01
C12:0	56.9	40.8	4.34	<0.01
C14:0	181	151	10.8	0.01
C16:0	572	602	35.1	0.39
Cis-9 C16:1	18.1	20.3	1.38	0.12
C18:0	138	130	10.4	0.44
trans-6-8 C18:1	3.54	4.74	0.28	<0.01
trans-9 C18:1	2.52	3.48	0.19	<0.01
trans-10 C18:1	5.71	7.18	0.69	0.04
trans-11 C18:1	10.3	10.6	1.11	0.77
cis-9 C18:1	234	271	12.9	0.01
cis-9, *cis*-12 C18:2	28.3	29.3	2.00	0.61
cis9, cis-12, *cis*-15 C18:3	3.49	3.43	0.28	0.85

[1]Treatments were a control diet or a diet containing a 60% palmitic 30% oleic fat supplement at 2.35% of diet DM.

Table A.7 Comparison of milk FA yield between treatments[1] by parity for Year 2

Summations of milk FA[4]	Treatments[2]		SEM	P Value
	CON	FAT		Trt
FA yield (g/d)				
De novo	385	363	18.9	0.25
Mixed	507	614	26.5	<0.01
Preformed	500	545	21.7	0.04
Selected individual FA (g/d)				
C4:0	40.0	45.7	2.25	0.01
C6:0	29.3	30.1	1.51	0.60
C8:0	18.1	17.2	0.93	0.34
C10:0	50.2	44.1	3.00	0.04
C12:0	59.4	51.0	3.88	0.03
C14:0	176	164	8.57	0.16
C16:0	488	593	25.4	<0.01
Cis-9 C16:1	19.0	21.5	1.39	0.08
C18:0	136	143	9.17	0.41
trans-6-8 C18:1	3.06	4.47	0.17	<0.01
trans-9 C18:1	2.43	3.45	0.13	<0.01
trans-10 C18:1	5.13	6.75	0.34	<0.01
trans-11 C18:1	10.5	12.7	0.88	0.02
cis-9 C18:1	228	263	11.9	0.01
cis-9, *cis*-12 C18:2	30.3	32.7	1.40	0.09
cis9, cis-12, *cis*-15 C18:3	3.49	3.28	0.16	0.21

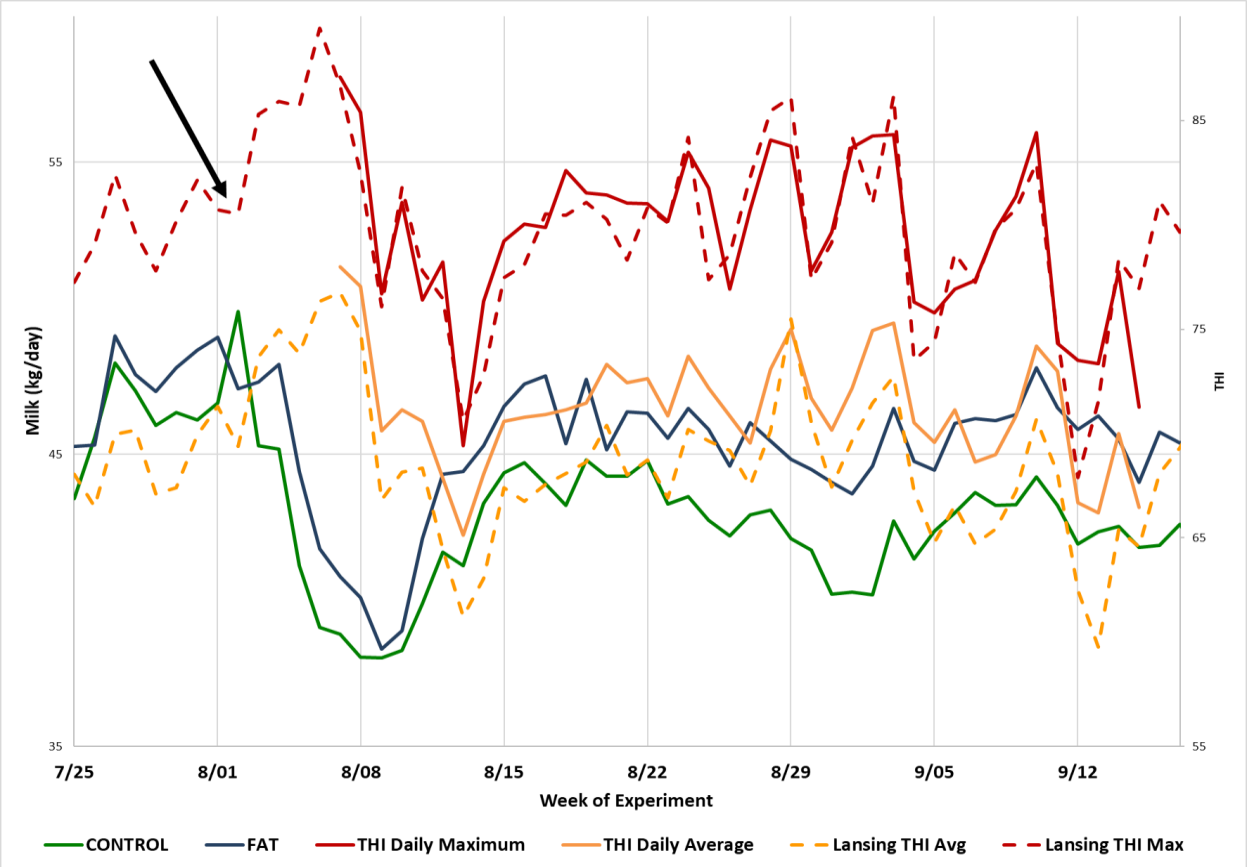
[1]Treatments were a control diet or a diet containing a 60% palmitic 30% oleic fat supplement at 2.35% of diet DM.

Table A.8 Difference in income per cow between treatments1 based on additional cost of supplement for Year 2

	Treatments		
	CON	FAT	Difference
Milk Income ($/cow/d)[2]	$14.27	$15.55	$1.28
Fat Supplement Cost ($/cow/d)[3]		$0.85	$0.85
Extra Revenue[4]			$0.43

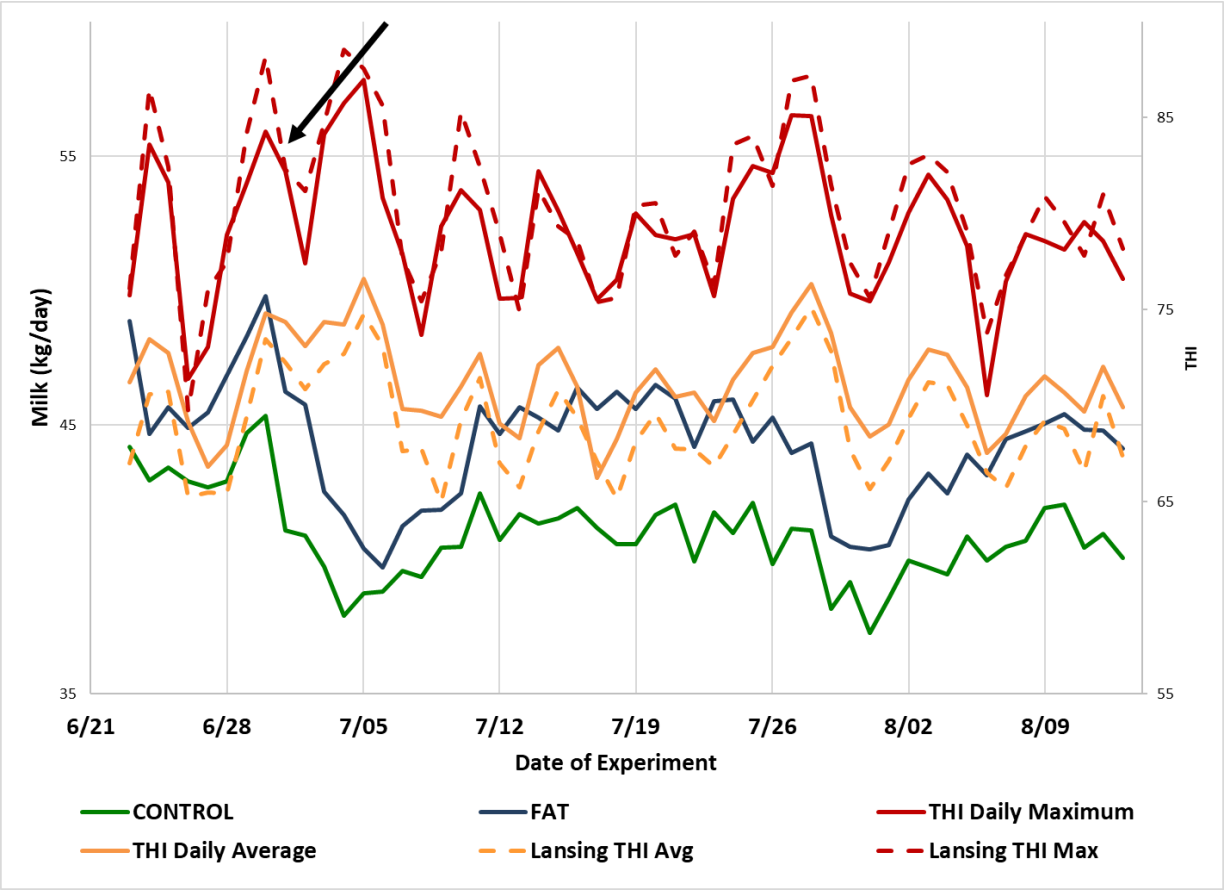
[1]Treatments were a control diet or a diet containing a 60% palmitic 30% oleic fat supplement at 2.35% of diet DM
[2]Milk income based on the average milk component values for each treatment and prices from USDA records from July and August 2023
[3]Supplement cost is based off of the average daily DMI of each treatment and the supplement cost of $1500/ton
[4]Revenue is calculated by subtracting the cost of the fat supplement from the milk income of each group

Figure A.1 Comparison of average daily milk yield by treatment and average and maximum daily THI for Year 1[1]



[1]Black arrow indicates start date for treatments in Year 1- August 2, 2022

Figure A.2 Comparison of average daily milk yield by treatment and average and maximum daily THI for Year 2[1]



[1]Black arrow indicates start date of treatments for Year 2- July 1, 2023