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EFFECT OF EXOGENOUS EMULSIFIER AND PALMITIC ACID USE IN TOTAL MIXED RATION ON MILK PRODUCTION, COMPOSITION, AND FATTY ACID PROFILE OF CONFINED DAIRY COWS

(Efeito do uso de emulsificante exógeno e ácido palmítico na ração totalmente misturada sobre a produção, composição e perfil de ácidos graxos do leite de vacas mantidas em confinamento)

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EFEITO DO USO DE EMULSIFICANTE EXÓGENO E ÁCIDO PALMÍTICO NA RAÇÃO TOTALMENTE MISTURADA SOBRE A PRODUÇÃO, COMPOSIÇÃO E PERFIL DE ÁCIDOS GRAXOS DO LEITE DE VACAS MANTIDAS EM CONFINAMENTO

Dissertação apresentada ao programa de Pós-Graduação em Ciência Animal, da Universidade do Estado de Santa Catarina, como requisito parcial para obtenção do título de Mestre em Ciência Animal, área de concentração em Produção Animal.
Orientador: Prof. Dr. André Thaler Neto.

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Mel, teu calor no meu colo me fez muita falta, mas espero que daí de cima tu tenhas visto onde chegamos.

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ABSTRACT

The objective of this study was to evaluate the supplementation of lactating dairy cows with palmitic acid (PA) in their total mixed ration (TMR), to assess whether it improves milk production, composition, and fatty acid profile, and also to investigate whether the inclusion of the exogenous soy lecithin emulsifier promotes a synergistic effect on these parameters. Sixty Holstein cows were used, selected according to days in milk (DIM), individual milk yield, and parity, and equally divided among 3 treatments: 1) Control (C – no fat); 2) Palmitic Acid without emulsifier (FW); and 3) Palmitic Acid with emulsifier (FE). They received a basal diet composed of corn silage, wheat straw, protein concentrate, and bicarbonate. The lipid supplement (89% C16:0) was supplied as part of the TMR, as was the emulsifier, subsequently added to the feed bunk in the corresponding groups and mixed with the aid of a fork. The experimental period totaled 21 days, divided into 14 day of adaptation and 7 days of data collection. Feed intake was measured for each group and milk yield individually from 3x/d milking. Milk samples were collected on the 3 milkings for 5 days, for analysis of composition and fatty acid profile. Statistical analysis was conducted using a mixed-effect model, considering treatment as a fixed effect and the animal as a random effect. Because of non-normality of the majority, the data were analysed using non-parametric comparison, firstly the Kruskal-Wallis test for the main variables. When $p \leq 0.10$ the paired Mann-Whitney test with Bonferroni correction was applied, with $P \leq 0.05$ considered as statistically significant, and between 0.05 and 0.10 considered a tendency towards significance. Neither PA supplementation nor the exogenous emulsifier were detected to affect milk yield. However, there was a tendency towards greater 3.5%FCM and ECM in the FW and FE groups. FE treatment improved lactose content and defatted dry extract, and reduced milk urea nitrogen content. An increase in unsaturated fatty acids (UFA), mainly monounsaturated (MUFA), also stearic acid, oleic acid and acetate was detected in milk, devoid of alterations in FA preformed (Pref), *de novo* (DeNov) and Mixed groups. PA and emulsifier had limited effects on overall production responses, so it remains to be investigated practical feeding strategies that can be easily applied in daily farm management, and the use of other supplemental doses of emulsifier.

Keywords: Nutrition; Intake; Fat profile; TMR; Holstein.

RESUMO

O objetivo deste estudo foi avaliar a suplementação de vacas lactantes com ácido palmítico na ração totalmente misturada (TMR), para analisar se o ácido palmítico aumenta a produção, composição e perfil de ácidos graxos do leite, além de investigar se a inclusão do emulsificante exógeno lecitina de soja promoveria um efeito sinergético nesses parâmetros. Foram usadas 60 vacas, selecionadas de acordo com os dias em lactação (DEL), produção individual de leite, e ordem de parto, divididas igualmente entre 3 tratamentos: 1) Controle (C – sem gordura); 2) Ácido palmítico sem emulsificante (FW) e; 3) Ácido palmítico com emulsificante (FE). Elas receberam uma dieta base composta de silagem de milho, palha de trigo, concentrado proteico e bicarbonato. O suplemento lipídico (89% de C16:0) foi fornecido compondo a TMR, bem como o emulsificante, posteriormente adicionados à pista nos lotes correspondentes e misturados com o auxílio de um garfo. O período experimental totalizou 21 dias, dividido em 14 dias de adaptação e 7 de coleta de dados. O consumo foi medido por cada grupo e a produção individual das 3 ordenhas diárias. Amostras de leite foram coletadas nas 3 ordenhas por 5 dias, para análise de composição e perfil de ácidos graxos. A análise estatística foi conduzida usando um modelo misto, considerando o tratamento como efeito fixo e o animal como efeito aleatório. Por causa da não normalidade da maioria, os dados foram analisados usando comparações não-paramétricas, primeiramente o teste Kruskal-Wallis para as principais variáveis. Quando $p \leq 0.10$ o teste pareado de Mann-Whitney com correção de Bonferroni foi aplicado, com ≤ 0.05 considerado estatisticamente significante, e entre 0.05 e 0.10 considerado uma tendência à significância. Nem a suplementação com ácido palmítico nem o emulsificante exógeno elevaram a produção de leite. Mas, houve uma tendência a aumentos em leite corrigido para fibra (3.5%FCM) e leite corrigido para energia (ECM) nos grupos FW e FE. O tratamento FE aumentou o teor de lactose e de extrato seco desengordurado (DDE), e reduziu o teor de nitrogênio ureico do leite (MUN%). Um aumento nos ácidos graxos insaturados, principalmente mono-insaturados, além de ácido esteárico, oleico e acetato foi visto, sem alterações nos grupos de ácidos graxos pré-formados, de novo e mistos. O ácido palmítico e o emulsificante tiveram efeitos limitados sobre respostas produtivas, permanecendo a necessidade de investigar estratégias nutricionais práticas que possam ser aplicadas no manejo diário das fazendas, além do uso de outras doses suplementais do emulsificante.

Palavras-chave: Nutrição; Consumo; Perfil de gordura; TMR; Holandês.

TABLES LIST

ABBREVIATIONS LIST

PA	Palmitic acid
DMI	Dry matter intake
FA	Fatty acid
TMR	Total mixed ration
3.5%FCM	3.5% Fiber corrected milk
ECM	Energy corrected milk
CLA	Conjugated linoleic acid
HF	Heart frequency
IBT	Internal body temperature
4%FCM	4% Fiber corrected milk
NEFA	Non-sterified fatty acids
SFA	Saturated fatty acids
MUFA	Mono-unsaturated fatty acids
PUFA	Poli-unsaturated fatty acids
RSPO	Rountable on Sustainable Palm Oil
WWF	World Wildlife Fund WWF
FAO	Food and Agriculture Organization of the United States
GIT	Gastrointestinal tract
VLDL	Very low density lipoprotein
MUN	Milk urea nitrogen
N	Nitrogen
DM	Dry matter
OM	Organic matter
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
TDM	Total dry matter
MM	Mineral matter
EE	Ether extract
THI	Temperature and humidity index

DDE	Deffated dry extract
ProdLact	Production of lactose in milk
Lact%	Content of lactose in milk
MUN%	Content of milk urea nitrogen
ProdFat	Production of fat in milk
Case%	Content of casein in milk
MTS%	Content of milk total solids
ProdMTS	Production of milk total solids
Fat%	Content of fat in milk
ProdP	Production of protein in milk
UFA	Unsaturated fatty acid
Stearic	Stearic acid
Oleic	Oleic acid
Acet	Acetate
Palm	Palmitic acid
Pref	Preformed fatty acids
Mixed	Mixed origin fatty acids
DeNov	De novo fatty acids
BHB	Beta-hydroxybutyrate
MM	Mineral matter
TDM	Total dry matter
MUN	Milk urea nitrogen

SUMMARY

INTRODUCTION

In dairy cattle farming, it is economically important to maximize the productive performance of animals, which directly depends on factors, such as genetic potential and the quality of nutrition provided. Diet plays a central role in this process, directly impacting milk production and economic returns for the producer. Corn is widely used as an energy source in diets for lactating cows; however, the growing concern about excessive starch and its negative effects on ruminal fermentation has driven the use of lipid supplements as a strategic alternative.

The inclusion of lipids in the diet can increase energy density without compromising the digestibility of the fibrous fraction, allowing for an increased proportion of forage and reducing the risk of ruminal disturbances caused by rapidly fermentable carbohydrates (BAUMAN et al., 2011). Among lipid supplements, sources with high concentrations of long-chain saturated fatty acids, such as C16:0 (palmitic acid) and C18:0 (stearic acid), have been widely studied due to their influence on milk production and fatty acid profile (LOFTEN et al., 2014; HARVATINE et al., 2006).

Evidence indicates that palmitic acid (PA) supplementation can increase milk production and its components without reducing dry matter intake (DMI) (MATHEWS et al., 2016). However, the effects of palmitic acid addition to the diet on productive performance are still inconsistent, with studies reporting that supplementation either increased DMI (MOSLEY et al., 2007), decreased DMI (LOCK et al., 2013; RICO et al., 2014; GIAGNONI et al., 2025), or did not alter DMI (PIANTONI et al., 2013; de SOUZA et al., 2017; de SOUZA et al., 2018) compared to a control group. Furthermore, the inclusion of certain types of fatty acids (FA) in the diet can alter the ingestive behavior of cows, promoting satiety and reducing voluntary intake (RICO et al., 2014).

Due to the wide variety of results with the use of lipidic supplements, greater clarity is sought regarding their effects on production and feed efficiency, as the profitability of their use is essential for this energy source to become a viable option for high-producing farms. When evaluating a lipid supplement, the inherent characteristics of the fat—such as its source, composition, and presentation form—are determining factors for its utilization. Furthermore, toxicity to ruminal microorganisms depends on the type of fat (GIAGNONI et al., 2025).

Another challenge associated with the use of large quantities of fat in ruminant diets is the limited physiological capacity for secreting endogenous emulsifiers, such as lysolecithin and bile salts, which are essential for lipid emulsification and digestion in the small intestine (DRACKLEY, 2000; CUNNINGHAM et al., 2004). The digestibility of fatty acids tends to decrease with increased lipid flow to the duodenum, especially when fatty acids are saturated or long-chain (BOERMAN et al., 2015; de SOUZA et al., 2020).

To optimize the utilization of supplemented fatty acids, it is necessary to improve the processes of emulsification, micelle formation, and intestinal absorption. Thus, the provision of exogenous emulsifiers can represent an effective strategy to expand lipid digestion capacity beyond physiological limits, resulting in greater absorption and potentially improved productive performance (de SOUZA et al., 2020).

Studies involving post-ruminal infusions of exogenous emulsifiers have demonstrated increases in the absorption of total fatty acids, C16 and C18, as well as an increase in milk fat secretion, including *de novo*, fatty acids formed in the mammary gland, with C4:0 to C14:0 and some C16:0; preformed, fatty acids from corporal mobilization of the adipose tissue from plasma or from the feed, half of the C16:0 and the others with more than 16C; mixed, some from de novo and some from mobilization, majoritarian C16:0 and C16:1*de novo*, mixed, and preformed components (BIONAZ et al., 2020; de SOUZA et al., 2020). Subsequently, dos Santos Neto et al. (2023) compared ruminal and abomasal infusions of an exogenous emulsifier (polysorbates-C18:1), observing similar positive effects, suggesting that ruminal protection of the emulsifier might not be necessary.

However, to date, there are no reports of experiments evaluating the provision of exogenous emulsifiers directly in the total mixed ration (TMR) of dairy cows, with the aim of verifying their effects on milk production, composition, and fatty acid profile, especially in association with supplements rich in palmitic acid (~89% C16:0). This gap reinforces the need to investigate practical feeding strategies that can be easily applied in daily farm management.

Given this context, the present study had the general objective of evaluating the effect of supplying fat composed primarily of palmitic acid (89% C16:0) and the exogenous use of a soy lecithin-based emulsifier on milk production, composition, and fatty acid profile in mid-lactation cows. Specifically, the aims were to assess whether palmitic acid supplementation improves milk production and verify whether it increases milk fat content, also to investigate whether the inclusion of soy lecithin promotes a synergistic effect on these parameters.

CHAPTER 1

1. LITERATURE REVIEW

1.1. Fat Supplementation in Dairy Cows

Fat supplementation in dairy cow diets constitutes a strategic practice, particularly recommended in systems with high-producing animals. When supplementing fat, it is essential to consider the type of fat, the quantities, and the balance, as supplementation must be carried out in a balanced manner to avoid animal health problems and ruminal dysfunction (GIAGNONI et al., 2025). The effects of fat supplementation in research are inconsistent, depending on the type of fat, and can maintain, reduce, or increase DMI (ALLEN, 2000). Lock et al. (2013) observed a decrease in DMI in the supplemented group with a C16:0-enriched (~85% C16:0) fat supplement. On the other hand, Lock and Souza (2017), Mosley et al. (2007), and Souza and Lock (2018a) observed an increase in DMI, also with a C16:0-enriched supplement. Furthermore, Mathews et al. (2016) observed an increase in milk production without suppressing dry matter intake in a continuous analysis. Furthermore, it is crucial to consider the interaction with other nutrients, ensuring a balanced diet in terms of proteins, carbohydrates, and fiber.

Some important reasons to supplement fat are:

1.1.1. Provides Concentrated Energy

Among the strategies adopted by nutritionists in dairy cow management, increasing the proportion of concentrates and fatty acid supplementation stand out to increase the dietary energy density (LOCK et al., 2013; LOCK and SOUZA, 2017; GHENO et al., 2024). Fat supplementation is particularly advantageous in situations of high energy demand, or when the intake of base diets, without supplementation and with conventional forages and concentrates is insufficient to meet the animals' requirements (PALMQUIST and JENKINS, 2017).

During the postpartum period, the high metabolic demand of lactation, combined with insufficient dry matter intake (DMI), results in a negative energy balance. In high-producing cows, especially in early and mid-lactation, it may be necessary to increase energy intake to meet nutritional requirements (AVILA et al., 2000). Dietary supplementation of fat can be an important source of energy to compensate for the reduction in DMI, particularly during heat stress (GHENO et al., 2024). Fat has a higher energy value than carbohydrates, providing approximately 2.25 times more metabolizable energy (Mcal/kg) (NASEM, 2021). Its inclusion in the diet contributes to an increase in energy density and total energy intake, which, depending on the type and amount of fat, can happen without impairing the digestibility of the fiber fraction, resulting in greater feed efficiency and increased milk production (JENKINS and MCGUIRE, 2006; LOCK et al., 2013). Cows supplemented with linseed oil, rich in linolenic fatty acid, produced 1.5kg more milk and 1.4kg more energy-corrected milk than non-supplemented cows, also, milk omega-3 increased by 53% when there was supplementation, together with de novo and long-chain fatty acids rise (GHENO et al., 2024).

1.1.2. Improves Milk Production

In general, fat supplementation can promote greater milk production (RABIEE et al., 2012), especially when using sources rich in saturated fat (whose bonds between atoms are all single) (LOCK et al., 2013). Relling and Reynolds (2007) demonstrated increases in milk fat (0.187 g/day) with the use of saturated fat, rich in C18:0 and C16:0, compared to the control group. LOCK et al. (2013) observed, with a C16:0-enriched fat supplement, a 1.65 kg increase in fat-corrected milk (3.5% FCM; $p = 0.10$) and a 1.4 kg/day reduction in dry matter intake, resulting in a better FCM/DMI ratio (1.505) compared to the control group (1.455). De Souza and Lock (2018a) reported an increase in milk yield (45.6–49.4 L; $p = 0.03$), and energy-corrected milk (ECM; 43.4–47.5; $p = 0.02$) with a C16:0 fat supplement, compared to the control, as well as an increase in DMI (28.4–30.3 kg/day; $p < 0.01$). Mathews et al. (2016) also reported increases of 2.9 kg/d in the 3rd week ($p = 0.07$) and 4.8 kg/d in the 7th week ($p < 0.05$), with a C16:0 fat supplement, without negatively impacting dry matter intake.

This increase in production is primarily attributed to the use of easily digestible fat sources, such as saturated fats or bypass fats, allowing for better utilization of supplemental fat without the action of ruminal biohydrogenation (LOCK et al., 2013).

What about negative effect of certain fats on milk yield?

1.1.3. Improves Reproductive Health

The energy deficit faced during the postpartum period, where energy demand exceeds intake, predisposes to the occurrence of metabolic diseases, a decline in production, and low reproductive efficiency (PALMQUIST and JENKINS, 2017). In this context, lipid supplementation helps prevent energy deficit, reducing negative impacts on metabolism and promoting reproductive performance. Fat supplementation rich in linolenic acid improved the conception rate at first artificial insemination (AI) ($p=0.015$), with the chance of pregnancy increasing 1.037 times for each kg of supplemental fat consumed (PEREIRA et al., 2022). Furthermore, Rodney et al. (2015) reported positive results on the fertility of cows supplemented with CLA fat during the transition period, with several studies indicating a change in days between calving and the next pregnancy, as described by de Veth et al. (2009), with a reduction from 151 to 117d in cows fed a ruminally protected conjugated linoleic acid (CLA) compared with unsupplemented ones.

1.1.4. Supports Metabolism

The provision of essential fatty acids, such as omega-3 and omega-6, contribute to the modulation of inflammatory processes, maintenance of cell integrity, improvement of immunity, and reduction of the risk of metabolic diseases. (GHENO et al., 2024). These fatty acids are also fundamental for the synthesis of prostaglandins and other lipid hormones linked to physiological functions, including reproduction (PALMQUIST and JENKINS, 2017). Dietary fat may also help to decrease the rapid mobilization of fatty acids and maintain plasma glucose concentration, especially during the transition period (de VETH et al., 2009; ALLEN, M. S., 2023).

Furthermore, the role of cholesterol, an essential precursor of steroid hormones, whose availability can be increased by fat supplementation, is accentuated (FERNANDES et al., 2012), and the major contributor of cholesterol for lipoprotein synthesis in enterocytes is the de novo synthesis by ruminal enterocytes (BIONAZ et al., 2020). Circulating cholesterol can be enhanced by the intake of supplemental fat, as showed by Mathews et al. (2016), in which cows receiving palmitic acid, compared to a non supplemental fat control group, had total cholesterol concentration in plasma 51% greater after 7 weeks, supposedly due to enhanced formation and lower turnover of low-density lipoproteins.

Heart rate (HF) and internal body temperature (IBT) can also vary in cows depending on the treatment, which can be attributed to the natural cow’s response of evaporation to dissipate heat and maintain homeothermy. The group supplemented with linseed oil showed higher HF in the morning and lower in the afternoon, 0.2ºC lower IBT in the afternoon, and 20 more minutes ruminating per day compared to non-supplemented cows (GHENO et al., 2024).

1.1.5. Milk Composition

Improvements in milk composition are relevant for the dairy industry. On-farm practices have an impact on milk quality and composition, which greatly influence milk processing (LANDRY et al., 2025). The fatty acid profile is influenced by the type of fat supplemented, which can increase nutritional quality and modify sensory characteristics, such as flavor and creaminess, due to variations in fatty acid chain length and degree of unsaturation, potentially transforming it into a "functional food," a term that originated in Japan to define foods with a specific physiological effect (PALMQUIST and JENKINS, 2017).

When supplements rich in saturated fatty acids, as C16:0 and C18:0, are used, there is a general increase in milk fat concentration and production, often accompanied by higher milk yield (RELLING and REYNOLDS, 2007; LOCK et al., 2013; GIRÓN et al., 2024). The inclusion of beneficial fatty acids, such as omega-3, highlights milk for human consumption, bringing benefits for the health and nutrition of consumers (GHENO et al., 2024). LOCK et al. (2013) reported an increase in 16-carbon fatty acid content and a 27% elevation in fat yield with supplementation of C16:0. Also, Girón et al. (2024) showed increases in milk fat (p=0.04) and milk fat content (p=0.02) in cows supplemented with PA in the immediate postpartum, without losing corporal condition.

A current discussion, evidenced by Harvatine (2021), is focussing on palmitic acid's melting properties of milk fat and its rate of desaturation compared to stearic acid. While the second one is converted approximately 50% to oleic acid in the mammary gland, palmitic acid has a much lower desaturase ratio, which provides palmitic with a greater impact on thermal properties of milk fat that could affect the characteristics of final dairy products. Palmitic acid supplements increase C16:0 in milk fat, generally a minor effect on short and medium-chain fatty acids than 18C fatty acids (HARVATINE, 2021), sometimes increasing the yield of mixed milk FA (dos SANTOS NETO et al., 2021).

An increase in milk efficiency was described by dos Santos Neto et al. (2021) and Landry et al. (2025) (ECM/DMI), where cows supplemented with palmitic acid had increases in the yields of milk, milk fat, lactose, milk protein, 3.5% FCM, 4% FCM, ECM, milk energy output, milk fat, and milk protein contents.

1.1.6. Response to thermal stress

Fat provides a lower heat increment per unit of energy compared to other energy sources (ALLEN, 2000), acting as an energy stabilizer in heat stress situations. This fact can compensate for the reduction in dry matter intake during hot periods, optimizing milk production, and reducing thermogenesis during ruminal fermentation (GHENO et al., 2024; ALLEN, 2000).

In summary, fat supplementation in dairy cows is a crucial strategy for enhancing production, providing additional energy, improving reproductive health, and improving milk quality. (MATHEWS et al., 2016; GHENO et al., 2024). The nutritionist's role in selecting the correct type of fat and determining the appropriate dosage and timing of supplementation is crucial to ensuring benefits without compromising the digestive and ruminal health of the animals (de SOUZA and LOCK, 2019; GIAGNONI et al., 2025). Cows under heat stress supplemented with 1.5% and 3% saturated fat acids, mainly C16:0, increased milk yield, SCM, fat, protein, lactose and total solids yield, without changing DMI. Also, cows receiving the higher SFA level may have been in an improved energy status, once they had slightly reduced plasma non-sterified fatty acids (NEFA) levels compared with cows non-supplemented (WANG et al., 2010).

1.2. Types of Fat Available on the Market and Their Differences

Fatty acids in milk can be classified by their origin in: *de novo*, fatty acids formed in the mammary gland, with C4:0 to C14:0 and some C16:0; preformed, fatty acids from corporal mobilization of the adipose tissue from plasma or from the feed, half of the C16:0 and the others with more than 16C; mixed, some from *de novo* and some from mobilization, majoritarian C16:0 and C16:1 (BIONAZ et al., 2020; de SOUZA et al., 2020).

Different types of fat are incorporated into dairy cow diets to optimize milk production, promote animal health and fertility, and ensure the quality of the milk produced. Dietary lipids can be found in esterified form – mono- and digalactoglycerides in forages and triglycerides in concentrated feeds. The ideal lipid supplement, according to RICO et al. (2014), is one that does not interfere with ruminal fermentation or dry matter intake, is highly digestible in the intestine, and increases milk production and its components. However, this scenario is utopian, making the evaluation of the effects of supplements enriched with different fatty acids fundamental (DE SOUZA and LOCK, 2018a), given that each type can trigger different metabolic responses.

Although many commercial supplements contain mixtures of fatty acids, products enriched with individual acids have become increasingly accessible (DE SOUZA and LOCK, 2019).

The following types are prominent in the market:

1.2.1. Saturated Fat

Saturated fats are commonly found in vegetable oils, such as palm oil and coconut oil, as well as in soybean meal. At room temperature, they are solid and exhibit greater resistance to oxidation, providing a longer shelf life for the product. Palm oil is particularly used due to its cost-effectiveness, high energy density, and resistance to ruminal biohydrogenation because of its inert passage (de SOUZA and LOCK, 2018a).

Researchers have demonstrated that palmitic acid supplementation can increase milk production and its components (MATHEWS et al., 2016; PIANTONI et al., 2013; de SOUZA and LOCK, 2018a; LANDRY et al., 2025). Although, in some research it has been demonstrated to have no effect on milk yield (LOCK et al., 2013). This inconsistency can be related to the reported effects of PA on DMI, which is inconsistent across studies. An increase (MOSLEY et al., 2007; de Souza and Lock, 2018a), a decrease (LOCK et al., 2013; RICO et al., 2014; GIAGNONI et al., 2025), or no changes (PIANTONI et al., 2013; MATHEWS et al., 2016; de Souza et al., 2017; de SOUZA et al., 2018) in DMI have been reported with PA supplementation. The increase in milk efficiency with dietary PA supplementation appears to be more consistent. Authors reporting a decrease in DMI saw no effect on milk yield (Lock et al., 2013). Landry et al. (2025) reported that DMI was not different between treatments, but PA increased milk yield and consequently ECM/DMI efficiency.

1.2.2. Unsaturated Fat

Unsaturated fats are derived from vegetable oils such as soybean, canola, sunflower, and linseed oil, as well as from marine sources like fish and algae, and can be added to rations in the form of the seed itself, or as free oils (BAUMAN et al., 2011; CASTRO et al., 2019). They possess double bonds between carbon atoms, which make them liquid at room temperature. Despite their metabolic benefits, they should be administered with caution to avoid digestive disorders, as a ruminal environment with a low pH, that can lead to an alternative biohydrogenation via, reducing milk fat; however, they have positive effects on cell health and milk quality (BAUMAN et al., 2011).

Supplementary soybean oil and linseed oil reduced the proportion of saturated fatty acids (SFA), and increased mono unsaturated fatty acids (MUFA) and poli unsaturated fatty acids (PUFA) content compared to control diet. The results were also advantageous for reproduction, because cows that received linseed oil tended to show a higher number of pregnant cows in the first insemination and a reduction in the number of protocols ($p=0.07$) (CASTRO et al., 2019). Treatment with n-3 PUFA increased its plasma concentrations, modulated plasma adiponectin concentrations and shortened the calving-to conception interval (PEREIRA et al., 2022).

1.2.3. "Bypass" or Inert Fat

Unlike monogastrics, where dietary fatty acids reach the intestine without modification, the high microbial activity in the rumen substantially modifies the fatty acids in ruminant diets, especially unsaturated ones, making supplementation with protected, or bypass, fat essential (BIONAZ et al., 2020). "Bypass" or inert fats are modified to resist ruminal action, being hydrolyzed only in the small intestine. This allows for the provision of high-density energy without impairing ruminal fermentation, even when using unsaturated sources. Such supplements generally include palmitic (C16:0), stearic (C18:0), and oleic (cis-9 C18:1) acids, which are the main components of milk fat and adipose tissue (DOUGLAS et al., 2007; DE SOUZA et al., 2018). More common sources of inert fats include oilseeds (cottonseed, whole soybeans), animal fats, palm oils, and their modifications. Calcium salts are frequently used to protect unsaturated fatty acids from ruminal biohydrogenation (RABIEE et al., 2012; ALLEN, 2000).

1.2.4. Omega-3 and Omega-6

In ruminant diets, the main source of alfa-linolenic acid (C18:3n-3) is forage and linseed oil, while linoleic acid (C18:2n-6) is found in the fat of most grains and of soybeans, safflower seed, and sunflower seed (CASTRO et al., 2019), and they also are the most abundant FAs present in forages C18:3 (60–70%) and C18:2 (~ 20%) (BIONAZ et al., 2020). Called as alpha-linolenic acid (omega-3) and linoleic acid (omega-6), they are essential fatty acids, polyunsaturated, and found in vegetable oils, seeds, and fish. They play a crucial role in serving as precursors to anti-inflammatory responses, maintaining hormonal balance, reproductive health, and milk quality, thereby benefiting both animals and the products intended for human consumption. (PALMQUIST and JENKINS, 2017; GHENO et al., 2024). Although milk fat with greater linoleic can be produced, it is not stable due to potential oxidation. Also, if supplementation of linoleic or linolenic acid is excessive, desaturation and chain elongation to arachidonic acid, the precursor of PGF2α, will be inhibited. PGF2α plays a crucial role in various physiological processes, particularly in reproductive physiology, it exerts its actions by binding to specific G-protein coupled receptors. (PALMIQUIST and JENKINS, 2017).

1.2.5. Palmitic Acid

Individual fatty acids can cause different effects in animals, and among the various fatty acids available for supplementation, recent research has focused specifically on palmitic acid (C16:0) (DE SOUZA and LOCK, 2018b). Approximately 50% of the palmitic acid present in the organism is used directly and synthesized as de novo in the mammary gland, while the remaining fraction, along with other long-chain fatty acids, is taken up from the bloodstream. It is a saturated fatty acid, the main constituent of palm oil, generally used as a supplement for dairy cows.

Palmitic acid supplementation increases milk production and fat concentration (RABIEE et al., 2012; PIANTONI et al., 2013; LOCK et al., 2013; MATHEWS et al., 2016; de SOUZA et al., 2017; de SOUZA and LOCK, 2018a), as well as productive efficiency, compared to non-supplemented diets (LOCK et al., 2013) or diets supplemented with other fat sources (RICO et al., 2014).

The literature reports that supplements with high levels of palmitic acid increase milk fat production (RELLING and REINOLDS, 2007) and the incorporation of C16:0 into the lipid fraction of milk, with this increase being linearly related to the concentration of C16:0 present in the diet (MOSLEY et al., 2007). Rico et al. (2014) did not observe an increase in milk production, but reported an increase in feed efficiency and changes in the milk lipid profile, as well as greater fatty acid digestibility compared to the control. Lock et al. (2013) showed a better FCM/DMI ratio (1.51) in cows supplemented with palmitic acid compared to the control group (1.46). PA increased milk yield and ECM without any changes in DMI, consequently improving milk efficiency, ECM/DMI (LANDRY et al., 2025).

The inconsistencies regarding the effect of palmitic acid on milk production and DMI among studies are due to variations in experimental design, such as C16:0 inclusion levels, lactation stage, duration of treatments, and energy level of control diets. Mathews et al. (2016) emphasize that the increase in the energy density of diets supplemented with C16:0 is only one of multiple factors influencing responses during lactation. For example, palmitic acid administration during the first three weeks postpartum resulted in higher energy intake, energy-corrected milk, and total milk yield (DE SOUZA et al., 2019). When supplied in the immediate postpartum and at peak lactation, an increase in lipolysis, weight loss, and a reduction in plasma insulin concentration were observed (DE SOUZA and LOCK, 2019; DE SOUZA et al., 2019).

Variations in responses to C16:0 supplementation suggest that factors inherent to the animal itself significantly influence the effects of the supplement. De Souza and Lock (2018a) observed, for example, an interaction between the parity of the animals and the effects of the lipid supplement: primiparous cows showed an increase in body weight and a reduction in preformed fatty acids, while multiparous cows maintained their weight and exhibited an increase in these fatty acids. Palmitic acid impacts dairy cow metabolism, increasing energy partitioning to milk regardless of the lactation stage, while reducing body energy reserves in early-lactating cows (GIRÓN et al., 2024).

1.3. Advantages of Using Palmitic Acid for Dairy Cows

1.3.1. Digestive Stability and Lower Oxidation

As a saturated fatty acid, palmitic acid exhibits greater resistance to oxidation, providing greater stability to the product when added to the ration. This characteristic is important in feeding systems where consistency and ingredient stability are essential. Boerman et al. (2017) supplemented increasing levels of stearic acid to dairy cows and did not observe an increase in production, possibly due to a reduction in fatty acid digestibility with high levels of this acid. De Souza et al. (2018) recorded, however, that increasing C16:0 supplementation promoted only a small reduction in fatty acid digestibility, with an increase in productive responses. The reduction in digestibility was three times greater for C18:0 compared to C16:0 (DE SOUZA et al., 2018).

1.3.2. Milk Yield

The results of Weiss and Pinos-Rodríguez (2009) indicate that the energy partitioning resulting from fatty acid supplementation is directly related to the timing of supplementation and interacts with the dietary fiber content. The co-supplementation of C16:0 and C18:0 in early lactation cows (21–126 days postpartum) resulted in increased energy intake, with a greater allocation to milk production in low-fiber diets. In high-fiber diets, the additional energy intake was directed towards replenishing body energy reserves. This interaction may have been caused by a limitation of milk precursors when the high-forage diet was fed. The low-forage diet likely provided more glucose precursors because of greater DMI and may have provided more amino acids. This would allow the additional energy provided by fat to be directed toward milk synthesis. With the high-forage diets, energy was not the limiting factor for milk production; therefore, the added energy when fat was supplemented was diverted to body stores.

When used in a balanced manner, palmitic acid can contribute to increased milk production, providing supplemental energy in situations where fresh feed intake is limited or energy demand is high. Its wide potential to increase milk fat concentration and production, as well as promote greater productive efficiency, is also noteworthy (LOCK et al., 2013). De Souza and Lock (2018a) observed an increase in dry matter intake, apparent energy, and energy available for milk in cows supplemented with palmitic acid.

1.4. Disadvantages of Using Palmitic Acid for Dairy Cows

1.4.1. Effect of Using Palmitic Acid for Dairy Cows

Excessive supplementation of palmitic acid, being a saturated fat, can result in milk with a less healthy lipid profile, showing a higher proportion of saturated fats. This aspect can affect milk quality for human consumption, especially in markets that value products with a healthier fatty acid profile (higher omega-3 content and lower saturated fat content) (PALMQUIST and JENKINS, 2017; GHENO et al., 2024).

1.4.1.1. Environmental Impact and Sustainability

Palm oil is the main source of palmitic acid; however, its unsustainable cultivation represents a significant environmental problem, especially in tropical regions such as Southeast Asia and parts of Africa. Organizations such as RSPO (Roundtable on Sustainable Palm Oil), WWF (World Wildlife Fund), and FAO (Food and Agriculture Organization of the United Nations) have been establishing strict sustainability standards for palm oil production. However, the adoption of these practices still represents a challenge, as not all producers or countries follow them, and the global market remains predominantly dominated by conventional methods.

1.4.1.2. Interference with Mineral Absorption

Dietary minerals must be absorbed through the epithelial cells of the gastrointestinal tract (GIT) to enter the bloodstream and be used by tissues, which can occur in any part of the GIT, but with a focus on the small intestine (GOFF, 2018). The absorption of minerals requires crossing the epithelial cells of the gastrointestinal tract, generally occurring in the small intestine (GOFF, 2018).

In ruminants, the surfaces of the rumen, reticulum, and omasum, composed of stratified epithelium, are not intended for nutrient absorption, but only for protection against abrasion (GOFF, 2018). *In vitro* research conducted by Schröder, et al. (2015) revealed that the presence of short-chain fatty acids in ruminal fluid favors the active transport of calcium, increasing the net flux of this mineral from 0.90 nmol/cm to 1.80 nmol/cm under such conditions. An excess of saturated fats can interfere with the absorption of minerals such as calcium and magnesium, as it alters digestibility in the gastrointestinal tract by not fragmenting and reaching the adequate intestinal contact surface for absorption. Palmitic acid, being a long-chain fatty acid, can reduce this contact surface and interact with minerals present in the digesta, without providing the facilitated absorption that short-chain fatty acids confer (RAHNEMA et al., 1994).

1.5. Use of Emulsifiers in Dairy Cow Feeding

Several factors limit the use of large quantities of fat in ruminant diets, including inhibitory effects on ruminal fermentation, low intestinal absorption at high intakes, and dietary imbalances that may reduce energy intake. Consequently, possible inconsistencies in feed intake and milk production may result from a combination of factors, such as differences in experimental design, level of fat inclusion in the diet, lactation stage, duration of supplementation, and energy status of the studied group, meaning that dietary energy density is only one aspect influencing lactation outcomes (MATHEWS et al., 2016).

Emulsification is the process of reducing fat droplets to a size that forms a stable suspension in an aqueous environment, with the aid of bile salts for micelle formation, allowing the action of enzymes present in pancreatic juice to proceed with lipid digestion and absorption (CUNNINGHAM et al., 2004). In ruminants, micelle formation is essential for this process and consequently, for improving fatty acid digestibility. Bile provides bile salts and lecithin, endogenous emulsifiers, and pancreatic juice provides enzymes to convert lecithin to lysolecithin, and bicarbonate to raise the pH. Once these components separate fatty acids from food particles and bacteria, enabling micelle formation, the transport of lipids by jejunal epithelial cells is facilitated. After the absorption of fatty acids and lysolecithin, fatty acids are re-esterified into triglycerides, forming chylomicrons and very low-density lipoproteins (VLDL) for transport via lymph to the bloodstream (LOCK et al., 2006).

Commercially, lecithin is predominantly derived from soybean grains; however, sources such as sunflower seeds and canola are also available. They act as emulsifiers, bulking agents, viscosity regulators, carriers, wetting agents, anti-splattering agents, and dispersants. Their amphiphilic nature allows them to accumulate at the oil-water interface, thereby reducing interfacial tension and facilitating emulsion formation (MCFADDEN, 2019). In addition, soy lecithins can be used as a source of choline for mid-lactation cows. Choline is essential in lipid transport because it constitutes VLDL, produced in the liver, and cannot be replaced by other phospholipids, as showed by Marchesini et al. (2012), who obtained the same milk yield and fat-corrected milk with soy lecithin and with microencapsulated choline chloride with hydrogenated vegetable oils.

1.5.1. Why Use Emulsifiers in Dairy Cow Diets?

1.5.1.1. Improve fat digestibility

The limited capacity of the intestine to secrete endogenous emulsifiers such as lysolecithin and bile salts (DRACKLEY, 2000) is one of the causes that reduce fatty acid digestibility when their flow to the duodenum increases, especially when they are saturated or long-chain (de SOUZA et al., 2020). Several researchers have previously tested the use of exogenous emulsifiers to supply this metabolic limitation and allow fatty acids to be provided to animals beyond their natural capacity to secrete endogenous emulsifiers. An increase in metabolic efficiency can result from the elevation of the energy supplement through lipid absorption, a theory supported by the apparent high digestibility of FA in moderate palmitic acid diets (FONTOURA et al., 2021).

1.5.1.2. Increase energy availability

The use of emulsifiers in dairy cow feeding contributes to increased fat absorption, allowing for greater energy utilization from lipid sources. This factor is especially important during periods of intense lactation, where cows have a higher caloric requirement to maintain milk production.

Abomasal infusion of exogenous emulsifier in animals supplemented with C16:0 and C18:0 led to an increase in the absorption of total fatty acids, 16-carbon and 18-carbon fatty acids (DE SOUZA et al., 2020). Lecithin supplementation demonstrated positive effects on nutrient utilization efficiency, as evidenced by a linear reduction in milk urea nitrogen (MUN). Marchesini et al. (2012) reported such a reduction of MUN in Holstein cows supplemented with soy lecithin, an effect observed, according to Fontoura et al. (2021), only in animals that received a moderate amount of palmitic acid, although both authors showed linear reductions in dry matter intake (DMI).

The improved nitrogen (N) utilization efficiency attributed to lecithin can be explained by the more efficient use of degradable protein by ruminal microorganisms, even though the conversion of dietary N into milk protein is relatively low (HUHTANEN et al., 2015), or even due to reduced protein intake resulting from linear decreases in DMI (FONTOURA et al., 2021). Excess crude protein in the diet constitutes one of the main reasons for the low efficiency of N use in milk synthesis (HUHTANEN et al., 2015). Thus, the benefit of emulsifiers extends to protein metabolism, as efficient N utilization allows for a reduction in dietary crude protein content and, consequently, in the costs associated with this ingredient.

1.5.1.3. Increase feed stability

The inclusion of emulsifiers promotes the homogeneous mixing of fats in feeds, both of plant and animal origin, preventing phase separation or the formation of solid lipid aggregates. This uniform distribution reduces the possibility of uneven intake by animals.

Additionally, the benefits of emulsifiers extend beyond the ruminal environment. In a comparative study, dos Santos Neto et al. (2023) evaluated ruminal and abomasal infusion of polysorbate-C18:1 and observed that both routes increased the digestibility of total, 16C, and 18C fatty acids, indicating no need for ruminal protection for this type of emulsifier. However, according to Fontoura et al. (2021), the ruminal degradation of unprotected lecithin and lysolecithin may limit intestinal efficacy, which is why rumen-inert lecithins are recommended in strategies aimed at increasing lipid digestibility. Given the diversity of emulsifiers, further research is needed to elucidate the effects of different formulations and compositions of these additives on fatty acid digestion.

1.5.1.4. Ensure Digestive Enzyme Function

The efficiency of lipid digestion essentially depends on micelle formation after a complex process of digestion and emulsification. Pancreatic juice and bile play a central role in this process, as highlighted by McFadden (2019), by providing digestive enzymes and bicarbonate. Among the enzymes involved are proteases, lipases, amylase, pancreatic lipase, and colipase, whose effective action depends on access to substrate binding sites. Thus, for phospholipases, pancreatic lipase, and colipase to perform their function correctly, it is essential that fats are previously emulsified and micellized, thanks to the action of bile salts and lysolecithin, whether endogenous or exogenous.

1.5.2. Differences in the Use of Different Fats in Combination with Emulsifiers

1.5.2.1. Saturated and "Bypass" Fats

Lecithin stands out as a prominent emulsifier by promoting greater solubility and stability of micelles, facilitating the intestinal absorption of long-chain saturated fatty acids (FONTOURA et al., 2021). Increasing the efficiency of fat utilization and reducing the risk of intestinal overload, it facilitates the breakdown of lipid capsules, allowing for a more controlled and efficient release of fatty acids in the small intestine, thereby improving the bioavailability of these nutrients (MCFADDEN, 2019).

1.5.2.2. Unsaturated Fats

Emulsifiers contribute to the homogenization of unsaturated fats in the feed, making the mixture more easily digestible and maximizing available energy and nutrient absorption. Oleic (C18:1) and linoleic (C18:2) fatty acids are amphiphilic, and an increase in their concentrations increases the solubility of stearic acid (C18:0). The presence of lysolecithin enhances this effect, suggesting that the hydrolysis of lecithin in the small intestine releases lysolecithin and unsaturated fatty acids, generating an additive effect on the digestibility of saturated fatty acids (MCFADDEN, 2019).

1.5.2.3. Mixtures of Saturated and Unsaturated Fats with Emulsifiers

When using a combination of saturated and unsaturated fats, the differences in digestibility of the two can complicate efficient fat absorption. Palmitic and stearic acids behave as non-polar solutes, whereas, oleic, linoleic, and lauric acids are amphiphilic, which can improve energy distribution and the utilization of both, once lecithin hydrolysis in the small intestine releases lysolecithin and unsaturated fatty acids (e.g., oleic or linoleic acids) and thus having an additive effect on saturated fatty acid digestibility (MCFADDEN, 2019).

Overall thesis objective? It would be good to add a short summary here of what research is missing from the literature…that leads to your objective

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CHAPTER 2

EFFECT OF EXOGENOUS EMULSIFIER AND PALMITIC ACID USE IN TOTAL MIXED RATION ON MILK PRODUCTION, COMPOSITION, AND FATTY ACID PROFILE OF CONFINED DAIRY COWS

EFFECT OF EXOGENOUS EMULSIFIER AND PALMITIC ACID USE IN TOTAL MIXED RATION ON MILK PRODUCTION, COMPOSITION, AND FATTY ACID PROFILE OF CONFINED DAIRY COWS

Abstract: The objective of this study was to evaluate the supplementation of lactating dairy cows with palmitic acid (PA) in their total mixed ration (TMR), to assess whether it improves milk production, composition, and fatty acid profile, and also to investigate whether the inclusion of the exogenous soy lecithin emulsifier promotes a synergistic effect on these parameters. Sixty Holstein cows were used, selected according to days in milk (DIM), individual milk yield, and parity, and equally divided among 3 treatments: 1) Control (C – no fat); 2) Palmitic Acid without emulsifier (FW); and 3) Palmitic Acid with emulsifier (FE). They received a basal diet composed of corn silage, wheat straw, protein concentrate, and bicarbonate. The lipid supplement (89% C16:0) was supplied as part of the TMR, as was the emulsifier, subsequently added to the feed bunk in the corresponding groups and mixed with the aid of a fork. The experimental period totaled 21 days, divided into 14 day of adaptation and 7 days of data collection. Feed intake was measured for each group and milk yield individually from 3x/d milking. Milk samples were collected on the 3 milkings for 5 days, for analysis of composition and fatty acid profile. Statistical analysis was conducted using a mixed-effect model, considering treatment as a fixed effect and the animal as a random effect. Because of non-normality of the majority, the data were analysed using non-parametric comparison, firstly the Kruskal-Wallis test for the main variables. When $p \leq 0.10$ the paired Mann-Whitney test with Bonferroni correction was applied, with $P \leq 0.05$ considered as statistically significant, and between 0.05 and 0.10 considered a tendency towards significance. Neither PA supplementation nor the exogenous emulsifier were detected to affect milk yield. However, there was a tendency towards greater 3.5%FCM and ECM in the FW and FE groups. FE treatment improved lactose content and defatted dry extract, and reduced milk urea nitrogen content. An increase in unsaturated fatty acids (UFA), mainly monounsaturated (MUFA), also stearic acid, oleic acid and acetate was detected in milk, devoid of alterations in FA preformed (Pref), *de novo* (DeNov) and Mixed groups. PA and emulsifier had limited effects on overall production responses, so it remains to be

investigated practical feeding strategies that can be easily applied in daily farm management, and the use of other supplemental doses of emulsifier.

Key-words: Nutrition; Intake; Fat profile; TMR; Holstein.

Introduction:

Maximizing the productive performance in dairy cattle directly depends on factors such as genetic potential and the quality of nutrition provided. Diet plays a central role in this process, directly impacting milk production and economic returns for the producer, that way, aiming to increase energy density, without compromising the digestibility of the fibrous fraction, the inclusion of lipids can be a viable option. Fat supplementation in dairy cow diets constitutes a strategic practice, particularly recommended in systems with high-producing animals, especially in early and mid-lactation, when it may be necessary to increase energy intake to meet nutritional requirements (AVILA et al., 2000), once, during the postpartum period, the high metabolic demand of lactation, combined with reduced dry matter intake (DMI), results in a negative energy balance (PALMQUIST and JENKINS, 2017; de SOUZA et al., 2018). Also, dietary supplementation of fat can be an important source of energy to compensate for the reduction in DMI during heat stress (GHENO et al., 2024).

When supplementing fat, it is essential to consider the type of fat, the quantities, and the balance, as supplementation must be carried out in a balanced manner to avoid animal health problems and ruminal dysfunction (GIAGNONI et al., 2025). Its inclusion in the diet can contribute to an increase in energy density and total energy intake, depending on the fat chosen, without impairing the digestibility of the fiber fraction, if given to a maximum of 7% of DM (NASEM, 2021), which results in greater feed efficiency and increased milk production (JENKINS and MCGUIRE, 2006; LOCK et al., 2013). In general, fat supplementation promotes an increase in milk production (RABIEE et al., 2012), and fatty acid profile (LOFTEN et al., 2014; HARVATINE et al., 2006), especially when using sources rich in saturated fat (whose bonds between atoms are all single) (LOCK et al., 2013), such as C16:0 (palmitic acid) and C18:0 (stearic acid). However, the effects of palmitic acid to the diet on productive performance are still inconsistent, with studies reporting that supplementation either increased DMI (MOSLEY et al., 2007), decreased DMI (LOCK et al., 2013; RICO et al., 2014; GIAGNONI et al., 2025), or did not alter DMI (PIANTONI et al., 2013; de SOUZA et al., 2020).

The general guideline for fat supplementation is that dietary fat should not exceed 6% to 7% of dietary dry matter (DM). A typical basal diet of forages and grains naturally contains about 2% to 3% fat. This leaves room for approximately 3% to 4% of supplemental fat (BIONAZ et al., 2020). Several factors limit the use of large quantities of fat in ruminant diets, including inhibitory effects on ruminal fermentation, low intestinal absorption at high intakes, and dietary imbalances that may reduce energy intake. Consequently, possible inconsistencies in feed intake and milk production may result from a combination of factors, such as differences in experimental design, level of fat inclusion in the diet, lactation stage, duration of supplementation, and energy status of the studied group, meaning that dietary energy density is only one aspect influencing lactation outcomes (MATHEWS et al., 2016).

Emulsification is the process of reducing fat droplets to a size that forms a stable suspension in an aqueous environment, with the aid of bile salts for micelle formation, allowing the action of enzymes present in pancreatic juice to proceed with lipid digestion and absorption (CUNNINGHAM et al., 2004). In ruminants, micelle formation is essential for this process and consequently, for improving fatty acid digestibility. Bile provides bile salts and lecithin, endogenous emulsifiers, and pancreatic juice provides enzymes to convert lecithin to lysolecithin, and bicarbonate to raise the pH. Once these components separate fatty acids from food particles and bacteria, enabling micelle formation, the transport of lipids by jejunal epithelial cells is facilitated (CUNNINGHAM et al., 2004). After the absorption of fatty acids and lysolecithin, fatty acids are re-esterified into triglycerides, forming chylomicrons and very low-density lipoproteins (VLDL) for transport via lymph to the bloodstream (LOCK et al., 2006). Commercially, lecithin is predominantly derived from soybean grains; however, sources such as sunflower seeds and canola are also available (MCFADDEN, 2019). One of the causes that reduce fatty acid digestibility when their flow to the duodenum increases, especially when they are saturated or long-chain (de SOUZA et al., 2020) is the limited capacity of the intestine to secrete endogenous emulsifiers such as lysolecithin and bile salts (DRACKLEY, 2000).

Several researchers have tested the use of exogenous emulsifiers to supply this metabolic limitation and allow fatty acids to be provided to animals beyond their natural capacity to secrete endogenous emulsifiers (e.g. references). An increase in metabolic efficiency can result from the elevation of the energy supplement through lipid absorption, a theory supported by the apparent high digestibility of FA in moderate palmitic acid diets (FONTOURA et al., 2021).

Due to the wide variety of results with the use of lipidic supplements, greater clarity is sought regarding their effects on production and feed efficiency, as the profitability of their use is essential for this energy source to become a viable option for high-producing farms. When evaluating a lipid supplement, the inherent characteristics of the fat—such as its source, composition, and presentation form—are determining factors for its utilization. Furthermore, toxicity to ruminal microorganisms depends on the type of fat (GIAGNONI et al., 2025).

Another challenge associated with the use of large quantities of fat in ruminant diets, especially long-chain saturated fatty acids, is the limited physiological capacity for secreting endogenous emulsifiers, such as lysolecithin and bile salts, which are essential for lipid emulsification and digestion in the small intestine (DRACKLEY, 2000; CUNNINGHAM et al., 2004). The digestibility of fatty acids tends to decrease with increased lipid flow to the duodenum, especially when fatty acids are saturated or long-chain (BOERMAN et al., 2015; de SOUZA et al., 2020). To optimize the utilization of supplemented fatty acids, it is necessary to improve the processes of emulsification, micelle formation, and intestinal absorption. Thus, the provision of exogenous emulsifiers can represent an effective strategy to expand lipid digestion capacity beyond physiological limits, resulting in greater absorption and potentially improved productive performance (de SOUZA et al., 2020).

However, to date, there are no reports of experiments evaluating the provision of exogenous emulsifiers directly in the total mixed ration (TMR) of dairy cows, with the aim of verifying their effects on milk production, composition, and fatty acid profile, especially in association with supplements rich in palmitic acid (~89% C16:0). Given this context, the objective of the present study was to evaluate the effect of supplying fat composed primarily of palmitic acid (89% C16:0) and the exogenous use of a soy lecithin-based emulsifier on milk production, composition, and fatty acid profile in mid-lactation cows. Specifically, the aims were to assess whether palmitic acid supplementation improves milk production and verify whether it increases milk fat content, also to investigate whether the inclusion of soy lecithin promotes a synergistic effect on these parameters.

Methodology:

All procedures used in this project were approved by the Ethics Committee of the State University of Santa Catarina (UDESC), under protocol No. 3324251124.

The study was conducted from November to December 2024 at Granja Risso, located in Irati, Santa Catarina, Brazil (latitude: 26º39'23" S, longitude: 52º53'32" W), in a compost bedded pack barn system, sectioned into three areas, with mechanical milking. The experimental design used was a randomized complete block design, consisting of 3 treatments: a control (C – no fat), and two supplemented with palmitic acid (PS – without emulsifier, and PC – with emulsifier). The experimental period lasted 21 days, with the first 14 days for adaptation, followed by 7 days of data collection. Sixty Holstein cows (60% primiparous) were selected from a larger herd and distributed into 3 homogeneous groups, based on days in milk (DIM) (153.3±63.5), individual milk production (45.8±7.8 kg/d), and parity (1.7±1.0). Milking was performed three times a day, at 6:00 AM, 2:00 PM, and 9:00 PM, in a low-line milking parlor with a 2x10 herringbone stall.

All groups received a base diet composed of corn silage, wheat straw, and concentrate (Table 1). The fat used (VitalEnergy®) was in the form of free fatty acids (89% C16:0, 7% C18:0, 2% C18:1, 1% C14:0, 1% C18:2) and was added into the TMR by hand (0.350 kg/cow/d). The emulsifier (POWERBOV®) had whole soy lecithin as its main component, enriched with medium-chain triglycerides, also incorporated into the TMR as top dress (0.01 kg/cow/d). The diets for all treatments were formulated according to NASEM (2021) to be isoproteic. Use of sBST occurred after the first milking, in a subcaudal manner, on day 11. Composed by bovine somatotropin, 1 syringe of 2g.

Table 1: Feed composition table, divided by treatment.

Variable	Control	Fat	Fat + emulsifier
Composition (%DM)			
Wheat Straw	1.7	1.7	1.7
Corn Silage	42.4	42.4	42.4
Cottonseed	7.5	7.5	7.5
Ground Corn Grain	14.3	14.3	14.3
Soybean Hulls	7.6	7.6	7.6
Soybean Meal 46%	14.3	14.3	14.3
DDGS, 32% CP%	7.8	7.8	7.8
Mineral Prexim	3.2	3.2	3.2
Soybean Hulls Topdress	1.2	0	0
Supplemental Fat Topdress	0	1.3	1.3
Emulsifier Topdress	0	0	0.04
Nutritional composition			
Total dry matter (%)	51.8	51.7	51.0
Mineral matter (%)	6.5	6.3	6.2
Crude protein (%DM)	14.6	15.7	14.5
Neutral detergent fiber (%)	35.2	30.9	34.8
Acid detergent fiber (%)	17.6	14.6	17.6
Ether extract (%)	4.9	5.7	5.1

The TMR was mixed according to the farm's operating procedures, being divided into 2 wagons due to the volume of feed and provided once a day after the first milking. The mixing in the wagon was done for the base diet, with the lipid supplement (FW and FE) and the emulsifier (FE) subsequently added to the corresponding groups in the feeding lane by one person, while simultaneously being mixed with the aid of a fork by another person.

1. Sample Collection

Samples of the TMR from each group were collected daily during the sampling period to compose a composite sample for each treatment, which were then frozen at -20°C until analysis. They were collected in 3 different points and mixed in a bucket to provide a sample that better represented the total. Silage samples were collected daily during the sampling period for dry matter (DM) determination, aiming to adjust the diet to be provided the following day, ensuring its standardization throughout the experiment.

Feed refusals were collected and weighed daily during the experiment, before feed delivery, to dictate the necessary adjustments to the TMR, resulting in an average intake of 27.9 kg for all treatments, targeting a maximum of 5% remaining in the feeder. An estimation of daily intake was obtained by subtracting the number of refusals from the total diet offered at the feeding lane. Cows had their daily milk production individually measured by digital panels and recorded throughout the experiment. Milk samples were obtained via automatic collectors at all milkings during d 16, 17, 18, 19, and 20 of the experimental period for subsequent analysis of composition and fatty acid profile by infrared spectroscopy. The daily values obtained per cow were the result of the average across the 3 milkings, proportional to the production at each milking. Sample preservation occurred using vials containing preservative, stored in cardboard boxes until shipment to the laboratory.

2. Laboratory Analysis

Milk samples were sent to Parleite – APCBRH, for physicochemical analysis of profile and composition. The contents of fat, protein, lactose, total solids, milk urea nitrogen (MUN), and fatty acid (FA) profile were obtained by Fourier-Transform Infrared (FTIR) Spectroscopy, using the Bentley 2000® equipment (Bentley Instruments Inc., Chaska, Minnesota, USA). In the FA profile, results were provided for saturated FA, monounsaturated FA, polyunsaturated FA, unsaturated FA, palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), *de novo* FA, mixed FA, and preformed FA, in the unit of g/100g of milk. Zootechnical data on days in milk (DIM), parity, and individual milk production were collected from Report R 3.1 of APCBRH, related to the collected dairy control.

TMR samples were mixed to result in one composite sample per treatment. The DM concentration was determined by drying it at 105ºC for 24 h. The ash was determined by combustion in a muffle furnace at 550ºC for 4 h, and the organic matter (OM) was determined by mass difference. They were analyzed NDF (Neutral Detergent Fiber), according to Mertens (2002) except that the samples were weighed into filter bags and treated with neutral detergent in ANKOM equipment (ANKOM Technology, Macedon NY, USA), and for for ADF (Acid Detergent Fiber), according to Method 973.18 of AOAC (AOAC 1997). For total dry matter (TDM) and mineral matter (MM), the method followed was that of Weende (HENNING, 1864). The ether extract (EE) was determined in a reflux system with ethyl ether at 180ºC over 4 h (Extrator de Óleos e Graxas MA491, Marconi, Brazil). The n-alkanes was determined according to Dove and Mayes (2006). Protein, in turn, was quantified according to Kjeldahl method (Method 984.13; AOAC 1997

3. Statistical Analysis

Several variables were calculated from the obtained values:

- Milk production corrected to 3.5% fat, in kg/cow/day (adapted from Sklan et al., 1992):

ProdCM35 = ((0.4324 × milk production) + (16.216 × fat production));

- Energy-corrected milk production (NASEM, 2021 – equation 20):

ProdCME = (9.29 × milk fat production/milk production) + (5.5 × total milk protein production/milk production) + (3.95 × milk lactose production/milk production);

- Daily energy excretion in milk, in Mcal/cow/day (NASEM, 2021):

EEM = Milk production x ((0.0929 × milk fat content) + (0.0563 × true milk protein content) + (0.0395 × milk lactose content));

- True milk protein content, in % (adapted from DePeters and Ferguson, 1992):

PV = (crude milk protein content x 0.94).

- Daily milk urea nitrogen excretion, in g/cow/day (adapted from Barros et al., 2017):

ProdNUL = (NUL × milk production/100).

- Somatic cell score (Shook, 1982): SCS = log2 (SCC/100.000) + 3.

Statistical analysis was conducted to evaluate the effect of different treatments (control, fat, and fat + emulsifier) on milk production, composition, and fatty acid profile of dairy cows, and the effect of milking shifts (morning, afternoon, and night) on milk production.

The first step involved assessing data normality, and since the vast majority of data, especially those related to milk productivity, did not show a normal distribution, a non-parametric analysis was chosen for all variables.

For the treatments, a mixed model was used, considering treatment as a fixed effect and the animal (cow) as a random effect, with repeated measures within cows across days. Additionally, for non-parametric comparison, the Kruskal-Wallis test was applied to the main variables. The model used for comparing the means was:

$$Y_{ij} = \mu + T_i + V_j + \varepsilon_{ij}$$

Where:

- Y_{ij} = observed value of the response variable (e.g., milk production, composition, fatty acids) for treatment i in animal j;
- μ = overall mean;
- T_i = fixed effect of treatment i (i=1,2,3 → control, fat, fat + emulsifier);
- V_j = random effect of animal j (j=1,2,...,n), to account for variability among cows and repeated measures within them;

- ε_{ij} = residual error associated with observation ij, assumed to be $\varepsilon ij \sim N(0,\sigma^2)$.

For variables with $p \leq 0.10$, the paired Mann-Whitney test with Bonferroni correction was applied, and superscript letters indicate differences. P-values equal to or less than 0.05 were considered statistically significant, and values between 0.05 and 0.10 were considered a tendency towards significance.

When the superscript letters were the same among treatments, it means that the Kruskal-Wallis test indicated a significant difference between treatments ($p < 0.05$) or a tendency ($p > 0.05$ and < 0.10), but multiple comparisons did not detect significant differences between pairs after correction.

4. Results and Discussion

No effect of palmitic acid supplementation, associated or not with the use of an emulsifier, was detected for milk yield (Table 2) (C - 43.98; FW - 44.85; FE - 44.83 L/d. p=0.16). In general, fat supplementation promotes an increase in milk production or its composition, especially when using easily digestible fat sources, such as saturated fats or bypass fats, allowing for better utilization of supplemental fat without the action of ruminal biohydrogenation (LOCK et al., 2013). Furthermore, a rise in almost 4% of EE was noted when supplementing 3.8%DM of palmitic acid over a control diet containing 2.8% of EE (MATHEWS et al., 2016). When supplements rich in saturated fatty acids are used, there is a general increase in milk fat concentration and production, often accompanied by higher milk yield (RELLING and REYNOLDS, 2007; LOCK et al., 2013; de SOUZA et al., 2020; GIRÓN et al., 2024). LOCK et al. (2013) reported an increase in 16-carbon fatty acid content and a 27% elevation in milk fat yield. Also, Girón et al. (2024) showed increases in milk fat and milk fat content in cows supplemented in the immediate postpartum, without losing corporal condition. Other researchers have additionally demonstrated increases in milk fat content (RELLING AND REYNOLDS, 2007; de SOUZA et al., 2020). Also, it is possible that fat globule size increases with milk fat yield, making the globules more susceptible to lipolysis (LANDRY et al., 2025). Also, *de novo* synthesis and overall bacterial fermentation are reduced when FA are increased in the diet, especially by fat supplementation (BIONAZ et al., 2020). Desaturation of C18:0 into *cis*-9 C18:1 by the mammary tissue has been suggested as a mechanism to maintain milk fluidity when secretion of saturated FA such as C16:0 increases (BARBANO and SHERBON, 1980).

Table 2: Effect of lipid source supplementation (high concentration of palmitic acid) associated or not with the use of an emulsifier (whole soybean lecithin, enriched with medium-chain triglycerides) on milk production characteristics, for the control (without added extra lipid source), fat, and fat + emulsifier groups (Median ± standard deviation, the respective P-value, and the comparison of means).

Variable	Unit	Control (median ± SD)	Fat (median ± SD)	Fat + emulsifier (median ± SD)	P-value
ProdMilk	kg/d	43,9 ± 8,3	44,8 ± 7,4	44,8 ± 7,8	0,164
ProdFCM35	kg/d	44,3a ± 9,7	47,9a ± 7,6[2]	47,9a ± 7,7[2]	0,058
ProdECM	kg/d	44,5a ± 9,4	47,6a ± 7,5[2]	47,9a ± 7,4[2]	0,084
EEM	Mcal/day	30,3b ± 6,5	32,7b ± 5,3[2]	32,9a ± 5,1[1]	0,062

Milk production (ProdMilk), 3.5% fat-corrected milk (ProdFCM35), Energy-corrected milk (ProdECM), Energy excretion in milk (EEM), Superscript number 1 (refers to 93 observations), Superscript number 2 (refers to 94 observations), Numbers without superscript (refers to 95 observations).

The lack of significance in milk yield can be due to a combination of factors, such as experimental design, level of fat inclusion in the diet, lactation stage, duration of supplementation, and energy status of the studied group, meaning that dietary energy density is only one aspect influencing lactation outcomes (MATHEWS et al., 2016), and further studies are needed. We used the label's value of the emulsifier, 10 g/d, and the diet of the PC group had 4.92%. Changes also weren't detected in milk yield with a dose up to 30 g/d of emulsifier in a diet with 4% FA, but de Souza et al. (2020) observed increased 16C digestibility, rises in 3.5% FCM, ECM, milk fat content, and milk fat yield. Fontoura et al. (2021) tested higher doses of emulsifier, starting on 33.5 g/d, over two treatments, moderate or high palmitic acid, with 2.4 and 2.6%FA, and also did not detect production responses, but MUN reduced in moderate PA diet, as well as DMI when increasing soy lecithin. This way lecithin increased feed efficiency (kg/kg DMI) of milk and ECM in moderated PA diet. This highlights the importance of focusing on the results when there is not a detected statistical difference in milk yield, been noted that other parameters unveil improvements in production and content. Higher doses of the emulsifier used in this experiment need to be tested, seen than, even with a considerable low dose compared to other studies, there were many trends towards effects. Those effects include rises in 3.5% FCM, ECM and milk fat production, and declines in milk protein and casein contents.

Table 3: Effect of lipid source supplementation (high concentration of palmitic acid) associated or not with the use of an emulsifier (whole soybean lecithin, enriched with medium-chain triglycerides) on milk composition, for the control (without added extra lipid source), fat, and fat + emulsifier groups (Median ± standard deviation, the respective P-value, and the comparison of means).

Variable	**Unit**	**Control (median ± SD)**	**Fat (median ± SD)**	**Fat + emulsifier (median ± SD)**	**P-value**
DDE%	%	9,1a ± 0,4	9,0a ± 0,5[2]	9,1a ± 0,5[1]	0,032
MTS%	%	12,9 ± 0,9	12,8 ± 0,8	12,9 ± 1,0	0,538
ProdMTS	kg/v/d	5,5 ± 1,1	5,7 ± 0,9	5,9 ± 0,9	0,105
Fat%	%	3,8 ± 0,7	3,8 ± 0,5[2]	3,8 ± 0,7[2]	0,980
ProdFat	kg/v/d	1,6a ± 0,4	1,7a ± 0,3[2]	1,7a ± 0,3[2]	0,086
Prot%	%	3,4a ± 0,3	3,3a ± 0,3	3,3a ± 0,3	0,082
ProtP	kg/v/d	1,4 ± 0,3	1,5 ± 0,2	1,5 ± 0,2	0,511
Lact%	%	4,7b ± 0,2	4,7b ± 0,3[2]	4,8a ± 0,3[2]	<0,001
ProdLact	kg/v/d	2,1a ± 0,4	2,1a ± 0,4	2,12a ± 0,4[2]	0,035
Case%	%	2,7a ± 0,2	2,6a ± 0,2	2,6a ± 0,2	0,075
MUN	mg/Dl	16,2b ± 2,9	16,5b ± 2,6	15,0a ± 2,6	<0,001

Defatted dry extract (DDE%), Milk total solids (MTS%), Milk fat contente (Fat%), Milk fat production (ProdFat), Milk protein content (Prot%), Milk protein production (ProtP), Milk lactose content (Lact%), Lactose production (ProdLact), Milk casein content (Case%), Milk urea nitrogen content (MUN), Superscript number 1 (refers to 93 observations), Superscript number 2 (refers to 94 observations), Numbers without superscript (refer to 95 observations).

Regarding milk composition, differences were detected in defatted dry extract (DDE%) (p=0.032), as well as in the production (ProdLact) (p=0.035) and content of lactose in milk (Lact%) (p=<0.001), and milk urea nitrogen content (MUN%) (p<0.001), for the fat and emulsifier group, compared to the control and fat groups. Cows supplemented with fat and emulsifier had 0,1% more lactose content and 0,8 and 0,5% less MUN concentration compared to those in the control group or only supplemented with fat (Table 3). These results could demonstrate positive effects caused by lecithin on nutrient utilization efficiency, as evidenced by a linear reduction in milk urea nitrogen (MUN) (Marchesini et al., 2012). Also, supplemented fat helps to decrease the rapid mobilization of fatty acids and maintain plasma glucose concentration (de VETH et al., 2009; ALLEN, M. S., 2023), which is the precursor of lactose, the component that determines the osmolarity of milk, as cows supplemented with 1.5% and 3% saturated fat acids increased milk yield and lactose, without changing DMI (WANG et al., 2010).

A tendency towards an effect of the treatments was also observed on milk fat yield (ProdFat) (p=0.086), milk protein content (Prot%) (p=0.082), and milk casein content (Case%) (p=0.075), also on the cows receiving fat and emulsifier (Table 3). In this study, no effect of treatment was detected for the content (MTS%) and production of total solids in milk (ProdMTS), milk fat content (Fat%), and milk protein production (ProdP) (Table 3).

The cows supplemented with fat + emulsifier showed a tendency towards an increase in 3.5% fat-corrected milk yield, by 3.58 kg/d (ProdFCM35), as well as energy-corrected milk (ProdECM) (p=0.084), and energy excretion in milk (EEM) (p=0.062) (Table 2). De Souza and Lock (2018a) reported an increase of 4.1 kg/d in energy-corrected milk (ECM) compared to the control, as well as de Souza et al., (2020) (1.6kg/d) and Landry et al., (2025) (3.05kg/d). Also, Lock et al. (2013) observed a 1.65 kg increase in fat-corrected milk (3.5% FCM) and a reduction in DMI, resulting in a better FCM/DMI ratio (1.505) compared to the control group (1.455), same as that reported by Landry et al. (2025) who, without a difference in DMI, reported that PA increased milk yield (+1.8 kg/d), and consequently ECM/DMI efficiency. We could not measure individual consumption, however, based on the literature, if there was a decrease in DMI by cows receiving fat + emulsifier, or if it was maintained, based on its higher production, and considering the measured increases in FCM and ECM, there could be better FCM/DMI ratio and ECM/DMI efficiency, representing a better use of the diet ingested by cows, driven by the exogenous emulsifier. The use of exogenous emulsifiers helps compensate for metabolic limitations and allows an increase in energy intake through lipid absorption, a theory supported by the apparent high digestibility of FA in moderate palmitic acid diets (FONTOURA et al., 2021). As demonstrated by Burch et al. (2021), a FA supplement blend providing ~60% C16:0 and 30% cis-9 C18:1 increased FA digestibility by 8.7 percentage units compared with a blend with ~60% C16:0 and 30% C18:0, which lacked C18:1 emulsifying activity. However, also, digestibility is affected by which fatty acid is supplemented. No reduction in FA digestibility was observed when the duodenal flow of C16:0 increased up to 500 g/d; however, it linearly decreased in the presence of C18:0 (BOERMAN et al. 2015), possibly because of the increased ability of palmitic acid to form micelles. We were not able to analyze feces; however, based on de Souza et al. (2020), the increase in FA intake linearly increased the amount of FA in feces, but at a lower extent as cows received higher doses of emulsifier, which raised total FA absorption.

Table 4: Effect of lipid source supplementation (high concentration of palmitic acid) associated or not with the use of an emulsifier (whole soybean lecithin, enriched with medium-chain triglycerides) on the milk fatty acid profile, for the control (without added extra lipid source), fat, and fat + emulsifier groups (Median ± standard deviation, the respective P-value, and the comparison of means).

Variable	**Unit**	**Control (median ± SD)**	**Fat (median ± SD)**	**Fat + emulsifier (median ± SD)**	**P-value**
MUFA	%	0,9b ± 0,1	0,9b ± 0,1	0,9a ± 0,2	0,020
PUFA	%	0,1 ± 0,9	0,1 ± 0,9	0,1 ± 0,9	0,763
SFA	%	2,4 ± 0,4	2,3 ± 0,3	2,3 ± 0,4	0,524
UFA	%	0,9b ± 0,1	0,9b ± 0,1	1,0a ± 0,2	0,034
Palm	%	1,2 ± 0,2	1,2 ± 0,2	1,1 ± 0,2	0,462
Este	%	0,31b ± 0,04	0,32b ± 0,04	0,34a ± 0,05	0,001
Oleic	%	0,7b ± 0,1	0,7b ± 0,1	0,8a ± 0,1	0,001
Pref	%	1,2 ± 0,2	1,2 ± 0,2	1,2 ± 0,2	0,438
Mixed	%	1,2 ± 0,2	1,2 ± 0,2	1,2 ± 0,2	0,466
DeNov	%	0,9 ± 0,2	0,9 ± 0,2	0,9 ± 0,2	0,537
Acet	%	0,05b ± 0,02	0,05b ± 0,02	0,07a ± 0,02	<0,001

Monounsaturated fatty acids (MUFA), Poliunsaturated fatty acids (PUFA), Saturated fatty acids (SFA), Unsaturated fatty acids (UFA), Palmitic acid (Palm), Stearic acid (Stearic), Oleic acid (Oleic), Preformed fatty acids (Pref), Mixed fatty acids (Mixed), *De novo* fatty acids (DeNov), Acetate (Acet), Superscript number 1 (refers to 93 observations), Superscript number 2 (refers to 94 observations), Numbers without superscript (refer to 95 observations).

The effect of high concentration palmitic acid supplementation, associated or not with an emulsifier, was also analyzed on the milk fatty acid profile. There were observed differences in C16:0 and C18:0, with a rise in the emulsifier treatment. On the other hand, C16:1, C18:1 cis, C18:2 and C18:3 were decreased in the fat treatments, compared to the control. Around 70% of the lipids of microbial origin leaving the rumen are neutral lipids produced by microorganisms partly via *de novo* synthesis using acetate and glucose to produce even-chain FA, mostly C18:0 and C16:0, and propionate for the synthesis of odd-chain FA. Also, around 20% of *de novo* synthesized FA by bacteria are monounsaturated FA (BIONAZ et al., 2020).

The percentage of monounsaturated fatty acids (MUFA) showed a statistical difference (p=0.020) to a higher extent, as did unsaturated fatty acids (UFA) (p=0.034), stearic acid (Stearic) (p=0.001), oleic acid (Oleic), and acetate (Acet) (p<0.001). The use of fat and emulsifier generated differences in the means between treatments for MUFA, UFA, Stearic, Oleic, and Acet, relative to the control and fat treatments. The concentration of polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA), palmitic acid (Palm), preformed fatty acids (Pref), mixed fatty acids (Mixed), and *de novo* fatty acids (DeNov) was not detected to be affected by fat supplementation, with or without emulsifier (Table 4).

Table 5: Effect of lipid source supplementation (high concentration of palmitic acid) associated or not with the use of an emulsifier (whole soybean lecithin, enriched with medium-chain triglycerides) on beta-hydroxybutyrate (BHB) content on two distinct dates during the experimental period, for the control (without added extra lipid source), fat, and fat + emulsifier groups (Median ± standard deviation).

Variable	Control (median ± SD)	Fat (median ± SD)	Fat + emulsifier (median ± SD)	P-value
BHB15	0,41 ± 0,13	0,39 ± 0,13	0,36 ± 0,15	0,328
BHB21	0,38 ± 0,11	0,38 ± 0,12	0,38 ± 0,16	0,868

Beta-hydroxybutyrate on day 15 (BHB15), Beta-hydroxybutyrate on day 21 (BHB21).

No effect of palmitic acid supplementation, associated or not with the use of an emulsifier, was detected on beta-hydroxybutyrate (BHB) content on days 15 and 21 (p=0.328 and p=0.868) (Table 5), considering that the cows were in mid-lactation, with the medians within the health range. Also, the values agree with the reference for the absence of ketosis (BHBA ≤ 1,1 mmol/L), a disease characterized by elevated ketone body levels in the bloodstream (Ospina et al., 2010; Poncheki, 2019).

5. Study Limitations

We encountered some limitations in this experiment's execution. First, the farm didn't have headlocks, which prevented us from measuring the individual intake from cows. Also, the number of animals available for forming homogeneous groups was small. Moreover, the farm's management made it impossible for us to prepare two, or even three different rations, one for each group. This brings us to the management we came up with, two people mixing the lipid supplement and the emulsifier in the feeding lane, one adding and the other mixing with the aid of a fork, making it impossible to avoid the possibility of human error.

6. Conclusion

In this experiment, neither PA supplementation nor the exogenous emulsifier were detected to elevate milk yield. However, there was a tendency towards have greaer 3.5%FCM and ECM in the FW and FE groups. Top dress supply of soy lecithin, together with palmitic acid, caused improvements in lactose content and DDE, and reduced MUN content. An increase in UFA, mainly MUFA, and Stearic, Oleic and Acet was detected, devoid of alterations in FA Pref, DeNov and Mixed groups. Under the conditions of this trial, PA and emulsifier had limited effects on overall production responses, which can be due to a combination of factors, such as experimental design, level of fat inclusion in the diet, lactation stage, duration of supplementation, and energy status of the studied group. So it remains to be determined to what extent the effects observed in the current study would reproduce in commercial settings, and reinforces the need to investigate practical feeding strategies that can be easily applied in daily farm management, and the use of other supplemental doses of emulsifier.

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