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INFLUENCE OF DIETARY FATTY ACIDS ON MILK FAT CONTENT AND COMPOSITION IN DAIRY RUMINANTS

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INFLUENCE OF DIETARY FATTY ACIDS ON MILK FAT CONTENT AND COMPOSITION IN DAIRY RUMINANTS

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Potential impact of this research

The study on lipid supplementation in the diets of cows, goats, and sheep has important scientific, economic, and social implications. By evaluating the fatty acid profiles of diets and lipid supplements used in feeding dairy ruminants, researchers can precisely predict and modulate milk fat production and composition, representing an advancement in optimizing dairy nutrition. From a scientific perspective, this research sheds light on how dietary fatty acid profiles directly impact milk fat production and milk fatty acid profile. By categorizing lipid-supplemented diets according to their fatty acid profiles, researchers and nutritionists can enhance their understanding of these effects, paving the way for specific dietary interventions aimed at improving milk component production. Economically, the ability to predict and regulate milk production through diet manipulation offers benefits to nutritionists and producers. Strategic adjustments in diets to achieve desired fatty acid profiles can enhance feed efficiency and improve the quality of dairy products. This can potentially open up new markets and lead to greater profitability in the dairy sector. This study also has potential implications for public health. Enhancing milk quality through supplementation with lipids containing specific fatty acid profiles facilitates the production of more nutritious dairy products with beneficial health effects, thereby improving consumer health. Furthermore, optimizing milk production promotes sustainability in the dairy sector by increasing production efficiency.

Impacto potencial desta pesquisa

O estudo sobre a suplementação lipídica nas dietas de vacas, cabras e ovelhas tem importantes implicações científicas, econômicas e sociais. Ao avaliar os perfis de ácidos graxos das dietas e suplementos lipídicos utilizados na alimentação de ruminantes leiteiros, os pesquisadores podem prever e modular com precisão a produção e a composição de gordura do leite, representando um avanço na otimização da nutrição dos laticínios. Do ponto de vista científico, esta pesquisa esclarece como os perfis de ácidos graxos dietéticos impactam diretamente a produção de gordura do leite e o perfil de ácidos graxos do leite. Ao categorizar dietas suplementadas com lipídios de acordo com seus perfis de ácidos graxos, os pesquisadores e nutricionistas podem aprimorar seu entendimento desses efeitos, abrindo caminho para intervenções dietéticas específicas que visem melhorar a produção de componentes do leite. Economicamente, a capacidade de prever e regular a produção de leite através da manipulação da dieta oferece benefícios para nutricionistas e produtores. Ajustes estratégicos nas dietas para alcançar perfis desejados de ácidos graxos podem aumentar a eficiência alimentar e melhorar a qualidade dos produtos lácteos. Isso pode potencialmente abrir novos mercados e levar a uma maior rentabilidade no setor de laticínios. Este estudo também tem possíveis implicações para a saúde pública. Melhorar a qualidade do leite através da suplementação com lipídios contendo perfis específicos de ácidos graxos facilita a produção de produtos lácteos mais nutritivos, com efeitos benéficos para a saúde, melhorando assim a saúde do consumidor. Além disso, otimizar a produção de leite promove a sustentabilidade no setor de laticínios, aumentando a eficiência da produção.

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BIOGRAPHY

WALTER BEDON GALLARDO was born on March 23, 1980, in the town of Picoy, Santa Leonor district, Huaura province, Lima, Peru. He graduated in Animal Science from the National University José Faustino Sánchez Carrión, Huacho, Peru, between the years 2001 and 2016. During his undergraduate studies, his research focused on the effect of body condition score on the productive performance of dairy cows. He continued his graduate studies at the Federal University of Mato Grosso, Sinop campus, Mato Grosso, Brazil, where he obtained a Master's degree in Animal Science 2020. During his Master's degree, he investigated the effect of lipid supplementation on the performance, metabolism, and fatty acid profile in the milk of dairy cows, under the guidance of Dr. André Soares de Oliveira. In April 2020, he began his Ph.D. in Animal Science at São Paulo State University, Jaboticabal, São Paulo, Brazil. During this period, his research has focused on quantitative analysis with a meta-analytic approach on the effect of dietary fatty acid profile and fat supplements on fat production and fatty acid profile in the milk of dairy ruminants, such as cows, sheep, and goats, under the guidance of Dr. Izabelle Auxiliadora Molina de Almeida Teixeira. In addition to his academic training, Walter Bedon Gallardo has worked as a contracted professor at the School of Animal Science at the National University José Faustino Sánchez Carrión, Huacho, Peru, between the years 2013 and 2017. He has also worked and provided consultancy services to dairy farms in the Huara Valley, Lima region, Peru, after his graduation period, between the years 2007 and 2017.

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SUMMARY

INFLUÊNCIA DE ÁCIDOS GRAXOS DIETÉTICOS NO TEOR E COMPOSIÇÃO DE GORDURA NO LEITE DE RUMINANTES LEITEIROS

RESUMO - A suplementação lipídica nas dietas dos ruminantes leiteiros é importante, não só para aumentar a densidade energética da dieta, mas também para fornecer vários ácidos graxos (AG) com funções fisiológicas bioativas. Esses AG podem influenciar a expressão gênica, a composição da microbiota e a composição do leite. No entanto, ainda existem controvérsias quanto aos efeitos dos suplementos de gordura na produção de gordura do leite e no perfil de AG do leite. A inconsistência nos resultados é atribuída à variedade de fontes lipídicas, cada uma com perfis de AG distintos. O objetivo principal desta meta-análise foi investigar o impacto do perfil de AG nas dietas de ruminantes leiteiros sobre seu desempenho produtivo e perfil de AG no leite. Especificamente, avaliamos como o perfil de AG da dieta e/ou suplementos lipídicos podem afetar a produção de gordura e o perfil de AG do leite. Outro objetivo do nosso estudo foi desenvolver modelos preditivos para a produção de gordura no leite e AG no leite com base nas características dos animais, dieta e suplementos lipídicos, utilizando uma abordagem de meta-regressão. Para realizar essas análises, foram desenvolvidos dois bancos de dados separados: um para avaliar os efeitos em vacas leiteiras lactantes, compreendendo 217 artigos revisados por pares abrangendo 515 médias de tratamento, e outro para avaliar os efeitos em cabras e ovelhas, compreendendo 144 artigos revisados por pares com um total de 331 médias de tratamento. O tamanho do efeito foi avaliado usando as diferenças médias brutas (RMD) entre dietas com (tratamento) e sem (controle) fontes lipídicas. As médias foram ponderadas pelo inverso da variância em um modelo misto, e a heterogeneidade foi analisada usando análise de subgrupos. As dietas com suplementos lipídicos em vacas e suplementos lipídicos em pequenos ruminantes foram agrupadas usando análise de clusters e caracterizadas pelo seu perfil de AG. As dietas contendo níveis intermediários e altos de ácidos graxos insaturados (AGI), classificadas como grupos 1, 2 e 3, mostram uma redução na ingestão de matéria seca (IMS), produção de gordura e proporção de gordura no leite, bem como no ácido palmítico, AG mistos e ácidos graxos saturados (AGS) no leite. Em contraste,

esses mesmos grupos demonstraram um aumento nos níveis de ácido oleico, cis9, trans11 CLA e AG pré-formados no leite de vacas leiteiras. As dietas ricas em AGS (grupo 4) mostraram efeitos opostos na maioria das variáveis mencionadas, exceto na IMS, onde não foi observado efeito. Da mesma forma, observou-se que a maioria das dietas avaliadas resultam em um aumento na produção de leite e uma diminuição na porcentagem de proteína no leite, exceto aquelas ricas em ácidos graxos poli-insaturados (AGPI, grupo 3). Vale ressaltar que todas as dietas avaliadas levaram a uma diminuição nos AG de novo no leite de vacas leiteiras. Ao avaliar a resposta de pequenos ruminantes leiteiros à suplementação lipídica, observou-se que suplementos ricos em ácido oleico (grupo 1) mostraram atividade significativa no perfil de AG dessas espécies de ruminantes. Esses suplementos não apenas aumentaram a produção de leite, mas também aumentaram os ácidos graxos eicosapentaenoico, docosapentaenoico e docosahexaenoico no leite, ao mesmo tempo que reduziram a proporção de proteína no leite de ovelhas leiteiras. Em cabras leiteiras, esses suplementos aumentaram a lactose, bem como trans-11 C18:1, C20:0 e cis9, trans11 CLA C18:2 AG no leite. Vale ressaltar que fontes de suplemento do grupo 1 (suplementos ricos em ácido oleico) diminuíram os AG cáprico, láurico, mirístico e palmítico, bem como AG de cadeia ímpar e AGS no leite, tanto em cabras quanto em ovelhas. Adicionalmente, observou-se um aumento nos níveis de AG esteárico e oleico no leite de ambas as espécies. Também desenvolvemos modelos usando uma abordagem de meta-regressão com o objetivo de prever tanto a produção de leite quanto a proporção de gordura no leite, juntamente com o perfil de AG no leite. Esses modelos consideram várias variáveis, incluindo características do animal, produção e dieta. Os modelos resultantes foram ajustados com alta precisão e exatidão, como evidenciado por um coeficiente de correlação de concordância superior ao limite de 0,90 e um erro quadrático médio inferior a 15,9%. Em conclusão, a classificação de suplementos lipídicos e/ou dietas com base em seus perfis de AG desempenha um papel importante na determinação de seus efeitos na produção de leite, componentes do leite e perfis de AG em ruminantes leiteiros. Também é importante notar que esses efeitos mostram variações entre diferentes espécies e podem ser previstos com alta precisão e exatidão ao considerar as características específicas do animal, o perfil de AG do suplemento

e o perfil de AG da dieta. Nossos modelos preditivos sugerem sua potencial aplicabilidade para otimizar a produção e composição do leite em ruminantes leiteiros.

Palavras-chave: ruminantes, ácidos graxos, gorduras na dieta, meta-análises, gordura do leite

INFLUENCE OF DIETARY FATTY ACIDS ON MILK FAT CONTENT AND COMPOSITION IN DAIRY RUMINANTS

ABSTRACT- Lipid supplementation in the diets of dairy ruminants is important, not only for increasing the energy density of the diet but also for contributing various fatty acids (FA) with bioactive physiological functions. These FA can influence gene expression, microbiota composition, and milk composition. However, controversies persist regarding the effects of fat supplements on milk fat production and milk FA profile. The inconsistency in results is attributed to the variety of lipid sources, each with distinct FA profiles. The ultimate objective of this meta-analysis was to investigate the impact of the FA profile in the diets of dairy ruminants on their productive performance and FA profile in milk. Specifically, we evaluated how the FA profile of the diet and/or lipid supplements can affect fat production and the FA profile of milk. The other objective of our study was to develop predictive models for milk fat production and milk FA based on the characteristics of the animals, diet, and lipid supplements using a meta-regression approach. To conduct these analyses, two separate databases were developed: one to assess the effects on lactating dairy cows, comprising 217 peer-reviewed articles encompassing 515 treatment means, and another to evaluate the effects on goats and sheep, comprising 144 peer-reviewed articles with a total of 331 treatment means. The effect size was assessed using the raw mean differences (RMD) between diets with (treatment) and without (control) lipid sources. Means were weighted by inverse variance in a mixed model, and heterogeneity was analyzed using subgroup analysis. Diets with lipid supplements in cows and lipid supplements in small ruminants were grouped using cluster analysis and characterized by their FA profile. Diets containing intermediate and high levels of unsaturated fatty acids (UFA), classified as groups 1, 2, and 3, show a reduction in dry matter intake (DMI), fat production, and fat proportion in milk, as well as in palmitic acid, mixed FA, and saturated fatty acids (SFA) in milk. In contrast, these same groups demonstrated an increase in the levels of oleic acid, cis9, trans11 CLA, and preformed FA in the milk of dairy cows. Diets rich in SFA (group 4) showed opposite effects on most of the mentioned variables, except for DMI, where no effect was observed. Likewise, it has been

observed that most evaluated diets result in an increase in milk production and a decrease in milk protein percentage, except for those rich in polyunsaturated fatty acids (PUFA, group 3). It is worth noting that all evaluated diets led to a decrease in *de novo* FA in the milk of dairy cows. When evaluating the response of small dairy ruminants to lipid supplementation, it was observed that supplements rich in oleic acid (group 1) showed significant activity in the FA profile of these ruminant species. These supplements not only increased milk production but also increased of eicosapentaenoic, docosapentaenoic, and docosahexaenoic fatty acids in milk, while reducing the proportion of milk protein of dairy sheep. In dairy goats, these supplements increased lactose, as well as trans-11 C18:1, C20:0, and cis9, trans11 CLA C18:2 FA in milk. It is noteworthy that supplement sources from group 1 (supplements rich in oleic acid) decreased capric, lauric, myristic, and palmitic FA, as well as odd-chain FA and SFA in the milk, in both goats and sheep. Additionally, an increase in the levels of stearic and oleic FA was observed in the milk of both species. We also developed models using a meta-regression approach with the aim of predicting both milk production and milk fat proportion, along with the FA profile of milk. These models consider various variables, including animal, production, and dietary traits. The resulting models were fitted with high precision and accuracy, as evidenced by a concordance correlation coefficient exceeding the threshold of 0.90 and a root mean square error of less than 15.9%. In conclusion, the classification of lipid supplements and/or diets based on their FA profiles plays an important role in determining their effects on milk production, milk components, and FA profiles in dairy ruminants. It is also important to note that these effects show variations among different species and can be predicted with high precision and accuracy by considering the specific characteristics of the animal, the FA profile of the supplement, and the FA profile of the diet. Our prediction models suggest their potential applicability to optimize milk production and composition in dairy ruminants.

Key words: ruminants, fatty acids, dietary fats, meta-analysis, milk fat

LIST OF FIGURES

CHAPTTER 2

LIST OF TABLES

THESIS STRUCTURE

Chapter 1 presents a literature review on lipid supplementation in dairy ruminants and its effects on milk fat, fatty acid profiles, and lipid metabolism in the mammary gland and rumen. Additionally, we covered the need and importance of developing prediction models to estimate milk fat and fatty acid profiles. This chapter was written following the guidelines of the Graduate Program in Animal Science at UNESP, Jaboticabal Campus.

Chapter 2 presents a meta-analysis examining the relationship between the dietary fatty acid profile and milk fat production, as well as the fatty acid composition in dairy cows. Additionally, it proposes predictive models for estimating milk fat and fatty acids. This chapter has already been published in the journal "Animals" following its guidelines. The reference citation of this paper is: Gallardo, W.B.; Teixeira, I.A.M.A. Associations between Dietary Fatty Acid Profile and Milk Fat Production and Fatty Acid Composition in Dairy Cows: A Meta-Analysis. Animals 2023, 13, 2063. https://doi.org/10.3390/ani13132063. However, in this thesis, modifications were made to the font style and margins to meet the graduate program's requirements.

Chapter 3 presents a meta-analysis investigating the effect of the fatty acid profile of fat supplements on milk fat production and fatty acid composition in small ruminant dairy animals, specifically sheep and goats. Additionally, predictive models have been proposed to estimate milk fat and fatty acids. This chapter was written following the guidelines of the Journal of Dairy Science. The authors of the article are Walter B. Gallardo and Izabelle A. M. A. Teixeira.

CHAPTER 1 – General considerations

INTRODUCTION

Fatty acids (FA) are carboxylic acids that serve as key components of fats and oil, with multiple biological functions. They are not only important sources of dietary energy, but they also play important structural and functional roles in cell biology (Sardesai, 2020, Cerone and Smith, 2021). Likewise, fats provide essential FA (linoleic and linolenic acids) that the animal cannot synthesize, facilitate the absorption of fat-soluble vitamins, and supply specific bioactive FA (Such as omega-3, omega-6 and conjugated linoleic acids). Traditionally, FA supplementation in lactating dairy ruminants aimed to increase ration energy density (Baumann et al., 2016). However, recent research has extended beyond energy, exploring the effects of FA on the transcriptome and regulation of milk fat synthesis.

The effects of lipid supplements in the diet of dairy ruminants have shown some inconsistency, which is understandable given the diverse nature of FA and their sources. Therefore, it is essential to group lipid sources based on similar characteristics to precisely measure the real effects of lipids across ruminants species.

Various studies, including those by Glasser et al. (2008); Rabiee et al. (2012); Hu et al. (2017); Weld and Armentano (2017); Mahdavi et al. (2019); dos Santos Neto et al. (2021) in dairy cows, Angeles-Hernandez et al. (2020), Vargas-Bello-Perez et al. (2021) in dairy sheep, and Marín et al. (2015) in dairy sheep and goats, have addressed the effects of different lipid supplements on dairy ruminants. These studies highlighted the lack of a uniform effect of lipid supplements, emphasizing the importance of the type of supplement, ruminant species, and FA profile. In a previous study carried out as part of a master's degree (Gallardo and Oliveira, 2020), we evaluated the impact of lipid supplementation classified according to their origin. Although we obtained interesting results regarding the effects of lipid supplements, there was considerable variability in the responses. Given the variation in FA profiles among supplement sources, classifying them according to their origin may not be the

best approach. Instead, grouping based on common characteristics such as the FA profile seems more appropriate.

Predictive models to estimate milk production and FA profile have been important for feeding management, particularly in dairy cows (Hermansen, 1995, Moate et al., 2008, Daley et al., 2022). However, these models must focus on specific supplement or diet types, considering their FA profile. Furthermore, specific models should be established for different species to account for inherent variations in lipid supplements and diets.

Therefore, this thesis aims to emphasize the importance of categorizing lipid sources and/or diets based on common characteristics, such as the FA profile. This categorization aids in determining their effects on fat production and the FA profile of milk in dairy ruminants, including cows, goats, and sheep. Additionally, we developed models to predict milk fat production and FA profile in the milk of various dairy ruminant species.

FATTY ACIDS IN RUMINANT NUTRITION

Between 1950 and 1990, significant advances were made in improving our understanding of lipid digestion, absorption, and metabolism in dairy cows (Bionaz et al., 2020). Ruminants primarily obtain lipids from forage, cereal grains, oilseeds, and supplemental fats. Lipid metabolism in ruminants differs from that observed in non-ruminants due to microbial fermentation of nutrients in the rumen (Bell, 1981). Bacterial biohydrogenation converts unsaturated FA (UFA) into saturated FA (SFA). This process is vital for the survival of rumen microorganisms. Excessive UFA supplementation can negatively affect fiber digestion in the rumen and the performance of the animal (Jenkins et al., 2008).

Ruminant diets usually contain approximately 3.0% fat, mostly derived from forage (Bionaz et al., 2020). However, high-producing dairy cows consume diets–5-6% fat through supplements and concentrates (Bionaz et al., 2020, Jenkins, 1993). Although this amount is low compared to that of monogastrics, it highlights the importance of fat in ruminants nutrition. Major benefits have been reported when supplementing with FA, which impact the transcriptome and exert nutrigenomic effects, underscoring the importance of these bioactive components in the diet of dairy cows (Osorio et al., 2016). Additionally, based on the supplied

FA profile and its interactions with dietary components and the physiological status of cows, FA supplementation can influence energy partitioning. This occurs through direct effects on the mammary gland and by affecting insulin concentration and tissue insulin sensitivity (Piantoni and Vandehaar, 2023). Several studies have shown that lipid supplementation in the diet of dairy cows modifies fat synthesis (Kliem and Shingfield, 2016, Bernard et al., 2018). While some sources increase the proportion of fat in milk, others have no effect or even decrease it. The divergence in results appears to be linked to the FA profile of the diet, which varies significantly depending on the type of lipid supplement used (Gallardo and Teixeira, 2023). Ghasemi et al. (2021) observed an increase in the proportion of milk fat in diets supplemented with palmitic acid, whereas diets containing calcium salts rich in linoleic acid showed no effect. Similarly, Hu et al. (2017) demonstrated that the effects of palmitic and stearic acid supplementation differ when given together or separately. In the first case, fat concentration increased, whereas in the second case, no effect was observed. Other studies evaluating various supplement sources revealed that each supplement had specific effects on the variables analyzed (Rabiee et al., 2012, Weld and Armentano, 2017). Disparities in results between ruminant species can be attributed to physiological variations and differences in the type of feed intake (Hofmann, 1989).

Plants and animal fats are the main sources of lipids in ruminant diets and are categorized into various types, including oilseeds, vegetable oils, animal fats, processed fats, fat blends, and fish oil (Table 1). The extent to which the FA composition of milk can be altered depends on factors such as genetics, days in milk, diet, and ruminant species. However, this feeding strategy is considered one of the most efficient ways to modify the FA composition of milk (Hanuš et al., 2018).

Milk FA are considered important nutritional components of the human diet and substantially affect human health (Hanuš et al., 2018). Adding fat supplements to the diet can modify the FA composition of milk, contributing to public health policies advocating reducing SFA consumption and increasing PUFA intake (Kliem and Shingfield, 2016).

Table 1. Lipid sources typically used in ruminant diets[1]

Fat source[2]	TFAs	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1 trans	C18:1 cis	C18:2	C18:3	Others
Oilseeds											
Cottonseed	18.26		0.69	23.91	0.55	2.33		15.24	56.48	0.19	0.61
Linseed	27	0.01	0.03	5.07	0.04	3.26	0.01	15.3	15.7	59.6	0.3
Rapeseed	34.4	0.01	0.05	4.91	0.19	1.54	0.01	59.9	20.5	9.8	2.6
Safflower	30.7		0.10	10.77		11.97		14.33	60.63	0.30	1.9
Soybeans	16.99	0.58	0.2	11.93	0.08	4.05		21.99	52.43	7.59	1.17
Flaxseed	33.41		0.16	5.74	0.18	4.3		18.8	14.15	55.95	0.64
Sunflower	37.2		0.1	5.2	0.1	4.1		39.4	47.9	0.4	2.8
Vegetable seed oils											
Cottonseed oil	88		0.83	25.97	0.57	3		20.16	48.93	0.1	0.44
Linseed oil	88	0.01	0.03	5.07	0.04	3.26	0.01	15.3	15.7	59.6	0.3
Rapeseed oil	88	0.01	0.05	4.91	0.19	1.54	0.01	59.9	20.5	9.8	2.6
Safflower oil	88		0.1	10.77		11.97		14.33	60.63	0.3	1.9
Soybeans oil	88	0.11	0.11	10.83	0.14	3.89		22.82	53.75	8.23	0.13
Flaxseed oil	88		0.16	5.74	0.18	4.3		18.88	14.15	55.95	0.64
Sunflower oil	88			7.33	0.09	10.65	0.59	43.39	35.49	0.79	1.67
Corn oil	88			11.08		1.55		26.95	58.95	1.1	0.38
Palm oil	88	0.2	1.1	44		4.5		39.2	10.1	0.4	0.1
Camelina oil	88		0.06	5.2	0.08	2.5	10.57	15.1	12.5	32	21.5
Animal fats and blends											
Tallow			3.7	24.9	4.2	18.9		36	3.1	0.3	
Yellow grease		0.1	0.7	14.26	1.43	8.23		43.34	26.25	2.51	
Choice white grease		0.1	1.57	22.04	5.03	9.95		42.45	13.17	0.97	
Processed fats											
CSSFA	85.5			85.5		3.7		6.9	1.2		
CSUFA	85.5		0.14	8.04		2.37		22.26	43.09	3.35	5.21
Prill fat	99		1.5	85.1		2.5		7.9			
Hydrogenated fats	99.5	0.5	1.24	46.3		47.4		1.2	0.07		
Fish oil											
Atlantic menhaden oil	88		7.3	19	9.1	4.2		13.2	1.3	1.3	42
Northem krill	88		4.6	18.4	3.5	2.2		14.7	1.8	1.3	50.3
Sardine	88		5.7	17.8	6.8	3.1		7.8	2.3	1.9	49.9
Anchovy	88		10.2	23.5	10.6	3.4		10.3	1.6	0.8	29.3

[1]The composition used in this study are based on the references from NASEM (2021), except for processed fats and fish oils. Information about processed fats was gathered from commercial sources, while data regarding fish oil were extracted from Shahidi's research (2007).

[2]CSSFA: calcium salts of saturated fatty acids, CSUFA: calcium salts of unsaturated fatty acids

The inclusion of vegetable oils or oilseeds in the diet of lactating cows significantly affects the FA composition of milk. Studies have shown that this practice decreases the proportion of medium-chain SFA, increases the levels of stearic acid, and reduces the total concentrations of SFA in milk (Shingfield et al., 2008). These changes in the FA composition of milk often coincide with a general decrease in the production of short/middle-chain FA such as lauric acid (C12:0) and myristic acid (C14:0), and palmitic acid (C16:0). It has been suggested that this phenomenon may be attributed to an increase in the supply of 16-carbon FA in the diet, which inhibits the transcription and activity of acetyl-CoA carboxylase in the mammary gland, thereby reducing the proportion of de novo FA (Barber et al., 1997).

Diet plays an important role in the metabolic and cardiovascular health of both ruminants and humans. Omega-3 and omega-6 FA have been the subject of intense scrutiny due to their beneficial health effects. Supplementation with bioactive lipids has been associated with an increased bioavailability of these FA, especially in certain types of cells (Kumar et al., 2019). Additionally, FA has bioactive effects on the transcriptome, regulating gene expression through transcription factors that act as transcriptional regulators (Osorio et al., 2016).

During digestion, FA released by lipolysis undergoes rapid hydrogenation facilitated by bacterial isomerases and reductases (Jenkins, 1993). The binding of FA to feed particles in the rumen enhances biohydrogenation, which is a crucial process in this organ. The rumen microbiota impacts dietary lipids, influencing the type and form of FA reaching the intestine. Various FA, especially polyunsaturated FA, may negatively affect the microbiota, and biohydrogenation is considered a mechanism to reduce the toxicity of these FA to bacteria (Maia et al., 2010).

The milk quality of dairy ruminants is closely related to their FA profiles. Specific modifications to this profile can enhance the production of dairy products with higher added value. However, incorporating dietary FA into the ruminant diet may have adverse effects such as oxidative stress, which can trigger homeostatic imbalances in the mammary gland. Therefore, it is crucial to explore the potential of various antioxidant components in food to counteract these effects (Kyriakaki et al., 2022).

Feeding plays a fundamental role in enhancing milk quality in dairy ruminants such as sheep, whose diets, especially pasture-based and formulated with appropriate concentrates, can influence milk fat and FA profiles. Factors such as the source and level of fiber also impact the effects of lipid supplements such as vaccenic acid and conjugated linoleic acid (CLA). Recent research suggests that essential oils may improve the FA composition by interfering with biohydrogenation (Nudda et al., 2014).

In recent decades, there has been growing interest in understanding the FA composition in ruminant milk supplemented with lipid sources, especially because of the presence of CLA isomers that have beneficial biological effects (Palmquist et al., 2005). However, inconsistent results from various lipid supplement sources have highlighted the need to standardize characteristics to obtain more consistent outcomes. In this regard, studies such as those by Rennó et al. (2013) have shown that including long chain FA in the diet of dairy cows can increase the biological value of milk, especially at the onset of lactation, as evidenced by higher concentrations of specific FA such as CLA C18:2 cis-9, trans-11.

The drive to enhance the biological value of animal products, particularly milk, stemmed from FAO's 2003 recommendations on the consumption of SFA and trans FA in humans. Ruminants, due to the presence of CLA in their products like milk, have gained interest because CLA has potential health benefits for humans, including cancer prevention, reduction of atherosclerosis, and enhancement of the immune response (Jenkins et al., 2008). Additionally, dietary FA influences body composition, milk fat synthesis, milk production, and other reproductive aspects of animals (Jenkins et al., 2008, Bionaz et al., 2020).

In this context, the next crucial step involves the ability to modulate or predict milk fat production and its FA profile. Given the relevance of FA in milk to human health, it is essential to develop tools that enable this modulation and prediction according to market demands. Several studies have proposed predictive models for milk fat production (Moate et al., 2008, Maxin et al., 2011, Daley et al., 2022). However, these models have primarily focused on measuring milk fat production, leaving a gap in predicting the specific FA profile of milk. It is important to address this point and consider the amount of fat present in the diet

and/or supplements as predictor variables for both milk fat production and milk FA profile.

Hermansen (1995) proposed predictive models for milk FA profiles. However, these models only predict the concentrations of individual fatty acids. Therefore, it is important to develop models that consider the lipid characteristics of diet and/or supplements as predictor variables. Additionally, these models should have the ability to predict FA according to their origin (de novo, mixed, and preformed), which would not only be more scientifically interesting but also more applicable on-farm.

OBJECTIVE

In this thesis, we aim to improve our understanding of the factors associated with the FA profile of the diet and/or supplements, and how these factors influence milk production, milk fat production, and FA profile in the milk of dairy ruminants, especially in cows, goats, and sheep. Likewise, we aim to predict both milk fat production and milk FA profile in these ruminant species.

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CHAPTER 2 - Associations between Dietary Fatty Acid Profile and Milk Fat Production and Fatty Acid Composition in Dairy Cows: A Meta-Analysis

Simple Summary: Supplementing dairy cow diets with lipids offers important benefits for meeting the energetic demands of the cow and influencing milk composition. However, the effects of lipids vary due to the large variety of lipid sources and fatty profiles. Using a comprehensive analysis, we highlighted the importance of the fatty acid profile of lipid-supplemented diets for predicting their impact on milk fat production and composition. Diets rich in saturated fatty acids increase milk fat production and proportion while reducing short- and medium-chain fatty acids in milk. Conversely, diets high in unsaturated fatty acids increase long-chain fatty acids in milk. Likewise, by considering animal production and diet characteristics, milk fat production and the fatty acid profile of milk can be predicted and modulated. These findings demonstrate the potential to optimize milk composition through targeted dietary interventions.

Abstract: This meta-analysis aimed to investigate the effect of dietary fatty acid (FA) profile on milk fat production and FA profile in dairy cows. The study also aimed to develop prediction models using a meta-regression approach. The database included 217 peer-reviewed articles on lactating dairy cows (n = 12,892), consisting of 515 treatment means. Effect size was assessed using the raw mean differences between diets with supplementary lipid sources and those without. Subgroup analyses were employed to assess heterogeneity. Diets rich in saturated FA (SFA) increased milk fat production and proportion, while reducing de novo FA in milk. Diets high in monounsaturated FA and polyunsaturated FA decreased mixed FA in milk. Most lipid-supplemented diets increase preformed FA in milk, except those rich in SFA. Prediction models were developed using meta-regression. Key predictors of milk fat production included neutral detergent fiber (NDF), dietary myristic acid, and milk production. Milk fat proportion was best predicted by dietary unsaturated FA, NDF, and forage. De novo FA in milk was predicted by dry matter intake (DMI) and dietary FA, while preformed FA was predicted by DMI, dietary oleic and linoleic acids. In conclusion, this study emphasizes the importance of the dietary FA profile in evaluating the effects of lipids on milk fat production and FA profile. Accurate and precise predictions of

milk fat production, proportion, and FA profile can be achieved by considering cow production and dietary characteristics.

Keywords: dietary fat; fatty acid profile; meta-regression; milk fat composition; milk fatty acid profile; dietary fatty acid profile; fatty acids; lipid-supplemented diets; dairy cows; meta-analytic approach

1. INTRODUCTION

The utilization of lipid supplements in dairy cow nutrition dates back to the late 19th and early 20th centuries [1–3]. Lipid supplementation was initially employed to augment the dietary energy density, thereby enhancing the productive performance, fertility, and energy status of lactating cows [4]. However, recent research demonstrates that lipids exert essential bioactive physiological functions beyond their role as energy sources [5]. These functions include the modulation of gene expression, microbiota composition, and milk components, highlighting the importance of lipid supplementation in modern dairy production.

Compared to protein and lactose, milk fat is a highly variable component that can range from 2.4 to 5.5 g per 100 g of raw milk [6], and is influenced by both nutritional and non-nutritional factors [7]. Milk fat originates from two primary sources [8]: de novo fatty acids (FA) synthesized in the mammary gland, with acetate and beta-hydroxybutyrate as precursors, and preformed FA from the blood derived from diet and body mobilization [8–10]. Nutrition is a key determinant of milk fat production and the FA profile of the milk [11], with effects that can be either positive or negative, depending on the type and source of lipids used (e.g., seeds, oils, animal fat, rumen protected fats, and by-products of marine origin) and their respective FA profiles [12–14]. Rabiee et al. [14] evaluated lipid sources grouped by origin, and reported that oilseeds decrease fat concentration but increase daily milk fat production. They also found that Megalac (calcium salts of palm fat, Church and Dwight Co. Inc., Princeton, NJ, USA) improves both milk fat composition and production, but other types of calcium soap decrease milk fat proportion and production. More recently, dos Santos Neto et al. [12] reported that palmitic acid calcium soaps increase daily production but do not affect the proportion of milk fat. Furthermore, the

supplementation of palmitic acid in different forms (e.g., oilseeds, protected fats, and oils) has varying effects on milk production and the FA profile of the milk [15].

There is increasing interest in better understanding the effects of dietary lipid supplements in dairy cows on milk fat production and the FA profile in milk. However, the wide variety of fat sources and their different FA profiles make it difficult to clearly understand their actual effects. One possible solution is to group lipid-supplemented diets according to their FA profile, which could allow for a more precise assessment of the effects and, ultimately, the modulation of milk fat production and milk FA profile. Based on this, in our study, we set two specific objectives: (1) to investigate how the FA profile of the lipid-supplemented diet, grouped by FA profile, affects the milk fat production and proportion, as well as the milk FA profile, and (2) to develop empirical models to predict milk fat production and FA profile, taking into account cow production and diet characteristics. To achieve this, we used meta-analytic approaches that enabled obtaining reliable and precise results.

2. MATERIALS AND METHODS

2.1. Literature Search

A database was developed based on peer-reviewed journal articles published between 2000 and 2021. We conducted a systematic search of Web of Science, Scopus, and PubMed using the following keywords: “fatty acids” AND “milk fat”. EndNote X9 was used to manage articles.

Articles that met all of the following eligibility criteria were included in the database: (1) studies with lactating cows (encompassing different breeds of dairy cows); (2) utilization of both control (without lipid supplementation) and treatment (with lipid supplementation) diets—studies involving ruminal, post ruminal, or intravenous infusions were not included; (3) reports of the treatment means and respective measures of dispersion (standard error (SE), standard error of the mean, standard error of difference, or coefficient of variation); (4) reports of the composition and/or FA profile of the lipid supplements and diets; and (5) publication as full-length articles in indexed journals with a JCR impact factor. Duplicate studies and those failing to meet the eligibility criteria were excluded. A

total of 217 peer-reviewed articles were included in this study (Figure 1 and Supplementary Material S1).

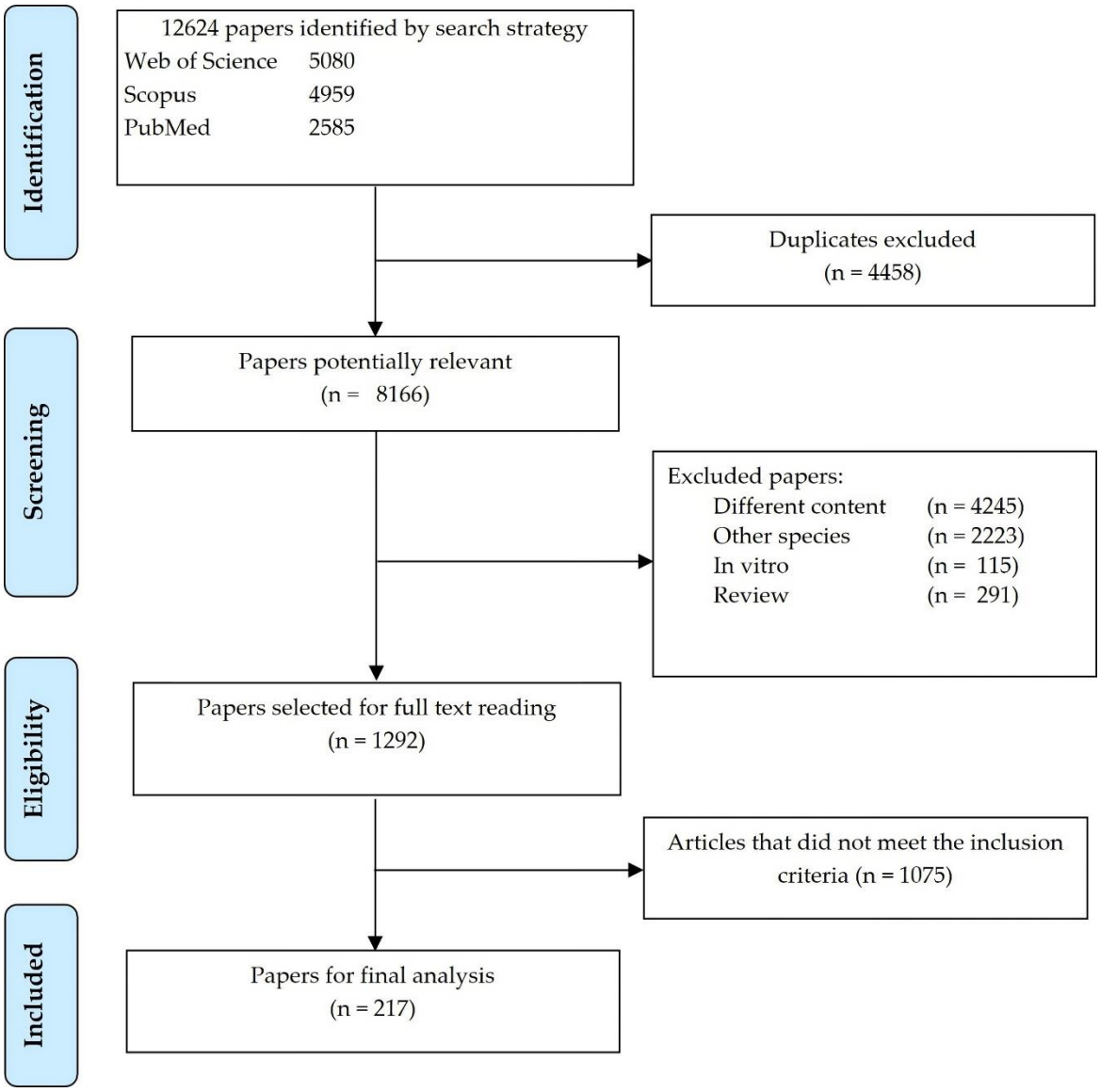

Figure 1. PRISMA flowchart detailing the search strategy and study selection for the meta-analysis.

2.2. Data Extraction

We extracted the following information from the selected articles: animal characteristics (body weight, days in milk (DIM), body condition score), intake and total digestibility of nutrients in the diet (dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), milk production and composition, FA profile of milk and diet and ruminal parameters (pH and volatile fatty acid (VFA)). Data on feed efficiency (ECM/DMI) were also extracted. In cases where the studies did not report feed efficiency, we initially calculated it using the following formula: ECM =

(0.327 × milk yield kg) + (12.95 × fat yield kg) + (7.2 × protein yield kg). The calculated ECM was then divided by DMI to obtain feed efficiency.

Additionally, we recorded information such as author names, year of publication, number of replicates per treatment, dietary source of lipids, and the amount of supplemented lipids. In the articles that only reported the composition of the diet ingredients, the FA profile of the diets was estimated accordingly. If the FA composition of a given ingredient was missing, it was estimated based on the feed library of NASEM [5]. The descriptive statistics of the composition of the diets are shown in Table 1.

Table 1. Chemical composition and fatty acid (FA) profile of diets with no lipid supplementation (control) and diets supplemented with lipids included in the meta-analysis

	Control Diets					Lipid-Supplemented Diets				
Nutrients	***n*** [5]	**Mean**	**SD** [6]	**Min** [7]	**Max** [8]	***n***	**Mean**	**SD**	**Min**	**Max**
Composition (g/kg of dry matter (DM))										
Forage diet	452	521.8	93.3	300.0	870.0	452	519.7	94.4	250.0	870.0
Crude protein (CP)	423	168.1	14.6	122.0	210.0	420	167.2	15.3	121.0	213.1
Neutral detergent fiber (NDF)	420	337.4	50.2	250.0	536.0	416	333.0	52.2	175.0	536.0
FA	515	26.0	9.40	8.0	87.0	515	47.6	14.3	13.8	97.4
C12:0	282	0.42	0.61	0.01	4.46	282	0.97	2.18	0.01	22.1
C14:0	390	0.28	0.48	0.02	4.47	389	0.57	1.02	0.04	8.5
C16:0	512	4.73	2.37	1.38	23.0	512	8.65	4.93	2.42	47.9
C16:1	328	0.16	0.11	0.01	0.78	333	0.35	0.34	0.02	2.99
C18:0	508	0.88	1.05	0.25	11.63	508	1.92	2.19	0.11	21.9
C18:1	511	4.40	2.86	0.22	30.35	515	8.9	5.90	0.32	61.0
C18:2	515	9.82	4.44	2.63	32.0	515	16.34	7.44	1.40	44.45
C18:3	510	3.29	2.59	0.05	18.1	508	6.58	6.25	0.01	39.3
SFA [1]	515	6.08	3.34	0.31	32.65	515	11.57	6.38	0.40	51.39
UFA [2]	515	17.66	6.76	5.99	51.29	515	33.72	13.01	8.42	83.57
MUFA [3]	515	4.47	2.88	0.22	30.35	515	9.13	5.88	0.38	61.0
PUFA [4]	515	13.19	5.19	3.96	33.3	515	24.6	10.84	3.5	78.14

[1]SFA (saturated fatty acids) = Σ (C12:0 + C14:0 + C16:0 + C18:0 + C20:0 + C22:0). [2]UFA (unsaturated fatty acids) = Σ (C16:1 + C18:1 + C18:2 + C18:3 + C20:4 + C20:5 + C22:6). [3]MUFA (monounsaturated fatty acids) = Σ (C16:1 + C18:1). [4]PUFA (polyunsaturated fatty acids) = Σ (C18:2 + C18:3 + C20:4 + C20:5 + C22:6). [5]n = number of observations. [6]SD = standard deviation. [7]Min = minimum. [8]Max = maximum.

2.3. Data Analysis

We employed two meta-analytic approaches to analyze the data: effect size meta-analysis and meta-regression. In the meta-analysis, we evaluated the

effect size of the diet with lipid supplementation compared to the control diet (without lipid supplementation) on milk production, milk components, nutrient intake and digestibility, milk FA profile, and ruminal parameters. The descriptive statistics of the collected variables are shown in Table 2. For the meta-regression, models were developed to predict milk fat production and proportion, as well as milk FA profile (de novo, mixed, and preformed) of dairy cows supplemented with lipids.

All statistical analyses were performed using R software (version 4.1.2; RStudio, 2021). Descriptive statistics and preliminary graphical evaluations were performed using the psych and ggplot2 packages, respectively.

2.3.1. Effect Size

The meta-analysis was performed using the rma function of the “metafor” package [16] of R Studio software (version 4.1.2; RStudio, 2021). The effects of lipid supplementation were evaluated by the raw mean difference (RMD) between experimental diets supplemented with lipids and control diets, weighted by the inverse of the variance [17]. To assess heterogeneity between studies, the $\chi 2$ test and the I2 statistic were used. The I2 represents the percentage of variation due to heterogeneity in the responses, with I^2 values < 25, 25 to 50, and >50% indicating low, moderate, and high heterogeneity, respectively [18]. For variables with moderate and high heterogeneity, a subgroup RMD analysis was conducted to identify potential sources of response heterogeneity [19]. The groups were established by cluster analysis of the effect size of milk fat proportion and fatty acid profile in lipid-supplemented diets using the NbClust package.

Table 2. Descriptive statistics of nutrient intake and digestibility, ruminal and parameters, production performance, and milk fatty acid (FA) profile in dairy cows fed diets with no lipid supplementation (control) and diets supplemented with lipids.

Item	Control Diets					Lipid-Supplemented Diets				
	n [1]	Mean	SD [2]	Min [3]	Max [4]	*n*	Mean	SD	Min	Max
Nutrient intake and digestibility										
DMI [5], kg/d	451	21.86	4.18	10.8	30.6	451	21.17	4.36	9.60	30.9
CP [6] intake, kg/d	86	3.33	0.83	1.85	5.15	86	3.22	0.96	1.80	6.50
NDF [7] intake, kg/d	116	7.53	1.47	4.26	10.5	116	7.10	1.46	3.65	10.2
DM [8] digestibility, %	113	67.34	3.83	58.2	74.7	113	67.43	4.03	58.1	75.3
CP digestibility, %	81	67.30	5.67	54.5	79.8	81	68.15	5.79	53.1	77.6
NDF digestibility, %	137	48.60	8.63	29.0	67.8	137	48.50	8.87	28.1	70.7
Ruminal fermentation										
Acetate, mol/100 mol	104	62.70	6.08	42.4	72.8	104	61.9	6.50	37.1	72.3
Propionate, mol/100 mol	104	20.44	3.55	13.9	35.2	104	21.0	3.63	12.8	35.3
Butyrate, mol/100 mol	102	11.73	2.19	8.03	19.3	102	11.72	2.29	6.4	18.6
Animal performance										
Body weight, kg	167	631.0	79.2	351	748	167	628.5	82.1	337	780
Days in milk, d	449	109	59.2	1	273	449	109	59.2	1	273
Milk production, kg/d	503	30.7	8.21	10.2	55.0	503	31.1	8.55	9.1	58.3
Feed efficiency, ECM [9]/DMI	417	1.42	0.25	0.77	2.70	417	1.44	0.26	0.62	2.72
Milk fat, kg/d	468	1.11	0.30	0.39	2.22	468	1.06	0.34	0.31	2.31
Milk protein, kg/d	458	0.98	0.25	0.33	1.66	458	0.97	0.26	0.31	1.85
Milk lactose, kg/d	363	1.48	0.44	0.46	2.81	363	1.49	0.45	0.48	2.87
Milk fat, g/kg	515	36.71	4.58	24.0	61.0	515	34.43	6.19	14.4	58.3
Milk protein, g/kg	504	32.09	2.36	26.3	40.4	504	31.63	2.4	26.0	41.4
Milk lactose, g/kg	429	47.3	2.24	41.3	55.0	429	47.2	2.25	39.5	55.3
Milk fatty acid profile, g/100 g FA										
C12:0	359	3.60	0.84	1.71	7.1	359	2.77	0.83	0.88	6.0
C14:0	371	11.7	1.77	6.44	16.7	371	9.90	1.91	3.9	15.1
C16:0	392	31.15	4.32	16.0	46.1	392	26.80	5.64	15.0	48.8
C18:0	395	10.10	3.17	3.1	24.5	395	12.0	4.13	1.95	30.3
C18:1	347	18.60	4.12	3.1	31.5	347	21.50	5.53	1.38	36.2
CLA [10] (cis 9, trans 11)	330	0.55	0.29	0.11	2.33	330	1.02	0.77	0.15	5.15
De novo	110	25.82	3.88	13.8	31.2	110	21.79	4.20	8.19	30.0
Mixed	97	33.90	5.17	22.3	51.0	97	32.72	6.62	20.8	51.2
Preformed	94	39.65	7.48	19.5	54.8	94	43.67	8.91	25.1	61.8

[1]n = number of observations. [2]SD = standard deviation. [3]Min = minimum. [4]Max = maximum. [5]DMI: dry matter intake. [6]CP = crude protein. [7]NDF = neutral detergent fiber. [8]DM = dry matter. [9]ECM = energy-corrected milk. [10]CLA = conjugated linoleic acid.

After cluster analysis, the groups were categorized as follows: Group 1 (n = 183; 35.5% of treatment means), Group 2 (n = 190; 36.9% of treatment means), Group 3 (n = 96; 18.6% of treatment means), and Group 4 (n = 46; 8.9% of

treatment means). These clusters were characterized by the amounts of fatty acids in the diet (g/kg DM). In general, the diets were characterized as follows: Group 1 consisted of diets with intermediate amounts of unsaturated FA (UFA) and polyunsaturated FA (PUFA) compared to other groups. Group 2 diets were low in saturated FA (SFA) and UFA. Group 3 diets were rich in UFA, mainly PUFA. The Group 4 diets were high in SFA and low in PUFA. The characteristics and composition of diets supplemented with lipid sources are shown in Table 3 and Figure 2.

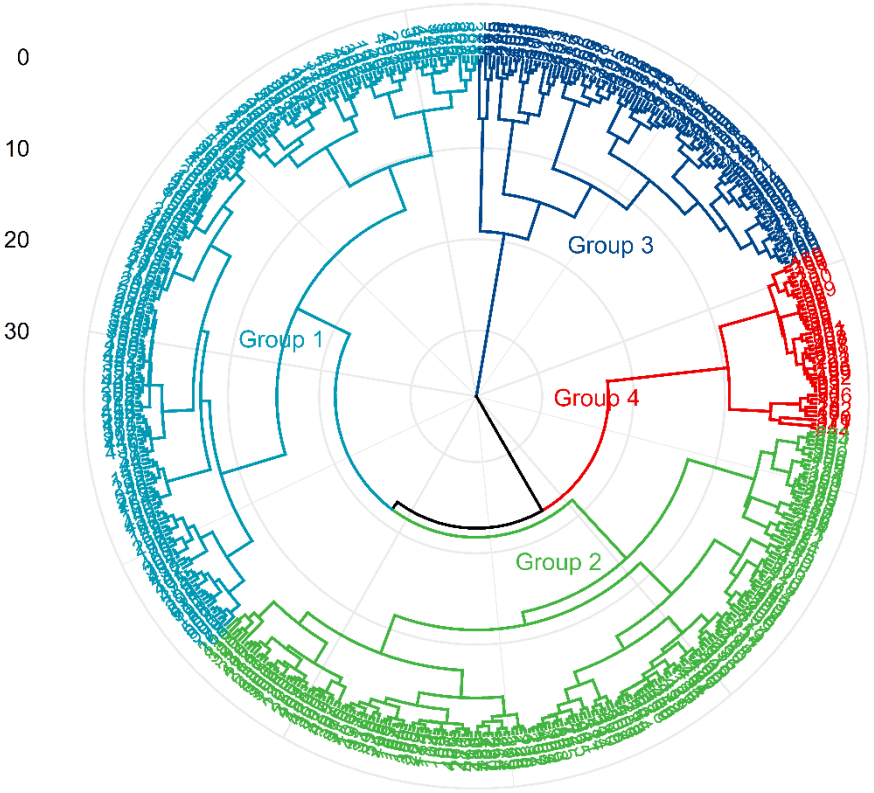

Figure 2. Distribution of diets with lipid supplements, grouped by cluster analysis, classified by the fatty acid profile of the diets (g/kg DM).

Table 3. Groups of diets supplemented with lipids categorized by the amount of fatty acid (FA) (g/kg DM).

Group of Diets	*n* [1]	Diet Characteristic [2]	FA Profile (g/kg of DM)
1	183	Intermediate amount of UFA	UFA 24–42, PUFA 19–34
2	190	Low in SFA and UFA	SFA < 15, UFA < 27, PUFA < 19
3	96	Rich in UFA high in PUFA	UFA > 42, PUFA > 34
4	46	Rich in SFA and low in PUFA	SFA > 15, UFA < 26, PUFA < 17

[1]n = number of observations. [2]SFA = saturated fatty acids. UFA = polyunsaturated fatty acids. PUFA = polyunsaturated fatty acids.

Outliers and publication bias, homoscedasticity, and the assumption of normality of the residuals were evaluated using residual plots and residual normality plots, respectively. Comparisons between lipid-supplemented and control diets with standardized residuals > 2.5 or <−2.5 and with Cook's distance > 5/n were removed from the dataset [19]. The presence of publication bias was assessed using a funnel plot [20] and Egger's regression test of asymmetry between the RMD and SE [21]. Cases showing asymmetry and publication bias were excluded from the analysis.

2.3.2. Meta-Regression

Predictive models for milk fat production and FA profile were developed considering dietary variables and animal characteristics. For this purpose, multiple linear regression with stepwise selection (bidirectional) was employed. A total of 24 variables were selected based on Pearson's correlation ($r \geq 0.2$; $p \leq 0.05$), corroborated by the biological coherence of the variables. These selected variables were used as candidate predictor variables in the development of prediction models. The multiple regression models were fit using maximum log-likelihood with the lme4 package. Study was included in the statistical model as a random effect. Data were weighted using the square root of the number of replicates per treatment.

In all generated models, the collinearity of the variables was assessed using the variance inflation factor (VIF) to control for overparameterization of the models. VIF values lower than 2.5 were considered to ensure minimal collinearity between the variables.

The models were evaluated using the Akaike information criterion (AIC), root mean square error (RMSE), and concordance correlation coefficient (CCC). The models were assessed by graphical and statistical analysis of the residual distribution using the cowplot and lmerTest packages.

3. RESULTS

3.1. Effect Size Meta-Analysis

Initially, we investigated the effect size (i.e., RMD) of including lipid supplements into the diets of dairy cows. When medium or high heterogeneity (I2 > 25%) was observed in the overall responses, we conducted subgroup analyses based on the FA profile of the diets. This approach allowed the assessment of the effect of different dietary FA profiles on milk production, milk fat production and proportion, and milk FA profiles.

Cows fed diets with lipid supplementation showed a reduction of 0.65 kg/d DMI ($p < 0.001$, Table 4) compared to the control group, leading to a decreased intake of CP by −0.14 kg/d and NDF by −0.46 kg/d. Although the overall effect of lipid supplementation on reducing DMI and CP and NDF intake was highly significant ($p < 0.001$), all responses showed high heterogeneity ($I^2 > 88\%$). However, in the subgroup analysis, we found that the diets with high PUFA content (Group 3) did not affect dry matter and crude protein intake (Figure 3a, 3b).

Table 4. Effect of lipid supplementation on the nutrient intake and digestibility, rumen parameters, production performance, and milk fatty acid profile of lactating dairy cows.

Item	*n* [1]	Control Means	Effect Size			Heterogeneity	
			RMD [2]	(95% CI)	*p*-Value	I^2%	*p*-Value
Nutrient intake and digestibility							
DMI [3], kg/d	451	21.86	−0.65	(−0.78, −0.51)	<0.001	88.3	<0.001
CP [4] intake, kg/d	86	3.33	−0.14	(−0.19, −0.09)	<0.001	99.9	<0.001
NDF [5] intake, kg/d	116	7.53	−0.46	(−0.57, −0.35)	<0.001	96.5	<0.001
DM [6] digestibility, %	113	67.3	0.32	(−0.07, 0.70)	0.10	84.0	<0.001
CP digestibility, %	81	67.3	0.75	(0.29, 1.21)	<0.01	55.2	<0.001
NDF digestibility, %	137	48.6	0.24	(−0.40, 0.89)	0.46	87.2	<0.001
Ruminal parameters							
Acetate, mol/100 mol	104	62.7	−0.56	(−0.92, −0.21)	<0.01	83.6	<0.001
Propionate, mol/100 mol	104	20.4	0.50	(0.18, 0.83)	<0.01	86.1	<0.001
Butyrate, mol/100 mol	102	11.7	−0.01	(−0.18, 0.15)	0.88	73.3	<0.001
Production performance							
Milk production, kg/d	503	30.7	0.48	(0.30, 0.65)	<0.001	79.9	<0.001
Feed efficiency, ECM [7]/DMI	417	1.42	0.04	(0.02, 0.06)	<0.001	26.2	0.99
Milk fat, kg/d	468	1.11	−0.05	(−0.07, −0.04)	<0.001	88.5	<0.001
Milk protein, kg/d	458	0.98	−0.004	(−0.01, 0.003)	0.25	68.7	<0.001
Milk lactose, kg/d	363	1.48	0.02	(0.01, 0.03)	< 0.01	64.0	<0.001
Milk fat, g/kg	515	36.71	−2.19	(−2.58, −1.80)	<0.001	98.9	<0.001
Milk protein, g/kg	504	32.09	−0.43	(−0.53, −0.32)	<0.001	97.4	<0.001
Milk lactose, g/kg	429	47.3	−0.01	(−0.01, 0.07)	0.73	92.3	<0.001
Milk fatty acid profile, g/100 g FA							
C12:0	359	3.6	−0.85	(−0.92, −0.77)	<0.001	98.9	<0.001
C14:0	371	11.7	−1.84	(−1.98, −1.69)	<0.001	98.4	<0.001
C16:0	392	31.15	−4.32	(−4.79, −3.86)	<0.001	99.7	<0.001
C18:0	395	10.1	1.87	(1.59, 2.14)	<0.001	99.3	<0.001
C18:1	347	18.6	2.88	(2.45, 3.31)	<0.001	99.5	<0.001
C18:2	367	2.3	0.22	(0.15, 0.29)	<0.001	99.2	<0.001
C18:3 *n*-3	364	0.47	0.096	(0.07, 0.12)	<0.001	99.6	<0.001
CLA [8] (cis 9, trans 11)	330	0.55	0.43	(0.37, 0.50)	<0.001	99.8	<0.001
CLA (trans 10, cis 12)	147	0.05	0.02	(0.01, 0.02)	<0.001	98.5	<0.001
SFA	222	67.2	−6.45	(−7.22, −5.68)	<0.001	99.5	<0.001
MUFA	146	26.0	5.22	(4.31, 6.13)	<0.001	99.9	<0.001
PUFA	146	3.79	1.10	(0.89, 1.31)	<0.001	99.9	<0.001
de novo	110	25.82	−3.98	(−4.61, −3.36)	<0.001	94.9	<0.001
Mixed	97	33.90	−1.10	(−2.15, −0.05)	<0.05	97.9	<0.001
Preformed	94	39.65	3.91	(2.44, 5.37)	<0.001	97.5	<0.001

[1]n = number of observations. [2]Raw mean difference. [3]DMI = dry matter intake. [4]CP = crude protein. [5]NDF = neutral detergent fiber. [6]DM = dry matter. [7]ECM = energy-corrected milk. [8]CLA = conjugated linoleic acid.

When evaluating the digestibility of nutrients, our findings indicate that, in general, lipid supplementation in the diet of dairy cows slightly increased CP digestibility ($p < 0.01$, Table 4) and had no effect on DM or NDF digestibility ($p = 0.10$ and 0.46, respectively, Table 4). However, when evaluating by subgroups, we observed that not all the diets followed the general response pattern to lipid supplementation. Specifically, diets rich in the PUFA (Group 3) increased CP digestibility, while diets low in SFA and UFA (Group 2) increased NDF digestibility (Figure 3e,f).

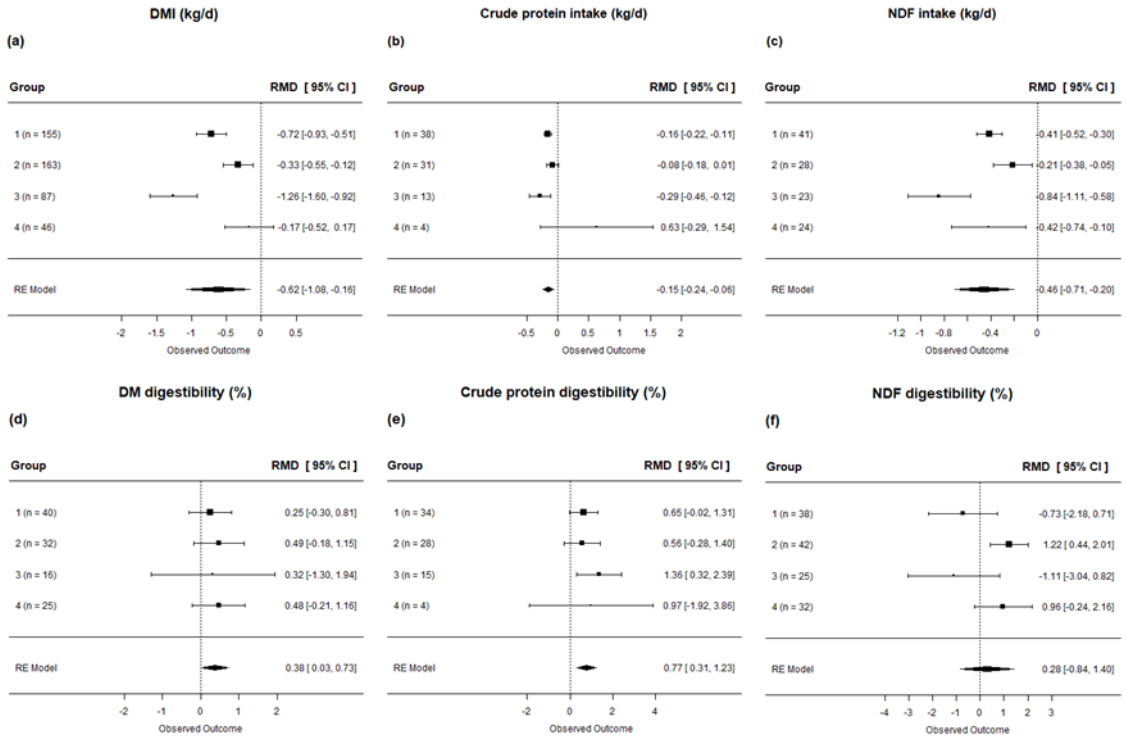

Figure 3. Forest plot of the effect of lipid-supplemented diets on nutrient intake [(a) DMI; (b) crude protein intake; (c) NDF intake] and digestibility [(d) DM; (e) crude protein; (f) NDF] in lactating dairy cows. Diets are grouped by fatty acid profile. The square represents the mean effect size of lipid source group and square size represents the relative weight of the lipid source group to the overall effect size. The lines connected to the squares represent the lower and upper 95% CI for the effect size. The diamond is the overall effect size with a 95% CI. The dotted vertical line represents a mean difference of zero (i.e., no effect), while negative or positive values denote the effect of the lipid source group.

We also evaluated the impact of lipid supplementation on ruminal parameters. Overall, lipid supplementation in dairy cow diets resulted in a decrease in acetate concentration ($p < 0.01$), an increase in propionate concentration ($p < 0.01$), and no effect on butyrate concentration ($p = 0.88$; Table 4). However, when evaluated by subgroup, we observed that only diets in Group 1 (intermediate levels of UFA) decreased the acetate concentration in the rumen. Additionally, diets in both groups 1 and 3 (diets with the highest levels of UFA inclusion) increased propionic acid concentration in the rumen of dairy cows supplemented with lipids (Figure 4a, b).

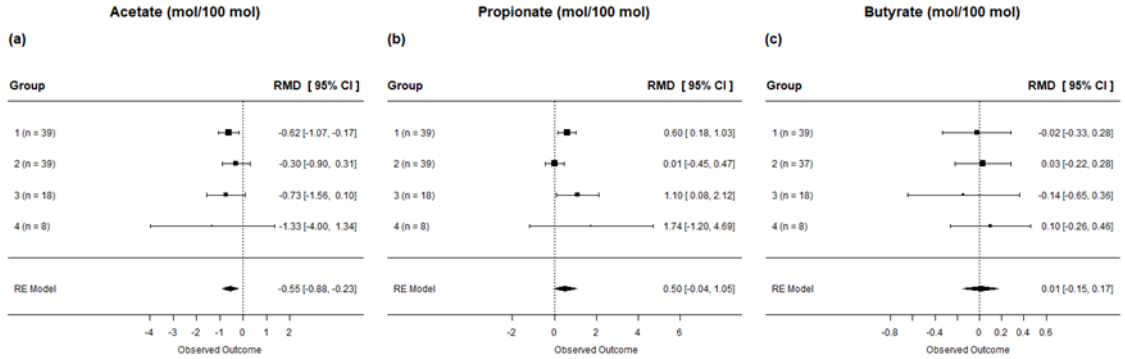

Figure 4. Forest plot of the effect of lipid-supplemented diets on rumen parameters in lactating dairy cows. Diets are grouped by fatty acid profile. [(a) acetate; (b) propionate; (c) butyrate]. The square represents the mean effect size of lipid source group and square size represents the relative weight of the lipid source group in the overall effect size. The lines connected to the squares represent the lower and upper 95% CI for the effect size. The diamond is the overall effect size with a 95% CI. The dotted vertical line represents a mean difference of zero (i.e., no effect), while negative or positive values denote the effect of the lipid source group.

In general, the inclusion of lipid supplements in dairy cow diets resulted in an increase in milk production by 0.480 kg/d compared to the control group ($p < 0.001$, Table 4). Additionally, we observed a 2.8% improvement in feed efficiency ($p < 0.001$, Table 4). It is important to note that the milk production showed high heterogeneity ($I2 = 79.9\%$, Table 4). Subgroup analysis revealed that not all diets led to an increase in milk production (Figure 5a). No effects on milk production were observed in cows supplemented with diets rich in UFA (Group 3). Regarding feed efficiency evaluation by subgroup, we found that only diets in Group 1 (characterized by intermediate levels of UFA) improved the feed efficiency of cows (Figure 5b).

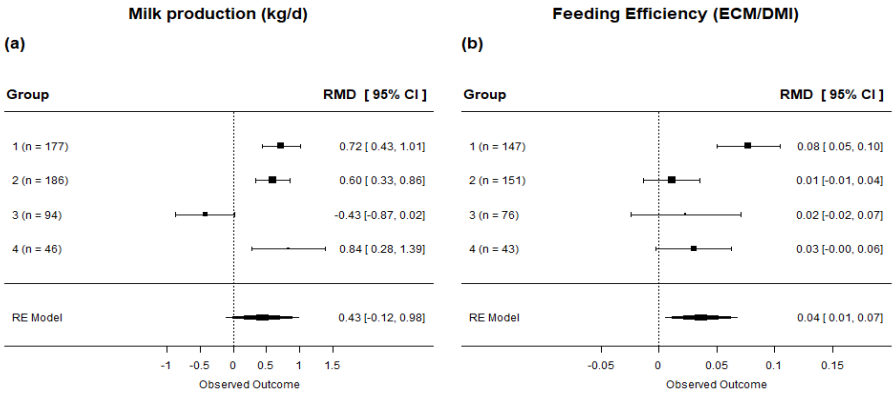

Figure 5. Forest plot of the effect of lipid-supplemented diets on production performance in lactating dairy cows. [(a) milk production; (b) feeding efficiency]. Diets are grouped by fatty acid profile. The square represents the mean effect size of lipid source group and square size represents the relative weight of the lipid source group in the overall effect size. The lines connected to the squares represent the lower and upper 95% CI for the effect size. The diamond is the overall effect size with a 95% CI. The dotted vertical line represents a mean difference of zero (i.e., no effect), while negative or positive values denote the effect of the lipid source group.

When assessing the milk components, in general, lipid supplementation in dairy cow diets decreased the daily milk fat production ($p < 0.001$, Table 4), had no effect on protein production ($p = 0.25$), and slightly increased lactose production ($p < 0.01$, Table 4). Furthermore, we observed a decrease in the proportion (g/kg) of milk fat and protein ($p < 0.001$, Table 4) when cows were supplemented with lipids. When evaluating the effects of lipid-supplemented diets on milk components according to their FA profile (i.e., subgroups), we observed that not all diets reduced milk fat production and proportion (Figure 6a, d). Diets rich in SFA (i.e., Group 4) increased milk fat production and proportion by 0.075 kg/d and 1.60 g/kg, respectively. When evaluating milk protein production by sub-group (Figure 6b), we observed that cows fed diets with high levels of UFA (group 3) de-creased protein production, whereas diets rich in SFA (group 4) led to increased daily protein production. In the present study, we also found that not all diets reduced the pro-portion of milk proteins (Figure 6e). Diets rich in UFA (group 3) had no effect on the pro-portion of milk protein. When evaluating milk lactose, we observed that diets rich in UFA (group 3) decreased lactose production (−0.035 kg/d; Figure 6c), while diets rich in SFA (group 4) decreased the proportion of lactose in milk (−0.41 g/kg; Figure 6f).

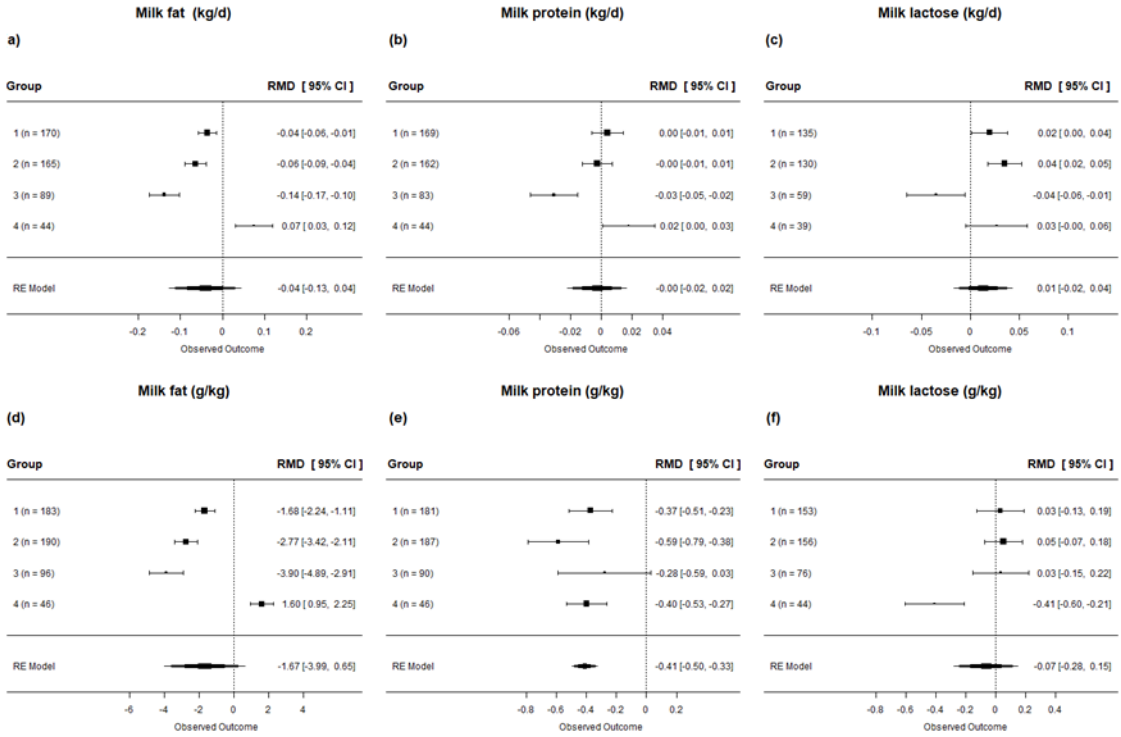

Figure 6. Forest plot of the effect of lipid-supplemented diets on the production [(a) milk fat; (b) milk protein; (c) milk lactose] and proportion [(d) milk fat; (e) milk protein; (f) milk lactose] of milk components in lactating dairy cows. Diets are grouped by fatty acid profile. The square represents the mean effect size of lipid source group and square size represents the relative weight of the lipid source group in the overall effect size. The lines connected to the squares represent the lower and upper 95% CI for the effect size. The diamond is the overall effect size with a 95% CI. The dotted vertical line represents a mean difference of zero (i.e., no effect), while negative or positive values denote the effect of the lipid source group.

When evaluating the FA profile of milk, we observed that, in general, the supplementation of cow diets with lipids led to a decrease in palmitic, myristic, and lauric ($p < 0.001$, Table 4). Myristic acid consistently decreased across all diets (Figure 7a). Palmitic acid showed a decrease, although it should be noted that not all diets resulted in a reduction in milk palmitic acid. The diets rich in SFA (group 4) increased the levels of palmitic acid in milk (Figure 7b). On the other hand, milk stearic acid, oleic acid (C18:1, cis9), and linoleic acid increased when cows were supplemented with lipids ($p < 0.001$; Table 4). When evaluated by subgroup, diets rich in UFA (group 3) had no effect on the levels of stearic acid and C18:1 cis 9 in milk (Figure 7c, d). Notably, there was a significant increase in the levels of cis-9, and trans-11 conjugated linoleic acid (CLA) and trans-10 and cis-12 CLA ($p < 0.001$; Table 4) in milk with lipids supplementation. However, when analyzed by sub-group, it was observed that diets rich in SFA (group 4) had

no effect on the levels of cis-9 or trans-11 CLA, while decreasing the levels of trans-10 and cis-12 CLA in milk (Figure 7e, f).

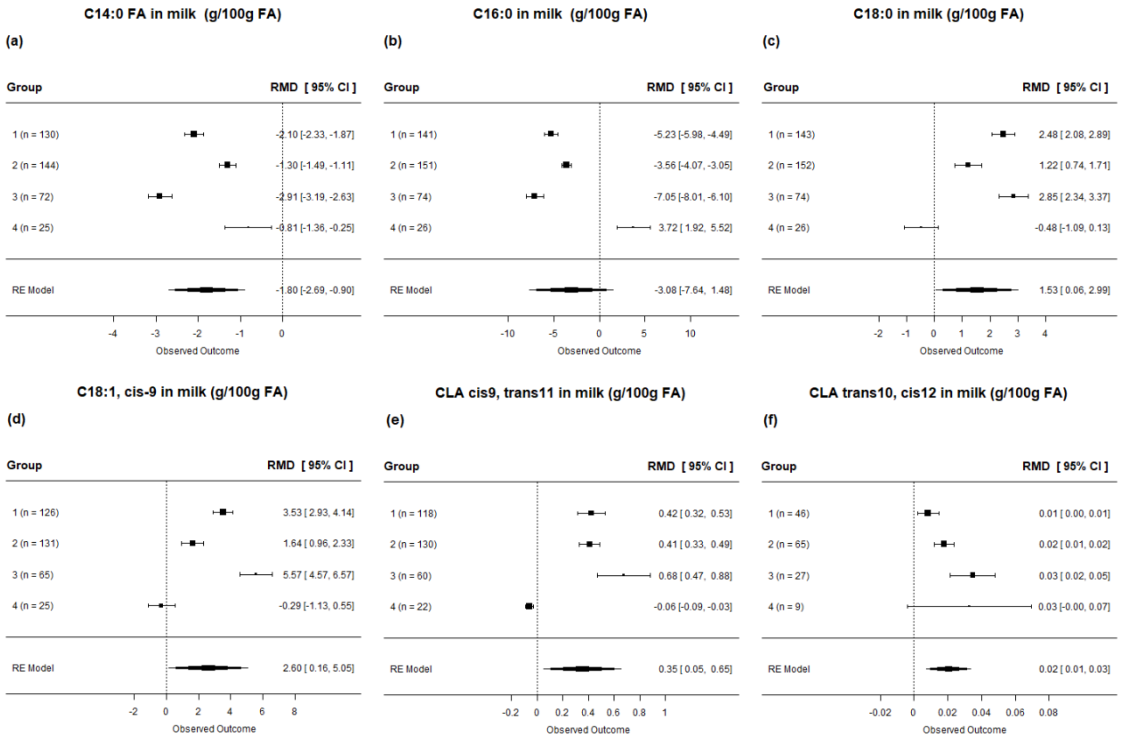

Figure 7. Forest plot of the effect of lipid-supplemented diets on fatty acid in milk [(a) C14:0; (b) C16:0; (c) C18:0; (d) C18:1; (e) CLA cis 9, trans 11; (f) CLA trans 10, cis 12] of lactating dairy cows. Diets are grouped by fatty acid profile. The square represents the mean effect size of lipid source group and square size represents the relative weight of the lipid source group in the overall effect size. The lines connected to the squares represent the lower and upper 95% CI for the effect size. The diamond is the overall effect size with a 95% CI. The dotted vertical line represents a mean difference of zero (i.e., no effect), while negative or positive values denote the effect of the lipid source group.

In general, lipid supplementation in dairy cow diets decreased de novo ($p < 0.001$) and mixed ($p < 0.05$) FA in milk; however, it increased preformed FA in milk ($p < 0.001$; Table 4). When evaluated by subgroup, we observed that all evaluated diets reduced de novo FA in milk (Figure 8a). Diets from Group 2 (Low in SFA and UFA) had no effect on mixed FA in milk, and diets rich in SFA (group 4) increased mixed FA and decreased pre-formed FA in milk (Figure 8b, c). We also observed that lipid-supplemented diets led to a reduction in SFA and an increase in monounsaturated fatty acids (MUFA) and PUFA in milk ($p < 0.001$; Table 4). However, when evaluated by subgroup, we observed that diets rich in

SFA (group 4) increased SFA, decreased MUFA, and had no effect on PUFA in milk (Figure 8d–f).

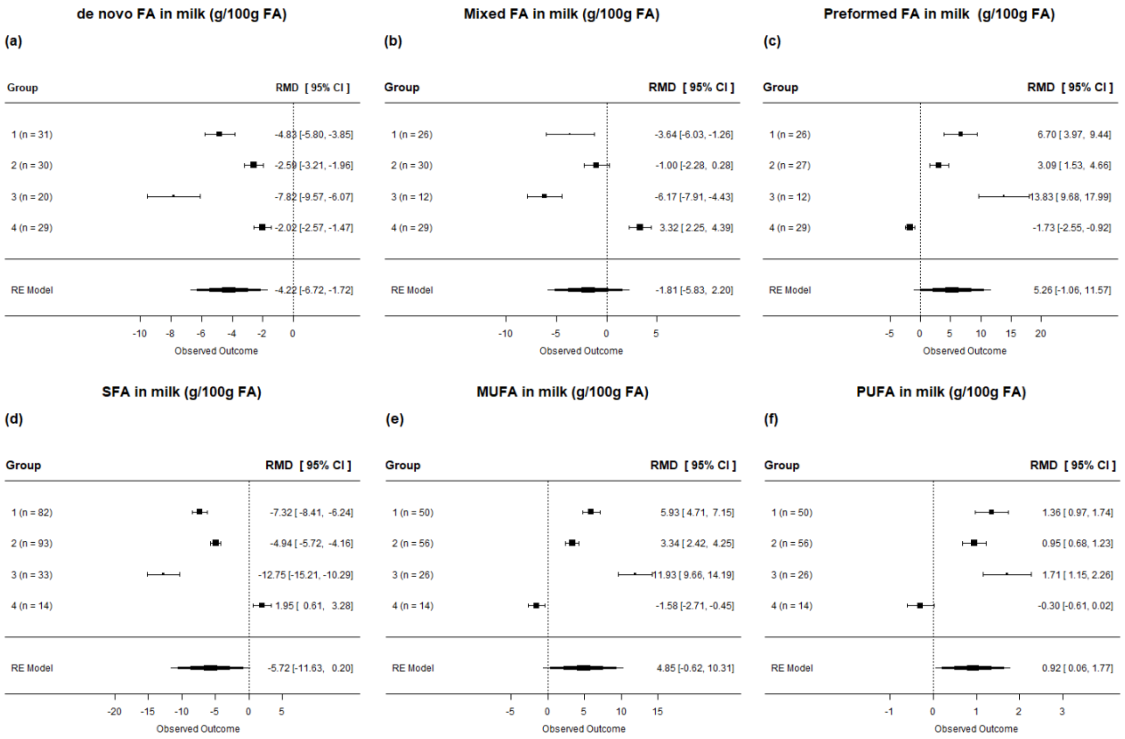

Figure 8. Forest plot of the effect of lipid-supplemented diets on milk fatty acids, according to their biological origin [(a) de novo FA; (b) mixed FA; (c) preformed FA; (d) SFA; (e) MUFA; (f) PUFA], in lactating dairy cows. Diets are grouped by fatty acid profile. The square represents the mean effect size of lipid source group and square size represents the relative weight of the lipid source group in the overall effect size. The lines connected to the squares represent the lower and upper 95% CI for the effect size. The diamond is the overall effect size with a 95% CI. The dotted vertical line represents a mean difference of zero (i.e., no effect), while negative or positive values denote the effect of the lipid source group.

3.2. Meta-Regression

A total of 24 variables were selected using Pearson's correlation ($r \geq 0.2$; $p \leq 0.05$), which were also supported by their biological coherence as candidate predictor variables in the development of prediction models of milk production and milk fat. A total of 16 models were selected to predict the different targeted production responses, and all models showed high accuracy and precision (CCC > 92%; RMSE < 7%).

Models 1 to 4 (Table 5) predict milk fat production (kg/d; CCC = 0.97, RMSE ≤ 7.0%). The simplest model considered dietary NDF, C14:0, and milk production as predictive variables. Alternatively, Models 2, 3, and 4 included dietary PUFA, C18:0, and UFA as predictor variables.

Models 5 to 8 (Table 6) predict the proportion of milk fat (g/kg), and all four models showed high CCC (≥0.93) and RMSE (≤6.37%). Model 6 considered dietary NDF, UFA, and forage as predictor variables. Model 7 predicts the milk fat concentration, considering milk production, DMI, NDF, and C16:0 in the diet. Models 6 and 8 are the more extensive versions of Models 5 and 7, respectively. In both cases, the dietary FA profile was also considered as a predictor variable (myristic and palmitic FA, respectively).

In this study, we also developed models to predict the FAs in milk based on their origin. Models 9 to 12 (Table 7) predict de novo milk FA (CCC ≥ 0.96, RMSE ≤ 6.18%). Model 9, the simplest model, considers the DMI and total FAs in the diet as predictor variables. Models 10, 11, and 12 included the variables from Model 9 and incorporated milk production, C18:0, and C14:0 in the diet, respectively, as predictive variables.

Two models were proposed to predict the mixed FA in milk (Table 8). The simplest model included dietary C16:0, C18:2, C18:3, and MUFA as predictor variables (i.e., Model 13). Model 14, in addition to the aforementioned variables, includes milk production and forage in the diet (CCC = 0.99, RMSE ≤ 3.14%).

Models 15 and 16 predict the preformed FA in milk (Table 9). Model 15, the simplest of the two, includes C18:1, C18:2, and DMI as predictors. Model 16, on the other hand, includes the proportion of fat in addition to these variables.

Table 5. The best-fit models for milk fat production (kg/d) in dairy cows

Milk Fat Production Models [1]	Intercept (kg/d)	NDF [2] (g/kg DM)	C14:0 [3] (g/kg DM)	MY [4] (kg/day)	PUFA [5] (g/kg DM)	C18:2 [6] (g/kg DM)	UFA [7] (g/kg DM)	Evaluation on Data [8]					
									n	AIC	CCC	RMSE	Mean Bias	Slope Bias
Model 1														
Estimate	−0.449	0.001	−0.031	0.036				Value	282	−260	0.97	0.07	−0.0004	0.039
SE	0.129	0.0003	0.009	0.002				%				6.98	0.004	2.23
p-value	<0.001	<0.001	<0.001	<0.001				*p*-value					0.91	0.01
Model 2														
Estimate	−0.302	0.001	−0.036	0.034	−0.003			Value	282	−257	0.97	0.07	−0.0004	0.037
SE	0.135	0.0003	0.009	0.002	0.001			%				6.81	0.005	2.18
p-value	<0.05	< 0.001	<0.001	<0.001	<0.001			*p*-value					0.91	0.01
Model 3														
Estimate	−0.332	0.001	−0.035	0.035	-	−0.03		Value	282	−253	0.97	0.07	−0.0005	0.038
SE	0.139	0.0003	0.009	0.002	-	0.001		%				6.93	0.005	2.21
p-value	<0.05	<0.001	<0.001	<0.001	-	<0.01		*p*-value					0.91	0.01
Model 4														
Estimate	−0.278	0.001	−0.037	0.034	-	-	−0.003	Value	282	−258	0.97	0.07	−0.0005	0.037
SE	0.136	0.0003	0.009	0.002	-	-	0.0008	%				6.82	0.005	2.18
p-value	<0.05	<0.001	<0.001	<0.001	-	-	<0.001	*p*-value					0.91	0.01

[1]All models were adjusted for random study effect. [2]NDF = neutral detergent fiber. [3]C14:0 = myristic acid. [4]MY = milk production. [5]PUFA = polyunsaturated fatty acid. [6]C18:2 = linoleic acid. [7]UFA = unsaturated fatty acid. [8]AIC = Akaike's information criterion. CCC = concordance correlation coefficient. RMSE = root mean square error.

Table 6. The best-fit models for the proportion of milk fat (g/kg) in dairy cows

Milk Fat Proportion Models [1]	Intercept (g/kg)	NDF [2] (g/kg DM)	UFA [3] (g/kg DM)	Forage (g/kg DM)	C14:0 [4] (g/kg DM)	MY [5] (kg/day)	DMI [6] (kg/day)	C16:0 [7] (g/kg DM)	Evaluation on Data [8]						
										n	**AIC**	**CCC**	**RMSE**	**Mean Bias**	**Slope Bias**
Model 5															
Estimate	22.12	0.019	−0.090	0.017					Value	409	2392	0.93	2.11	−0.04	0.10
SE	2.62	0.007	0.021	0.004					%				6.14	0.04	6.39
p-value	<0.001	<0.05	<0.001	<0.001					*p*-value					0.67	0.001
Model 6															
Estimate	22.36	0.025	−0.105	0.014	−1.209				Value	303	1782	0.93	2.16	−0.02	0.10
SE	2.91	0.008	0.024	0.004	0.279				%				6.37	0.006	6.33
p-value	<0.001	<0.01	<0.001	<0.01	<0.001				*p*-value					0.89	0.001
Model 7															
Estimate	17.27	0.036	-	-	-	−0.291	0.657		Value	378	2212	0.94	2.01	−0.04	0.09
SE	3.87	0.008	-	-	-	0.073	0.127		%				6.06	0.03	5.65
p-value	<0.001	<0.001	-	-	-	<0.001	<0.001		*p*-value					0.72	0.001
Model 8				-											
Estimate	16.08	0.036	-	-	-	−0.333	0.718	0.133	Value	378	2212	0.94	2.06	−0.03	0.09
SE	3.88	0.008	-	-	-	0.074	0.128	0.051	%				6.01	0.03	5.64
p-value	<0.001	<0.001	-	-	-	<0.001	<0.001	<0.05	*p*-value					0.74	0.001

[1]All models were adjusted for random study effect. [2]NDF = neutral detergent fiber. [3]UFA = unsaturated fatty acid. [4]C14:0 = myristic acid. [5]MY = milk production. [6]DMI = dry matter intake. [7]C16:0 = palmitic acid. [8]AIC = Akaike's information criterion. CCC = concordance correlation coefficient. RMSE = root mean square error.

Table 7. The best-fit models for de novo fatty acids in milk (g/100 g FA) of dairy cows

De novo FA in Milk Models [1]	Intercept (g/100 g FA)	DMI [2] (kg/day)	FA [3] (g/kg DM)	MY [4] (kg/day)	C18:3 [5] (g/kg DM)	C14:0 [6] (g/kg DM)	Evaluation on Data [7]					
								n	**AIC**	**CCC**	**RMSE**	**Mean Bias**	**Slope Bias**
Model 9													
Estimate	14.20	0.546	−0.11				Value	104	482	0.97	1.02	0.006	0.036
SE	2.46	0.08	0.03				%				4.69	0.003	1.96
p-value	<0.001	<0.001	<0.001				*p*-value					0.95	0.16
Model 10													
Estimate	14.76	0.803	−0.119	−0.170			Value	102	472	0.97	1.01	0.002	0.04
SE	2.41	0.13	0.027	0.071			%				4.62	0.001	1.99
p-value	<0.001	<0.001	<0.001	<0.05			*p*-value					0.98	0.16
Model 11													
Estimate	13.55	0.56	−0.12	-	0.10		Value	104	482	0.97	0.99	0.005	0.04
SE	2.41	0.079	0.026	-	0.039		%				4.58	0.003	2.01
p-value	<0.001	<0.001	<0.001	-	<0.05		*p*-value					0.95	0.15
Model 12													
Estimate	12.50	0.589	−0.13	-	-	3.99	Value	58	285	0.96	1.27	0.005	0.05
SE	3.74	0.14	0.036	-	-	1.64	%				6.18	0.001	2.59
p-value	<0.01	<0.001	<0.001	-	-	<0.05	*p*-value					0.97	0.23

[1]All models were adjusted for random study effect. [2]DMI = dry matter intake. [3]FA = fatty acid. [4]MY = milk production. [5]C18:3 = linolenic acid. [6]C14:0 = myristic acid. [7]AIC = Akaike's information criterion. CCC = concordance correlation coefficient. RMSE = root mean square error.

Table 8. The best-fit models for mixed fatty acids in milk (g/100 g FA) in milk of dairy cows

Mixed FA in Milk Models [1]	Intercept (g/100 g FA)	Milk Fat (g/kg)	C16:0 [2] (g/kg DM)	C18:2 [3] (g/kg DM)	C18:3 [4] (g/kg DM)	MUFA [5] (g/kg DM)	Forage (g/kg DM)	Evaluation on Data [6]						
									n	AIC	CCC	RMSE	Mean Bias	Slope Bias
Model 13														
Estimate	44.24	0.197	0.236	−0.187	−0.215	−0.345	−0.027	Value	78	411	0.99	1.03	−0.01	0.02
SE	5.01	0.075	0.076	0.066	0.092	0.091	0.011	%				3.06	0.01	1.58
p-value	<0.001	<0.05	<0.01	<0.01	<0.05	<0.001	<0.05	*p*-value					0.92	0.27
Model 14														
Estimate	38.92	-	0.284	−0.241	−0.235	−0.354		Value	78	404	0.99	1.06	−0.02	0.02
SE	1.86	-	0.077	0.066	0.095	0.095		%				3.14	0.02	1.99
p-value	<0.001	-	<0.001	<0.001	<0.05	<0.001		*p*-value					0.89	0.22

[1]All models were adjusted for random study effect. [2]C16:0 = palmitic acid. [3]C18:2 = linoleic acid. [4]C18:3 = linolenic acid. [5]MUFA = monounsaturated fatty acid. [6]AIC = Akaike's information criterion. CCC = concordance correlation coefficient. RMSE = root mean square error.

Table 9. The best-fit models for preformed fatty acids in milk (g/100 g FA) of dairy cows

Preformed FA in Milk Models [1]	Intercept (g/100 g FA)	C18:2 [2] (g/kg DM)	DMI [3] (kg/d)	C18:1 [4] (g/kg DM)	Milk Fat (g/kg)	Evaluation on Data [5]						
							n	AIC	CCC	RMSE	Mean Bias	Slope Bias
Model 15												
Estimate	53.33	0.332	−0.833	0.500		Value	90	522	0.99	1.46	0.007	0.02
SE	4.37	0.08	0.16	0.13		%				3.33	0.002	1.85
p-value	<0.001	<0.001	<0.001	<0.001		*p*-value					0.96	0.20
Model 16												
Estimate	64.91	0.282	−0.951	0.483	−0.220	Value	90	521	0.99	1.39	−0.001	0.02
SE	6.24	0.08	0.16	0.12	0.09	%				3.17	0.0004	1.59
p-value	<0.001	<0.001	<0.001	<0.001	< 0.05	*p*-value					0.99	0.24

[1]All models were adjusted for random study effect. [2]C18:2 = linoleic acid. [3]DMI = dry matter intake. [4]C18:1 = oleic acid. [5]AIC = Akaike's information criterion. CCC = concordance correlation coefficient. RMSE = root mean square error.

4. DISCUSSION

Our findings indicate that lipid-supplemented diets have an impact on both milk fat production and milk FA profile. Diets rich in SFA increase milk fat production and pro-portion, while diets rich in MUFA and PUFA negatively affect milk fat production and proportion. Therefore, it is essential to group lipid-supplemented diets based on their FA profile to enhance our understanding of milk fat production responses and milk FA pro-file. Furthermore, depending on diet and animal characteristics, it is possible to predict and manipulate milk fat production.

4.1. Meta-Analysis—Effect Size

In general, it is known that lipid supplementation in dairy cow diets increases milk production [12–14]. However, there are reports suggesting that certain lipid sources have no effect on milk production [22–24]. Consistent with these findings, our study showed that diets rich in UFA had no effect on milk production. This result may be attributed to the observed decrease in DMI and NDF intake in cows fed such diets, especially when UFA are included in amounts exceeding 5% of FA content in DM.

The negative effect of diets with lipid supplements on DMI was lower than that re-ported in previous studies (a decrease of 1.1 kg/d by Weld and Armentano [25] and 0.875 kg/d by [14]). Lipid supplementation may have led to an increase in cholecystokinin (CCK) secretion due to the elevated intestinal flow of FA, which, in turn, stimulates the secretion of pancreatic lipase and bile, resulting in reduced abomasum emptying. In addition, CCK is known to act as a satiety signal in the central nervous system [26]. Further-more, the decrease in DMI can be attributed to the increased energy density of the diet when lipids are added, allowing the animal to meet its energy requirement with lower feed intake. Another factor that could explain the overall decrease in DMI is the enhanced digestibility of crude protein and NDF with certain lipid sources, as demonstrated in this study.

In general, lipid-supplemented diets for dairy cows alter both milk fat production and proportion [12,14,24]. Rabiee et al. [14] reported that, overall, lipid supplementation had no effect on milk fat production and proportion. However, when

considering types of supplements, they found that oilseeds increase milk fat production but decrease the per-centage of milk fat, and calcium soaps rich in SFA increase both milk fat production and proportion. Conversely, dos Santos Neto et al. [12] reported that palmitic acid calcium soaps improve milk fat production but have no effect on the proportion of milk fat. In this regard, our study supports these findings, as the variations in responses align with the variations in the FA profile of the diet. However, diets rich in MUFA result in a decrease in milk fat. The decrease in milk fat with diets rich in MUFA and PUFA can be attributed to the inhibitory effect of trans FA (e.g., mainly trans-10, cis-12 CLA) in the mammary gland, which are produced during the ruminal biohydrogenation of UFA from the diet. The negative action of trans-10 and cis-12 CLA on milk fat synthesis is due to the decreased activity of SREBP1, an important transcription factor involved in the expression of genes related to de novo FA synthesis and desaturation of long-chain FA [27].

The proportion of milk protein reduction in our study aligns with previous studies [12,14,24]. The negative effect of dietary fat on milk protein proportion may be attributed to SREBP1 activity. According to Osorio et al. [9], increased expression of SREBF1 (a gene related to SREBP1) increases mTOR expression, while inhibiting SREBP1 has the opposite effect. However, there are conflicting findings in the literature. Hu et al. [13], who investigated dietary saturated free FAs, and Oliveira et al. [23], who evaluated soybean oil supplementation, reported no effect of these lipid sources on milk protein proportion. In our study, Group 3 (diets rich in UFA, high in PUFA) showed no effect on milk protein pro-portion. These divergent results regarding the effects of lipid-supplemented diets on the milk protein proportion highlight the need for a better understanding of the bioactive functions of FA in dairy cows.

The decrease in de novo FA in milk of dairy cows supplemented with dietary lipids, as observed in our study, is consistent with previous studies [12,28]. This can be explained by the fact that de novo FA are synthesized in the mammary gland, and their synthesis depends on precursors such as acetate and butyrate, which decrease when cows are supplemented with lipids, as shown herein. The decrease in de novo FA in milk can also be attributed to the inhibitory effect of CLA in the mammary gland. Our study revealed a significant increase in the levels of cis-9, trans-11 and trans-10, cis-12 CLA in all diets evaluated. The decrease in de novo FAs in milk is accompanied

by the reduced synthesis of lauric, myristic, and, to a greater extent, palmitic acid. Studies with a similar approach to ours, such as Mahdavi et al. [24] and dos Santos Neto et al. [12], also reported a decrease in 12- to 16-carbon FA.

Preformed FA increased with most diets evaluated in our study (Groups 1, 2, and 3). These findings are consistent with those reported by dos Santos Neto et al. [12] and Shepardson and Harvatine [22]. However, other studies indicate that sources with high SFA content do not affect the synthesis of preformed FA in milk [29,30]. In contrast to these results, our study demonstrates that diets with high levels of SFA (Group 4) show reduced preformed FA. The discrepancy in results may be attributed to the FA profile of the diets used in different studies. Oleic acid and stearic acid are the two FA with the highest pro-portion and variation in preformed FA in milk [31]. In the present study, these two FA exhibited the greatest increase compared to other preformed FA in milk.

In our study, the presence of UFA in the diets had a negative impact on the mixed FA in milk. These results are in agreement with studies that evaluated various vegetable oils and reported a decrease in 16-carbon FA in milk [23,24,32]. This result can be attributed to the higher levels of trans-10 and cis-12 CLA in the mammary gland, which are produced due to the high levels of UFAs in the diet derived from the lipid supplements.

4.2. Meta-Regression

Traditional models typically consider animal and diet characteristics as predictors of milk production [33]. However, incorporating specific dietary FA, such as PUFAs and C18:2, as predictive variables can provide valuable insights. High levels of dietary FA are known to increase the energy density of the diet, leading to improved milk production.

The relationship between forage, dietary fiber intake, and milk fat is well established, making them suitable candidate variables for predictive models of milk fat production and proportion. Interestingly, Model 6 revealed a negative relationship between C14:0 and milk fat. This effect may be attributed to potential competition between C14:0 and other FA in the mammary gland, affecting acyl-CoA activation and

subsequent esterification into triglycerides [34]. Recognizing that dietary FA serve as both energy substrates and bioactive compounds with physiological functions highlights the importance of including them in milk component prediction models. Maxin et al. [35] demonstrated the accuracy and precision of short-chain and long-chain FA as predictors of milk fat production, although their models were based on in vitro studies. In a recent study, Daley et al. [36] identified C16:0, C18:2, and C18:3 as predictors of milk fat production. These findings, combined with the present study, emphasize the significance of considering the FA profile of the diet as predictive variables of both milk fat production and proportion.

In this study, we also developed models to predict the FA in milk according to their biological origin. The FA in milk can originate from the uptake of preformed FA from plasma (>C16:0) or from de novo synthesis in the mammary gland (<C16:0). Additionally, there is a group of mixed FA (16-C FA) that involves both origins. The inclusion of DMI in Model 9 was expected, as it is directly related to the production of VFA, which are the main substrates for the de novo synthesis of FA. The inclusion of dietary FA is also considered appropriate, because they have an influence on the production of trans-FA, which inhibits de novo FA synthesis. Considering milk production as a predictor variable also makes sense because the volume of milk produced is directly related to the amount of fat produced. The models developed to predict the mixed FAs in milk (Models 13 and 14) have a greater number of predictor variables compared to the other models. This is understandable, considering that this type of FA has two origins, involving biosynthesis in the mammary gland and uptake from plasma.

For predicting preformed FAs, the simplest model (Model 15) includes DMI and the FA profile (i.e., C18:1 and C18:2) as predictor variables. These variables are relevant as they are associated with the subsequent uptake of FA from plasma. The presence of C18:1 and C18:2 is essential for the formation of preformed FA and depends on factors such as the amount ingested, the biohydrogenation process, and the formation of trans-FA. The availability of these FA and the changes they undergo in the biohydrogenation process play a role in determining the increase or decrease in preformed FA in milk. Likewise, the presence and amount of trans-FA (cis-9, trans-11

or trans-10 and cis-11 CLA) regulate the synthesis of other types of FA, especially de novo FA.

5. CONCLUSIONS

Our results highlight the importance of classifying lipid-supplemented diets based on their FA profile to measure their impact on milk fat production and proportion. Additionally, our findings demonstrate that milk fat can be predicted with accuracy and precision by considering diet, intake, and production characteristics. To the best of our knowledge, this is the first study to quantitatively examine the effects of lipid-supplemented diets, characterized by their FA profile, on milk fat production and proportion. Furthermore, our study utilizes the characteristics of the diets and production performance to predict the milk FA profiles based on their biological origin. We suggest that further studies evaluate the effects of lipid supplementation during different stages of lactation, in particular at the onset of lactation, as our study focused on cows after the lactation peak (DIM = 115).

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CHAPERT 3 - Impact of dietary lipid supplementation on milk fat production and milk fatty acid profile in dairy goats and sheep

ABSTRACT: The main objective of this study was to evaluate the effects of dietary lipid supplementation on milk production, milk fat production and proportion, and milk fatty acid (FA) profile in small ruminants (sheep and goats) using a meta-analytical approach. Our database comprised 144 peer-reviewed papers, including 81 goat studies (192 observations) and 63 sheep studies (139 observations) published between 2000 and 2022. The study consisted of two parts: an effect-size meta-analysis and a meta-regression. Firstly, we conducted an effect-size meta-analysis to evaluate the effect of lipid supplements, categorized according to their FA profile, on milk production, milk fat, and milk FA profile. The effect size was determined using the raw mean difference between the lipid-treated and control groups (without lipids). Means were weighted by the inverse variance in a mixed effect model, and heterogeneity was analyzed through subgroup analysis of lipid sources, based on the FA profile. In general, the incorporation of lipid supplements into the diets of small ruminantsresulted in distinct responses between sheep and goats. Lipid supplements rich in oleic acid showed an increase in milk production and a reduction in the proportion of milk protein in sheep, while also increasing lactose proportion in goats. None of the evaluated lipid sources affected the fat concentration in the milk of either species. Lipid supplements rich in oleic acid (group 1) reduced capric, lauric, myristic, and palmitic acids in milk, but increased stearic and oleic acids in both species. The same lipid supplements (group 1) decreased milk odd-chain FA (FA; C15:0 and C17:0) in both species while increasing milk long-chain FA in dairy sheep. Secondly, we developed prediction models using meta-regression approach to predict milk fat production and proportion and milk FA profile considering variables such as ruminant species, milk producction, dry matter intake, FA in the lipid supplements, and total FA in the diet. The developed models showed high accuracy and precision, with a concordance correlation coefficient greater than 0.90 and root mean square of error below 15.9%. In conclusion, the classification of lipid supplements according to their FA profile plays an important role in determining their effect on milk production, milk components, and the milk FA profile in goats and sheep. These effects vary between species and can be accurately

and precisely predicted based on animal characteristics, FA profile in the supplement, and total FA in the diet. The robustness of the prediction models suggests their potential application in optimizing milk production and composition in small ruminants.

Key words: dietary fat, fatty acids, meta-analysis, meta-regression, small ruminants

INTRODUCTION

Milk production in small ruminants plays an important role in the dairy industry of numerous countries, supplying essential nutrients for human consumption. Milk is rich in protein, fat, vitamins, and minerals, and its nutritional and economic value is largely dependent on its nutritional composition. Among these milk components, milk fat is of considerable importance, as it not only influences the taste and aroma of dairy products, but also determines the price of milk in most markets.

The fatty acid (FA) composition of milk fat varies considerably among individuals and small ruminant breeds (Nudda et al., 2020a) and it is influenced by factors such as age, stage of lactation, and nutrition (Palmquist et al., 1993; Bauman and Griinari, 2003). Modulating milk fat composition through nutrition is a commonly employed strategy for enhancing milk quality and value (Nadeem, 2015; Bernard et al., 2018; Hanuš et al., 2018). Dietary lipid supplementation has proven effective in achieving this goal (Bauman and Griinari, 2003; Shingfield et al., 2010). However, it is important to note that lipid-supplemented diets can result in diverse dietary FA profiles, leading to varying impacts on fat production and the FA profile of milk (Della Badia et al., 2022; Lévesque et al., 2022). Despite this body of knowledge, a comprehensive understanding of the effects of dietary lipid supplementation on milk fat composition and FA profiles in small ruminants is lacking. One of the main reasons for this research gap lies in the diversity of lipid sources, each with variable FA profiles (Gallardo and Teixeira, 2023a). To address this research gap, a comprehensive analysis of the existing scientific literature on the effects of dietary lipid supplementation in small ruminant dairy animals is necessary.

Our objective was to conduct a meta-analytical study on dietary lipid supplementation in small ruminants, specifically focusing on its effects on milk fat

composition and milk FA profiles. By improving our understanding of the effects of this nutritional strategy on milk fat composition, we aim to develop predictive empirical models that can be used by the dairy industry to optimize nutrition and enhance the quality and value of small ruminant dairy products.

MATERIALS AND METHODS

Literature search

A database was constructed using peer-reviewed journal papers from Web of Science and Scopus. The search encompassed papers published between 2000 and 2023, using the keywords “dietary fat* OR lipids OR fatty acids” AND “goats OR sheep” NOT “meat”, to retrieve relevant literature. The management of papers was performed using EndNote X9.

The database included papers that met all the following criteria: 1) studies conducted on lactating goats or sheep; 2) use of experimental control diets (without supplementation of lipid sources) and experimental treatment diets (with dietary supplementation of lipid sources). Treatments involving ruminal infusions, post-ruminal infusions, or intravenous infusions were not considered; 3) reporting of treatment means and respective measures of dispersion [SE, SEM, standard error of the difference (SED)]; 4) provision of information on the FA profile of the lipid supplement; and 5) complete research papers published in journals indexed with a journal citation report impact factor. All duplicate literature and those that did not meet the eligibility criteria were excluded (Figure 1). After applying the inclusion and exclusion criteria, a total of 331 observations corresponding to 144 peer-reviewed articles (81 papers on goats and 63 papers on sheep) were included in this study.

Data extraction

From the selected papers, we collected the following information: animal characteristics (BW and DIM), DMI, milk production, milk components, milk FA profile, and dietary FA profile.

Additionally, we collected information on authors, year of publication, dietary source of lipids, and amount of lipids supplemented. For papers reporting only the composition of the diet ingredients without specifying the FA profile, we estimated the

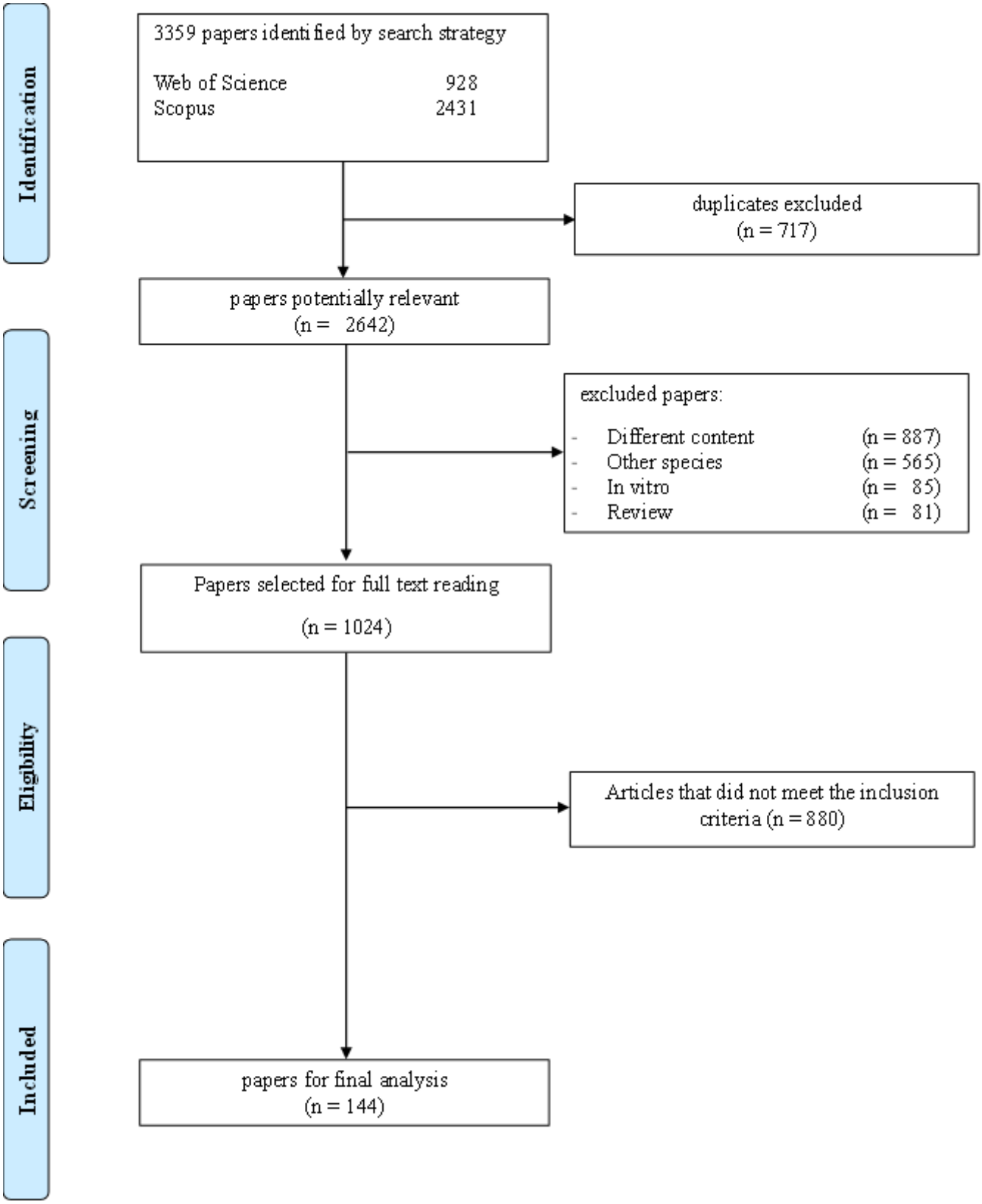

Figure 1: PRISMA flowchart of the papers included in this study

FA profile of the diets using the NASEM (2021) feed library.

Data analysis

We employed two meta-analytic approaches to data analysis: effect size meta-analysis and meta-regression. In the meta-analysis, we assessed the effect size of the lipid-supplemented diet compared to the control diet (no lipid supplementation) on milk production, milk components, DMI, and milk FA profile. In the meta-regression, we employed the same set of variables previously mentioned to develop prediction models for milk fat proportion and production, as well as the milk FA profile of small ruminants supplemented with lipids.

All statistical analyses were performed using R software (version 4.2.2) (R Core team, 2020). Descriptive statistics and preliminary graphical evaluations were performed using the psych and ggplot2 packages, respectively.

Effect Size Meta-Analysis. Meta-analysis was performed using the rma.mv function of the "metafor" package (version 3.8.1) (Viechtbauer, 2010), which is used for multilevel analysis. The effects of lipid supplementation were evaluated by the raw mean difference (RMD) between experimental diets supplemented with lipids and diets without lipid supplementation (control), weighted by the inverse of the variance using the method proposed by DerSimonian and Laird (1986). To assess heterogeneity among studies, we employed the $\chi 2$ test (Q) and the I2 statistic, which measures the percentage of variation due to heterogeneity in responses (Higgins et al., 2003). Values of I^2 <25, 25 to 50, and >50% were considered indicative of low, moderate, and high heterogeneity, respectively (Higgins et al., 2003). In cases of medium and high heterogeneity (calculated with the “var.comp” function from the dmetar package), we performed subgroup RMD analyses to identify possible sources of heterogeneity in the responses (Oliveira et al., 2017). Subgroup analyses were conducted based on ruminant species and FA profile of the lipid sources. Forest plots of the subgroup analysis were generated using the “forestplot” package.

Table 1. Chemical composition and fatty acid (FA) profile of diets (g/kg of DM) with no lipid supplementation (control) and diets supplemented with lipids included in the meta-analysis

	Control diets					Lipid-supplemented diets				
Nutrients	n	Mean	SD	Min	Max	n	Mean	SD	Min	Max
Goats										
CP	144	156.9	25.6	98.0	214	141	158.4	25.2	96.0	227
NDF	124	368.0	81.5	232	773	121	369.9	84.0	239	754
FA	189	21.5	7.51	10.2	52.1	189	44.5	16.8	17.5	112.5
C12:0	109	0.26	0.45	0.02	2.06	109	0.56	0.99	0.01	6.02
C14:0	149	0.20	0.19	0.02	1.11	151	0.47	0.40	0.03	2.14
C16:0	174	3.93	1.88	0.80	13.1	186	7.5	5.11	0.41	34.4
C16:1	130	0.16	0.19	0.01	1.23	130	0.43	0.68	0.02	6.6
C18:0	174	0.61	0.39	0.10	2.75	186	1.74	2.06	0.40	14.03
C18:1	174	3.19	1.95	0.71	14.3	186	8.18	5.95	1.51	37.9
C18:2	175	7.83	3.57	1.86	20.8	187	15.0	10.9	0.93	106.5
C18:3	173	2.82	1.92	0.1	11.2	177	7.27	7.01	0.1	37.7
SFA[1]	174	4.91	2.45	0.90	16.4	186	10.0	6.9	1.09	41.3
UFA[2]	175	14.0	5.6	4.28	35.8	187	30.6	16.8	5.16	166.8
MUFA[3]	174	3.30	1.95	0.71	14.3	186	8.48	5.93	1.51	37.9
PUFA[4]	175	10.7	4.26	3.50	23.9	187	22.2	13.9	0.93	144.0
Sheep										
CP	101	163.9	19.2	120.8	205	101	161.9	18.8	117.0	208
NDF	96	350.5	80.6	226	534	95	347.5	89.6	194	545
FA	139	19.9	5.4	11.1	40	139	44.0	15.5	16.0	104
C12:0	84	0.20	0.24	0.02	1.44	85	0.48	0.68	0.04	4.81
C14:0	120	0.21	0.18	0.01	0.94	120	0.51	0.45	0.05	2.64
C16:0	136	4.07	1.81	0.32	20	139	8.28	4.22	0.52	26.3
C16:1	109	0.25	0.56	0.02	3.52	112	0.5	0.54	0.02	3.22
C18:0	136	0.62	0.28	0.21	2.7	139	2.11	4.53	0.44	41.2
C18:1	136	2.95	1.69	0.75	11.19	139	7.67	5.9	1.72	48.0
C18:2	136	8.21	4.13	1.72	41.0	139	15.1	7.33	0.20	41.5
C18:3	136	3.01	2.09	0.09	15.8	136	7.16	6.03	0.27	36.5
SFA[1]	136	5.03	2.14	0.81	24.0	139	11.1	6.73	2.56	49.7
UFA[2]	136	14.4	6.59	6.18	69.5	139	30.7	12.2	6.70	77.3
MUFA[3]	136	3.15	2.01	0.99	12.7	139	8.07	6.01	2.07	48
PUFA[4]	136	11.2	5.13	2.05	56.8	139	22.6	10.45	0.20	62.2

[1]SFA = Σ (C12:0 + C14:0 + C16:0 + C18:0 + C20:0 + C22:0).
[2]UFA = Σ (C16:1 + C18:1 + C18:2 + C18:3 + C20:4 + C20:5 + C22:6).
[3]MUFA = Σ (C16:1 + C18:1).
[4]PUFA = Σ (C18:2 + C18:3 + C20:4 + C20:5 + C22:6).

Initially, lipid supplements were grouped according to their origin. The lipid supplements consisted of lipid mixtures (a combination of two or more lipid sources), calcium soaps, supplements rich in CLA, fish oil, oilseeds, hydrogenated fats, algae,

and vegetable oils. However, due to the diverse FA profiles within the lipid supplement groups, we chose to regroup the lipid supplements through a cluster analysis. This analysis considered the FA profile of the lipid supplements and the effect size of the lipid supplements on the milk fat proportion, using the NbClust package. Regrouping resulted in three clusters: group 1 (n = 33; 10.0%), group 2 (n = 101; 30.5%), and group 3 (n = 197; 59.5%). In general, the lipid supplements in Group 1 were characterized by high levels of oleic acid, whereas those in Group 2 exhibited high concentrations of linoleic acids. Group 3 supplements were distinguished by high levels of palmitic acid and low levels of MUFA. Additional characteristics of the supplemented lipids are presented in Table 2 and Figure 2.

To assess outliers and publication bias, we examined homoscedasticity using plots of residuals versus predictions and checked the assumption of normality of residuals by residual normality plots. Comparisons between lipid supplementation and control with standardized residuals >2.5 or < -2.5 and Cook's distance > 5/n were removed from the data set (Oliveira et al., 2017). Publication bias was assessed using a funnel plot (Light and Pillemer, 1986) and Egger's regression asymmetry test between RDM and SE (Egger et al., 1997). If asymmetry and biased studies we identified, they were excluded from the analysis.

Meta-Regression. Meta-regression was employed to determine the dietary variables, FA profile of the lipid supplement or animal characteristics that influence the prediction of milk fat production and milk FA profile. To accomplish this, a multiple linear regression with a stepwise (two-way) backward approach was used. A total of 26 variables were selected using Pearson's correlation ($r \geq 0.2$; $P \leq 0.05$), which was corroborated by the biological coherence of the variables. These selected variables were subsequently used as potential predictor variables in the development of the prediction models. The multiple regression model was adjusted for the maximum likelihood using the lme4 package. The study effect was included in the statistical model as a random effect, and the data were weighted using the square root of the number of replicates per treatment.

Table 2. Fatty acid profile in each group (g/100g FA) of lipid supplements in the diets of goats and sheep

Groups	Specie	Obs.	Characteristics	FA profile
1	Goat	17	Rich in acid oleic	MUFA > 60.0, PUFA < 31.0
	Sheep	16	(> 59 g/100g FA)	MUFA > 60.0, PUFA < 27.0
2	Goat	65	Rich in acid linoleic	MUFA > 20.0 < 31.0, PUFA > 52.0
	Sheep	36	(> 33 g/100g FA)	MUFA > 20.0 < 31.0, PUFA > 52.0
3	Goat	110	High in acid palmitic and low in MUFA	SFA > 10.0, MUFA < 23.0, PUFA > 19.0
	Sheep	87	(Mean 18.0 g/100g FA)	SFA > 10.0, MUFA < 28.0, PUFA > 21.0

To control for over-parameterization of the models, the collinearity of the variables was assessed using the variance inflation factor (VIF). VIF values less than 2.5 were considered acceptable, indicating minimal collinearity between variables.

The models were evaluated using Akaike Information Criterion (AIC), Root Mean Square Error (RMSE), and Correlation Coefficient of Concordance (CCC; Lin, 1989). The models were evaluated using cowplot and lmerTest packages.

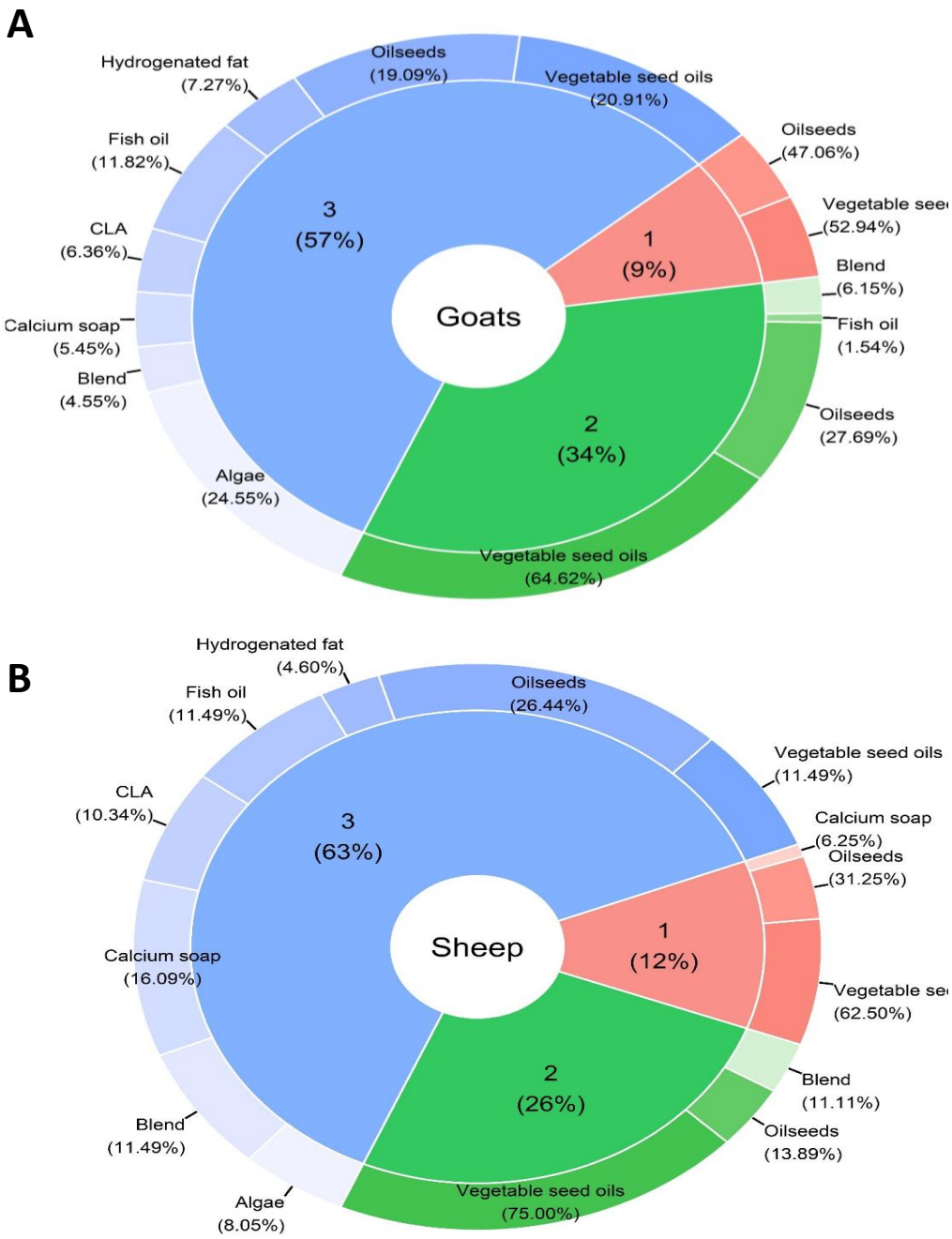

Figure 2. Distribution of lipid supplements evaluated for each species: (A) goats and (B) sheep. The groups were obtained using cluster analysis, considering the fatty acid profile of the lipid supplements and the effect size of the proportion of milk fat. Lipid supplements are classified into three groups, including various lipid sources and both species (sheep and goats). Group 1 supplements were characterized by high levels of oleic acid, group 2 had high concentrations of linoleic acid, and group 3 had high levels of palmitic acid and low levels of monounsaturated fatty acids.

RESULTS

Effect size Meta-analysis

We first evaluated the effect size (i.e., RMD) in a multilevel meta-analysis investigating the impact of including lipid supplements in the diet of small dairy ruminants. Given the high heterogeneity ($I > 75\%$, Supplementary Table S1) in the responses, an analysis was carried out by subgroups of species and then of lipid sources, categorized by their FA profile within each species, to estimate the real effect of the lipid supplements on the analyzed variables.

In general terms, the incorporation of lipid sources in the diet of small ruminants did not affect milk production in goats, but an increase was observed (0.06 kg/d, $P < 0.01$) in dairy sheep (Table 3). When analyzing the effect of different lipid sources within each species, it was observed that only the supplement sources rich in oleic acid (group 1) contributed to the increase in milk production in dairy sheep (Figure 3A).

The inclusion of dietary lipid sources resulted in a decrease in DMI in goats (-0.04 kg/d, $P < 0.05$), while no significant effect was observed in dairy sheep (Table 3). However, when examining the effect of the different lipid sources within each species, it was evident that none of the sources had a noticeable effect on DMI in dairy goats and sheep (Figure 3B).

Lipid supplementation in the diets of small ruminants increased daily milk fat and lactose production in goats ($P < 0.01$ and $P < 0.05$, respectively). However, no effects were observed on any milk component production in dairy sheep (Table 3). When analyzing the impact of different lipid sources within each species, none of the lipid source groups influenced milk fat in both dairy goats and sheep (Figures 3C and 3D).

Table 3. Effects of lipid supplementation on feed intake, ruminal fermentation, and milk production and composition in dairy small ruminants

Item	Goats					Sheep				
	Control means	n	Effect Size			Control means	n	Effect Size		
			RMD	(95% CI)	P-value			RMD	(95% CI)	P-value
DMI and digestibility										
DMI, kg/d	1.94	127	-0.04	(-0.07, -0.01)	<0.05	2.58	75	-0.04	(-0.08, 0.01)	0.11
CP intake, kg/d	0.28	30	-0.02	(-0.04, 0.002)	0.07	0.38	3	-0.07	(-0.12, -0.01)	<0.05
NDF intake, kg/d	0.74	23	-0.09	(-0.13, -0.04)	<0.01	1.18	3	-0.19	(-0.28, -0.09)	<0.001
EE intake, kg/d	0.06	27	0.04	(0.02, 0.05)	<0.001	0.07	8	0.07	(0.04, 0.10)	<0.01
DM digestibility, %	65.6	40	0.70	(-0.83, 2.23)	0.36	69.5	4	-1.33	(-5.58, 2.91)	0.53
CP digestibility, %	65.7	31	1.14	(0.73, 3.00)	0.22	67.6	6	2.00	(-1.87, 5.87)	0.30
NDF digestibility, %	52.7	33	1.29	(-0.30, 2.90)	0.11	51.3	5	0.35	(-3.42, 4.13)	0.85
EE digestibility, %	65.9	26	4.73	(0.73, 8.74)	0.02	65.6	4	-1.82	(10.4, 6.73)	0.66
Ruminal fermentation, mol/100mol										
Acetate	62.6	33	-0.61	(-.265, 1.43)	0.55	71.3	6	0.25	(-5.73, 6.23)	0.93
Propionate	21.0	33	1.94	(0.87, 3.01)	<0.001	25.5	6	-1.86	(-4.95, 1.22)	0.23
Butyrate	13.6	33	-0.43	(-1.19, 0.34)	0.27	14.0	6	0.10	(-2.18, 2.39)	0.93
Production, kg/d										
Milk yield	2.06	187	-0.014	(-0.06, 0.03)	0.55	1.44	135	0.06	(0.01, 0.11)	<0.01
Milk fat	0.08	151	0.005	(0.001, 0.008)	<0.01	0.09	113	0.002	(-0.002, 0.005)	0.42
Milk protein	0.07	148	0.001	(-0.001, 0.002)	0.75	0.08	113	0.001	(-0.001, 0.003)	0.18
Milk lactose	0.10	106	0.003	(0.001, 0.005)	<0.05	0.08	59	0.002	(-0.001, 0.005)	0.22
Milk composition, %										
Milk fat	36.9	192	1.93	(0.63, 3.23)	<0.01	63.3	139	-1.12	(-2.64, 0.41)	0.15
Milk protein	32.6	189	0.07	(-0.27, 0.42)	0.67	53.5	138	-1.00	(-1.42, -0.59)	<0.001
Milk lactose	45.1	155	0.57	(0.24, 0.89)	<0.001	48.8	90	-0.17	(-0.59, 0.25)	0.43

[1]RMD = raw mean difference.

In general, lipid supplementation in the diets of small ruminants resulted in a significant increase in the proportion of fat and lactose in goat milk ($P < 0.01$ and $P < 0.001$, respectively) and a decrease in the proportion of protein in dairy sheep ($P < 0.001$). When examining lipid supplement groups within each species, we found that lipid supplements rich in oleic acid (group 1) were responsible for the decrease in milk protein proportion in sheep (Figure 3E) and an increase in milk lactose proportion in dairy goats (Figure 3F).

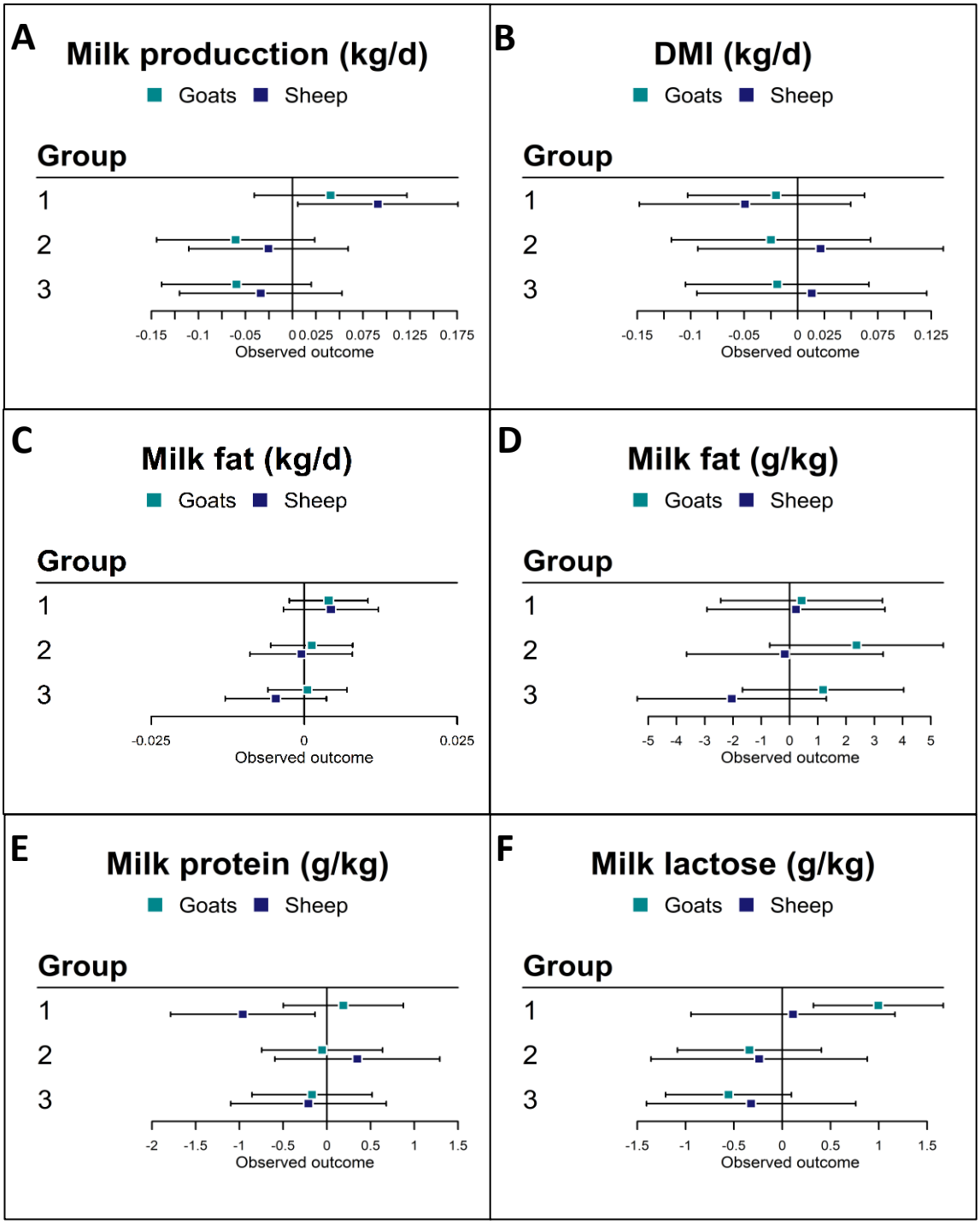

Figure 3. Forest plot showing subgroup analysis by lipid supplements within small ruminant species on milk production (A), DMI (B), milk fat production (C), milk fat proportion (D), milk protein proportion (E), and milk lactose proportion (F) in dairy small ruminants (goats and sheep). The square represents the mean effect size of the lipid source group. Group 1 supplements were characterized by high levels of oleic acid, group 2 had high concentrations of linoleic acid, and group 3 had high levels of palmitic acid and low levels of monounsaturated fatty acids. The lines connected to the squares represent the lower and upper 95% CI for the effect size. The vertical line represents a mean difference of zero (i.e., no effect), while negative or positive values denote the effect of the lipid source group.

The analysis of the FA profile in the milk of small ruminants revealed that lipid supplementation in their diets led to a decrease in capric (C10:0), lauric (C12:0), myristic (C14:0), and palmitic (C16:0) acids in both goats and sheep ($P < 0.001$, Table 4). Furthermore, an increase in stearic and oleic acids was observed in goat's milk, with no effects in sheep (Table 4). When examining the supplemented lipid sources within each species, we observed that lipid sources rich in oleic acid (group 1) resulted in a decrease in capric, lauric, myristic, and palmitic acids in both goats and sheep (Figures 4A, 4B, 4C, and 4D). The same lipid supplements (group 1) increased the concentrations of stearic and oleic acids in the milk of both species (Figures 4E and 4F). On the other hand, lipid supplements rich in linoleic (group 2) and palmitic (group 3) acids increased the concentration of palmitic acid in milk only in dairy goats (Figure 4D) and decreased the concentration of stearic and oleic acids in both goats and sheep (Figures 4E and 4F).

We also evaluated the effect of dietary lipid supplements on pentadecanoic acid (C15:0), palmitoleic acid (C16:1), margaric acid (C17:0), linoleic acid (C18:2 n-6), linolenic acid (C18:3), and vaccenic acid (C18:1 trans 11) in the milk of sheep and goats (Table 4, Figure 5). Odd-chain FA (C15:0 and 17:0) were negatively affected by lipid sources rich in oleic acid (group 1) in both goats and sheep (Figures 5A and 5C). The same lipid sources (group 1) decreased palmitoleic acid and increased vaccenic acid in goat milk (Figures 5B and 5F). In contrast, lipid sources rich in linoleic acid (group 2) increased palmitoleic acid in the milk of goats and linoleic acid in both goats and dairy sheep (Figures 5B and 5D). Lipid sources rich in palmitic acid (group 3) increased palmitoleic acid in goats and linolenic acid in both goats and sheep (Figures 5B and 5E).

Table 4. Effects of lipid supplementation on milk fatty acid (FA) profile in small dairy ruminants

Item	Goats					Sheep				
	Control means	n	Effect Size			Control means	n	Effect Size		
			RMD	(95% CI)	P-value			RMD	(95% CI)	P-value
(g/100 g FA)										
C4:0	2.21	146	0.12	(0.07, 0.18)	<0.001	2.92	100	0.08	(0.013, 0.15)	0.02
C6:0	2.31	141	-0.02	(-0.09, 0.05)	0.54	2.64	107	-0.16	(-0.25, -0.08)	<0.001
C8:0	2.83	152	-0.14	(-0.23, -0.06)	<0.01	2.68	107	-0.20	(-0.30, -0.09)	<0.001
C10:0	9.68	165	-1.22	(-1.53, -0.91)	<0.001	8.78	108	-1.42	(-1.80, -1.05)	<0.001
C12:0	4.74	165	-0.96	(-1.17, -0.76)	<0.001	5.17	115	-0.96	(-1.21, -0.71)	<0.001
C14:0	10.9	165	-1.48	(-1.78, -1.16)	<0.001	11.3	115	-1.17	(-1.55, -0.79)	<0.001
C15:0	0.92	144	-0.12	(-0.17, -0.09)	<0.001	1.04	102	-0.15	(-0.19, -0.10)	<0.001
C16:0	28.9	165	-3.47	(-4.27, -2.67)	<0.001	26.7	116	-2.36	(-3.29, -1.42)	<0.001
C16:1	0.72	158	-0.03	(-0.09, 0.05)	0.47	1.0	97	-0.03	(-0.12, 0.06)	0.48
C17:0	0.69	134	-0.12	(-0.16, -0.08)	<0.001	0.62	92	-0.08	(-0.12, -0.03)	<0.001
C18:0	8.90	179	1.75	(1.04, 2.45)	<0.001	8.34	120	0.14	(-0.69, 0.97)	0.73
C18:1 cis-9	17.8	167	1.56	(0.74, 2.39)	<0.001	17.0	107	0.50	(-0.49, 1.49)	0.32
C18:1 trans-10	0.27	65	0.45	(0.08, 0.82)	0.02	0.68	65	1.33	(0.96, 1.70)	<0.001
C18:1 trans-11	1.02	114	2.23	(1.67, 2.80)	<0.001	1.56	91	2.21	(1.60, 2.83)	<0.001
C18:2 n-6	2.37	152	0.05	(-0.096, 0.196)	0.50	2.68	108	0.15	(-0.023, 0.315)	0.09
C18:3 n-6	0.50	166	0.13	(0.035, 0.227)	<0.01	0.62	114	0.21	(0.10, 0.33)	<0.001
C20:0	0.28	126	0.026	(-0.004, 0.055)	0.08	0.28	82	0.035	(-0.002, 0.072)	0.06
C20:5	0.07	91	0.018	(-0.016, 0.052)	0.29	0.06	65	0.128	(0.086, 0.170)	<0.001
C22:0	0.09	75	0.007	(-0.004, 0.017)	0.21	0.14	66	0.017	(0.005, 0.028)	<0.01
C22:5	0.09	61	0.027	(-0.043, 0.097)	0.45	0.08	52	0.18	(0.104, 0.260)	<0.001
C22:6	0.04	72	0.09	(-0.035, 0.222)	0.15	0.06	63	0.34	(0.207, 0.482)	<0.001
cis 9, trans 11(CLA)	0.53	154	0.68	(0.49, 0.87)	<0.001	0.77	96	0.89	(0.65, 1.12)	<0.001
trans 10, cis 12(CLA)	0.10	67	0.057	(0.025, 0.090)	<0.001	0.08	68	0.034	(0.003, 0.066)	0.03
SFA	71.9	128	-5.44	(6.77, -4.11)	<0.001	69.7	77	-5.95	(-7.60, -4.29)	<0.001
MUFA	22.4	119	4.40	(3.24, 5.55)	<0.001	23.9	70	4.38	(2.94, 5.83)	<0.001
PUFA	3.80	117	1.12	(0.67, 1.57)	<0.001	4.88	67	1.72	(1.16, 2.29)	<0.001
De novo	37.3	24	-5.85	(-9.47, -2.24)	<0.01	39.1	18	-6.06	(-10.0, -2.12)	<0.01
Mixed	31.1	20	-3.12	(-6.60, 0.36)	0.08	27.8	9	-2.10	(-6.85, 2.66)	0.37
Preformed	31.5	24	9.27	(5.15, 13.4)	<0.001	34.6	17	8.90	(4.07, 13.7)	<0.001

[1]RMD = raw mean difference.

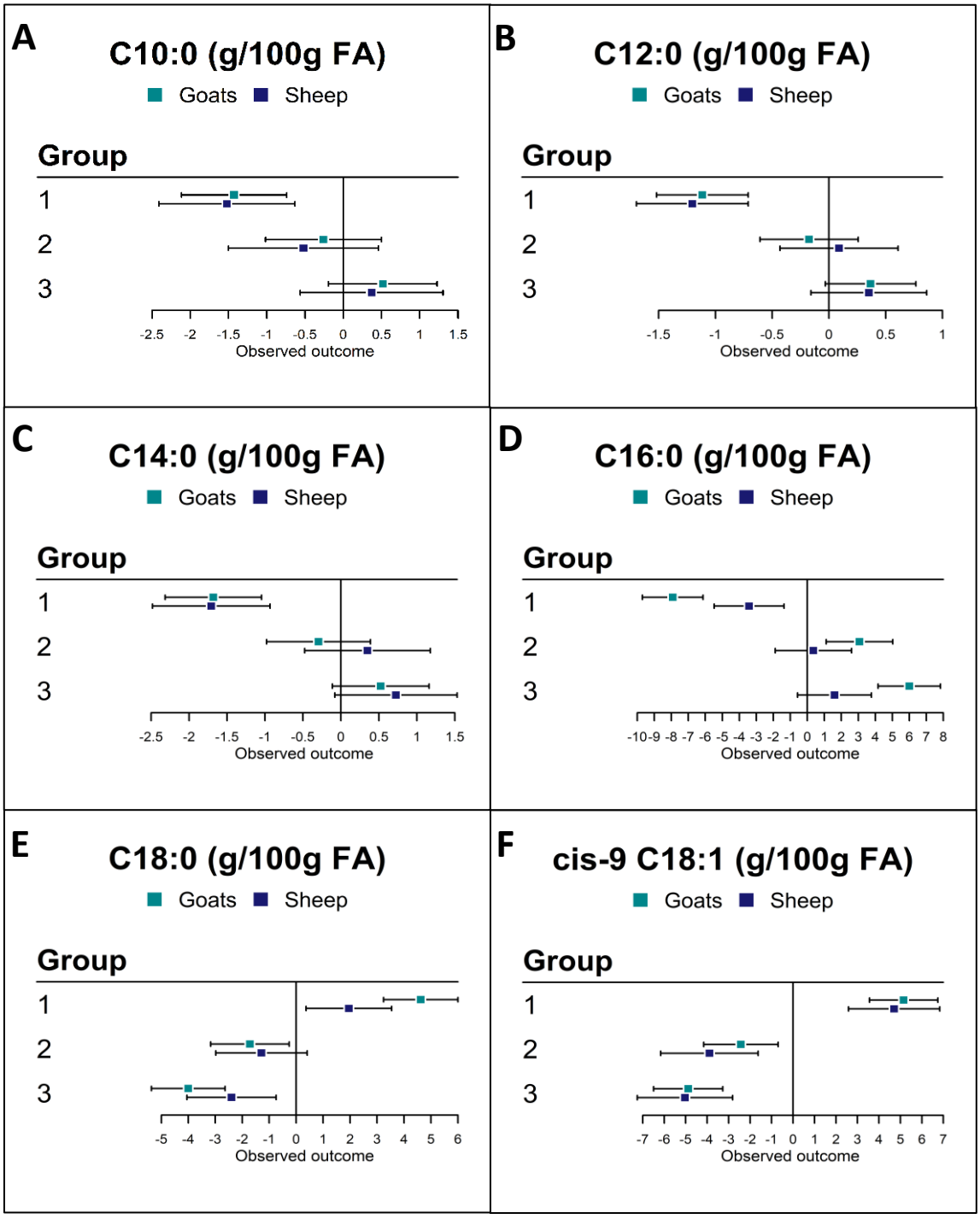

Figure 4. Forest plot showing subgroup analysis by lipid supplements within small ruminant species on the six major fatty acids in milk of dairy goats and sheep. C10:0 (A), C12:0 (B), C14:0 (C), C16:0 (D), C18:0 (E), cis-9 C18:1 (F). The square represents the mean effect size of the lipid source group. Group 1 supplements were characterized by high levels of oleic acid, group 2 had high concentrations of linoleic acid, and group 3 had high levels of palmitic acid and low levels of monounsaturated fatty acids. The lines connected to the squares represent the lower and upper 95% CI for the effect size. The vertical line represents a mean difference of zero (i.e., no effect), while negative or positive values denote the effect of the lipid source group.

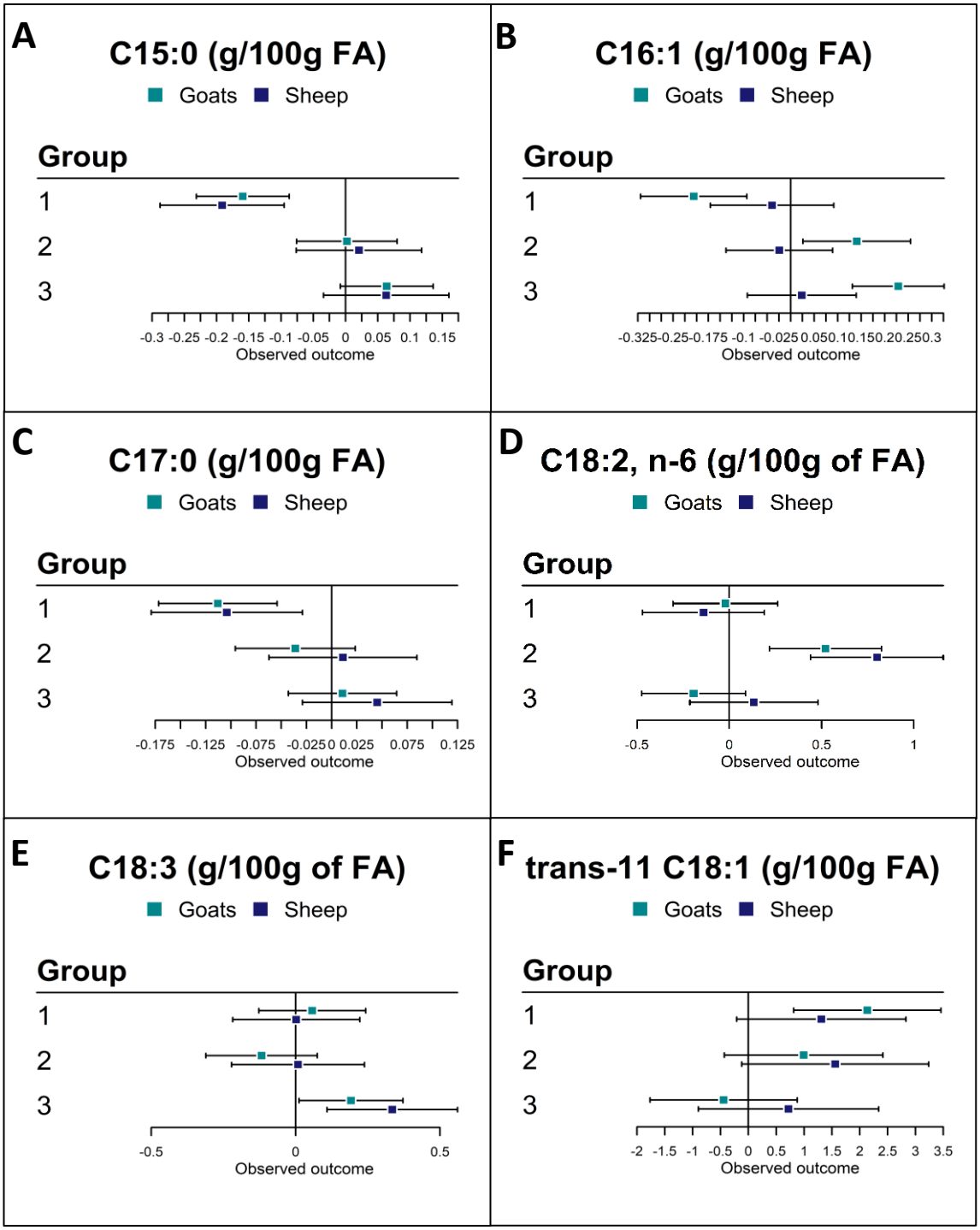

Figure 5. Forest plot showing subgroup analysis by lipid supplements within small ruminant species on the concentration of milk C15:0 (A), C16:1 (B), C17:0 (C), C18:2 (D), C18:3 (E), and C18:1, trans-11 (F) in dairy goats and sheep. The square represents the mean effect size of the lipid source group. Group 1 supplements were characterized by high levels of oleic acid, group 2 had high concentrations of linoleic acid, and group 3 had high levels of palmitic acid and low levels of monounsaturated fatty acids. The lines connected to the squares represent the lower and upper 95% CI for the effect size. The vertical line represents a mean difference of zero (i.e., no effect), while negative or positive values denote the effect of the lipid source group.

We also evaluated the impact of dietary lipid supplements on FA with more than 20 carbons (arachidic acid (C20:0), eicosapentaenoic acid (C20:5), behenic acid (C22:0), clupanodonic acid (C22:5), and docosahexaenoic acid (C22:6)) in the milk of small ruminants (Table 4, Figure 6). The positive effect of dietary lipid sources rich in oleic acid (group 1) in the concentration of C20:5, C22:5, and C22:6 in the milk of dairy sheep was notable (Figures 6B, 6D, and 6E).

Finally, we evaluated the effect of lipid supplements in the diet of small ruminants on milk FA, considering their origin. We observed a decrease in de novo FA ($P < 0.01$), an increase in preformed FA ($P < 0.001$), and no effect of mixed FA (Table 4). However, we were unable to perform a subgroup analysis of lipid sources because of the limitation in the number of observations in some groups. When examining milk FA according to their degree of saturation, we observed a decrease in SFA and an increase in MUFA and PUFA in the milk of dairy sheep and goats fed lipid supplements ($P < 0.001$; Table 4). In subgroup analysis, we found that lipid sources rich in oleic acid (group 1) decreased SFA (Figure 7A) and increased MUFA in goats and sheep (Figure 7B). Likewise, these lipid supplements (group 1) increased milk PUFA in goats (Figure 7C). Lipid supplements rich in linoleic (group 2) and palmitic (group 3) decreased milk MUFA in goats (Figure 7B) and increased milk PUFA in sheep (Figure 7C).

Meta-Regression

In this study, we also aimed to predict milk fat and milk FA in small ruminants fed lipid supplements. We selected 20 candidate variables as potential predictors using Pearson's correlation analysis ($r \geq 0.2$; $P \leq 0.05$) and their biological coherence.

Models 1 and 2 (Table 5), which were developed to predict milk fat production (kg/d), demonstrated both high accuracy and precision (CCC = 0.98, RSME < 8.2%). The simplest model (Model 1) included small ruminant species, milk production, and linoleic acid in the supplement as predictor variables. Model 2, in addition to the variables of Model 1 included palmitic acid of the lipid supplement (g/100 g FA) as an additional predictor (Table 6). Model 3, which was the only model developed to predict the proportion of milk fat (g/kg), demonstrated high accuracy and precision (CCC = 0.98, RMSE = 6.05%). This model included ruminant species as well as linoleic and palmitic acids in the lipid supplement as predictor variables (Table 5).

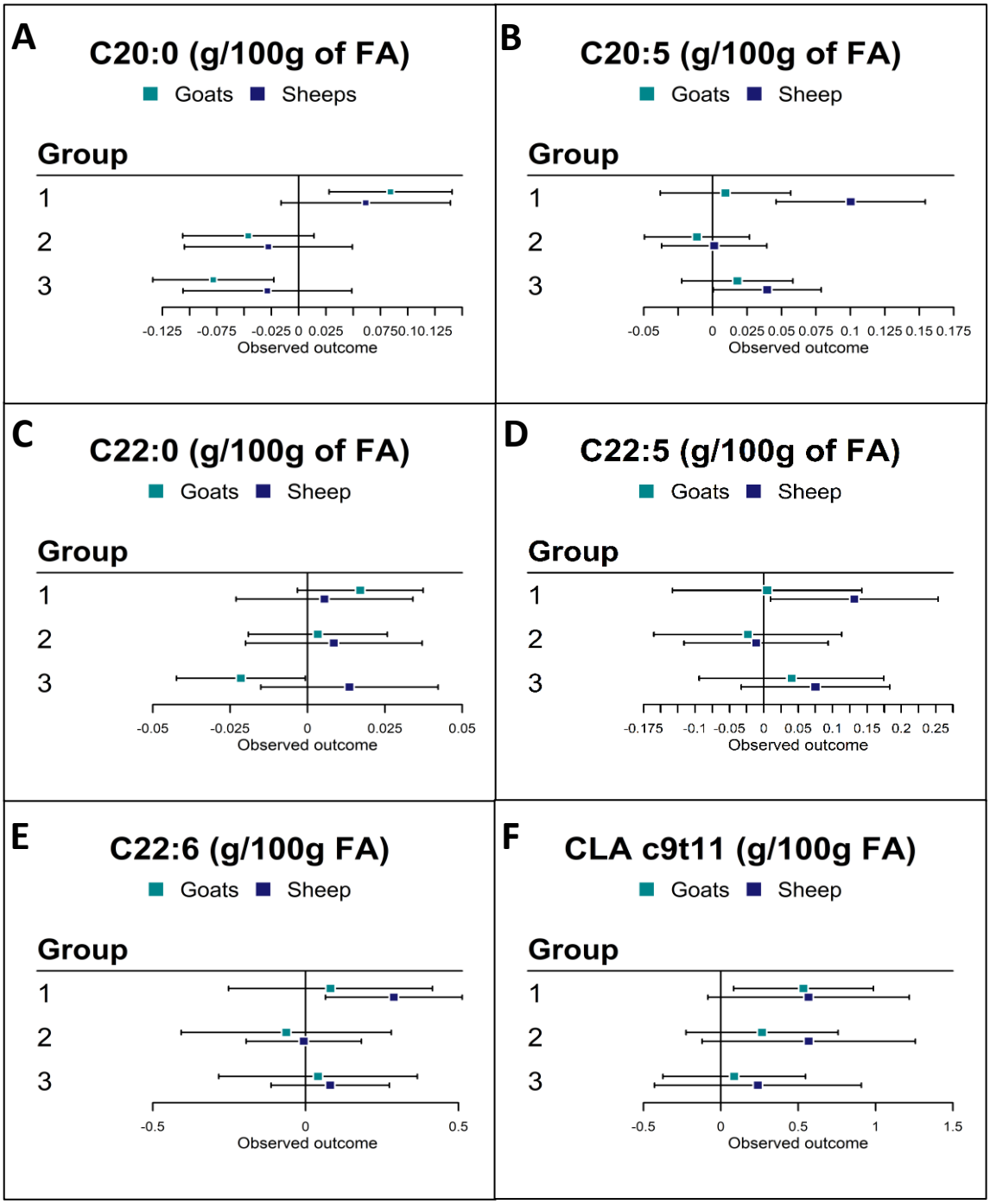

Figure 6. Forest plot showing subgroup analysis by lipid supplements within small ruminant species on long-chain fatty acids and conjugated linoleic acid in milk C20:0 (A), C20:5 (B), 22:0 (C), C22:5 (D), C22:6 (E), and CLA cis-9, trans-11 (c9t11) (F) of dairy goats and sheep. The square represents the mean effect size of the lipid source group. Group 1 supplements were characterized by high levels of oleic acid, group 2 had high concentrations of linoleic acid, and group 3 had high levels of palmitic acid and low levels of monounsaturated fatty acids. The lines connected to the squares represent the lower and upper 95% CI for the effect size. The vertical line represents a mean difference of zero (i.e., no effect), while negative or positive values denote the effect of the lipid source group.

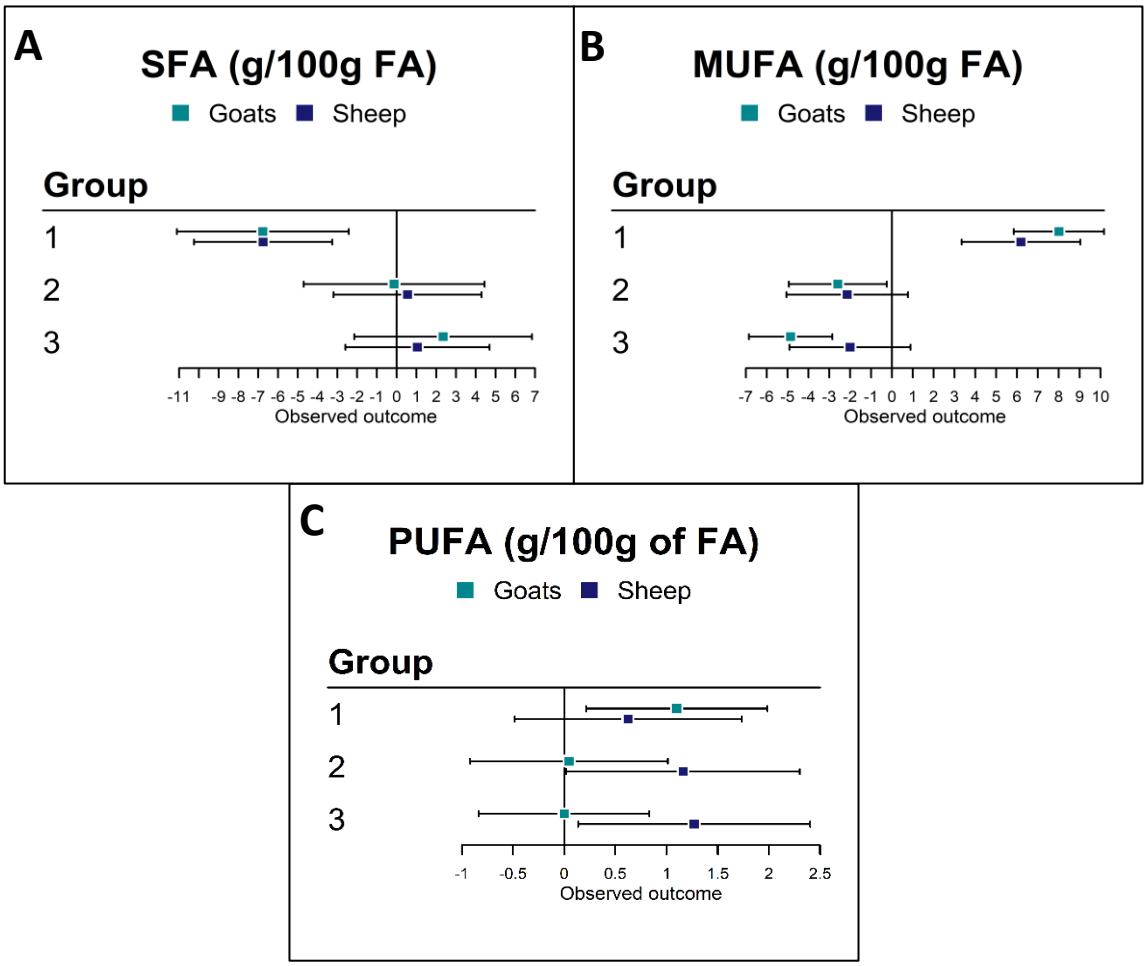

Figure 7. Forest plot showing subgroup analysis by lipid supplements within small ruminant species on fatty acids in milk of dairy small ruminants (goats and sheep): SFA (A), MUFA (B), PUFA, (C). The square represents the mean effect size of the lipid source group. Group 1 supplements were characterized by high levels of oleic acid, group 2 had high concentrations of linoleic acid, and group 3 had high levels of palmitic acid and low levels of monounsaturated fatty acids. The lines connected to the squares represent the lower and upper 95% CI for the effect size. The vertical line represents a mean difference of zero (i.e., no effect), while negative or positive values denote the effect of the lipid source group.

Models 4 and 5 were developed to predict the concentration of myristic acid in milk (g/100 g of FA). Both models demonstrated high accuracy and precision (CCC ≥ 0.90, RSME < 7.2%). Model 4, which is the simplest model, incorporated dietary FA and DMI as predictor variables to estimate milk myristic acid. On the other hand, Model 5, in addition to the variables present in Model 4, included linoleic acid in the supplement as an additional predictor variable (Table 6). Model 6, developed to predict palmitic acid content in the milk of small ruminants, also showed high accuracy and

precision (CCC = 0.93, RMSE = 6.49%). This model incorporated dietary FA, palmitic acid, and stearic acid in the lipid supplement as predictor variables (Table 6).

Models 7 and 8 (Table 7) were developed to predict the proportion of stearic acid (g/100 g FA) in milk. Both models exhibited high accuracy and precision (CCC ≥ 0.93, RSME < 15.9%). Model 7, which is the simplest model, includes oleic acid and PUFA in the supplement as predictor variables for estimating stearic acid in milk. Model 8 incorporated DMI as an additional variable in addition to the variables present in Model 7 (Table 7). Finally, models 9 and 10 (Table 7) were developed to predict the proportion of oleic acid in milk (g/100 g FA). Both models showed high accuracy and precision (CCC ≥ 0.93, RSME < 11.3%). Model 9, the simpler of the two, incorporated small ruminant species and linoleic acid in the supplement as predictor variables for oleic acid in milk. Building on model 9, model 10 added milk production as a predictor variable (Table 7).

DISCUSSION

The main objective of this study was to evaluate the effects of lipid supplementation in the diet of small ruminants (goats and sheep) on milk fat production, as well as on their FA profile. Our results indicate that the effect of lipid supplements varies according to animal species and is directly related to the FA profile of the supplement. Furthermore, we found that milk fat and FA profile of milk can be predicted based on the characteristics of the animals, diet, and the type of supplement used. These findings are of great importance in establishing nutritional strategies for milk production in small ruminants and may contribute to the development of more efficient and profitable practices in the dairy sector.

Table 5. Best-fitting models for milk fat production (kg/d) and milk fat proportion (g/kg) of dairy small ruminants

Milk fat models[1]	Intercept (k/d or g/kg)	Ruminant species	Milk production (kg/d)	C18:2 supp. (g/100 FA)	C16:0 supp. (g/100 FA)	Evaluation on data [2]						
							n	AIC	CCC	RMSE	Mean bias	Slope bias
Milk fat production, kg/d												
Model 1												
Estimate	-0.0039	0.0342	0.0383	0.0001		Value	264	-1390	0.98	0.007	0.0001	0.020
SE	0.0050	0.0042	0.0020	0.00005		%				8.18	0.003	1.30
P-value	0.39	<0.001	<0.001	<0.05		*P*-value					0.93	0.06
Model 2												
Estimate	-0.0085	0.0348	0.0384	0.0002	0.0002	Value	264	-1379	0.98	0.007	0.0001	0.019
SE	0.0052	0.0042	0.0019	0.00005	0.00008	%				8.07	0.003	1.25
P-value	0.10	<0.001	<0.001	<0.01	<0.01	*P*-value					0.92	0.07
Milk fat proportion, g/kg												
Model 3												
Estimate	36.4	23.44		0.054	0.069	Value	334	2332	0.98	2.93	-0.009	0.02
SE	1.63	2.11		0.021	0.032	%				6.03	0.001	1.29
P-value	<0.001	<0.001		<0.05	0.05	*P*-value					0.95	0.04

[1] Models were adjusted for random effect of study.
[2] AIC = Akaike's information criterion; CCC = concordance correlation coefficient; RMSE = root mean square error.

Meta-Analysis—Effect Size

Lipid supplementation is a widely used nutritional strategy aimed at enhancing the quality and quantity of milk produced in ruminants, mainly by increasing energy intake (Chilliard, 1993). Beyond their role as energy source, FA have recently been considered as bioactive molecules with specific physiological functions (Savoini et al., 2019). The effects of lipid supplementation, however, depend on several factors, including the type, quantity, and form of lipid used, as well as the animal species, breed, physiological state, and production level (Palmquist et al., 1993). As a result of these diverse factors, findings regarding the impact of lipid supplementation on small ruminant diets are often contradictory. Therefore, grouping lipid supplements based on their FA profile is important for determining the actual effects of lipid supplementation on milk production and percentage in dairy goats and sheep.

Our results indicate that lipid supplementation does not present significant differences between most of the fat sources evaluated within the species in terms of milk production, except for the supplement sources of group one (sources rich in oleic acid) that increased milk production in sheep. These findings are consistent with the results reported by Marin et al. (2015) in a study conducted on goats and sheep, as well as Vargas-Bello-Pérez et al. (2021), who focused exclusively on sheep. These results are likely linked to the lack of effect of lipid supplementation on DMI and nutrient digestibility in dairy goats and sheep, as evidenced in our study. In contrast, Ángeles-Hernandez et al. (2020) reported increases in milk production when evaluating sheep supplemented with dietary fat sources, which aligns with the responses observed with supplements sources rich in oleic acid (Group 1) in our study. The positive effects reported by Angeles-Hernandez et al. (2020) and Group 1 in our study support the idea that lipid supplements with a specific FA profile can enhance milk production in dairy sheep.

Table 6. Best-fitting models for milk C14:0 and C16:0 (g/100g of FA) in dairy small ruminants

Milk FA models[1]	Intercept (g/100g FA)	FA in diet (g/kg DM)	DMI (kg/d)	C18:2 supp. (g/100 FA)	C16:0 supp. (g/100 FA)	C18:0 supp. (g/100 FA)	Evaluation on data [2]					
								n	AIC	CCC	RMSE	Mean bias	Slope bias
C14:0 model													
Model 4													
Estimate	10.47	-0.053	0.579				Value	178	598	0.90	0.66	0.0002	0.14
SE	0.629	0.008	0.218				%				7.12	0.0001	8.55
P-value	<0.001	<0.001	<0.01				*P*-value					0.99	0.001
Model 5													
Estimate	10.58	-0.049	0.614	-0.016			Value	178	598	0.91	0.65	0.0003	0.134
SE	0.612	0.008	0.212	0.005			%				6.96	0.0001	7.96
P-value	<0.001	<0.001	<0.01	<0.01			*P*-value					0.99	0.001
C16:0 model													
Model 6													
Estimate	25.95	-0.092			0.175	0.052	Value	280	1455	0.93	1.62	0.007	0.09
SE	0.802	0.016			0.016	0.017	%				6.49	0.002	5.59
P-value	<0.001	<0.001			<0.001	<0.01	*P*-value					0.94	0.001

[1] Models were adjusted for random effect of study.

[2] AIC = Akaike's information criterion; CCC = concordance correlation coefficient; RMSE = root mean square error.

Table 7. Best-fitting models for milk C18:0 and C18:0 cis-9 (g/100g of FA) in dairy small ruminants

Milk FA models[1]	Intercept (g/100g FA)	Ruminant specie[2]	DMI (kg/d)	C18:2 supp. (g/100 FA)	C18:1 supp. (g/100 FA)	PUFA supp. (g/100 FA)	Milk production (kg/d)	Evaluation on data[3]					
									n	AIC	CCC	RMSE	Mean bias	Slope bias
C18:0 model														
Model 7														
Estimate	5.563				0.071	0.056		Value	299	1533	0.94	1.43	0.008	0.103
SE	0.635				0.011	0.009		%				14.7	0.003	7.40
P-value	<0.001				<0.001	<0.001		*P*-value					0.93	0.001
Model 8														
Estimate	6.33		-0.013		0.071	0.059		Value	242	1263	0.93	1.53	0.013	0.109
SE	0.837		0.006		0.012	0.009		%				15.8	0.007	7.21
P-value	<0.001		<0.05		<0.001	<0.001		*P*-value					0.89	0.001
C18:1 cis-9 model														
Model 9														
Estimate	18.49	-2.29		0.049				Value	274	1567	0.94	1.94	-0.008	0.092
SE	0.714	1.008		0.013				%				10.5	0.002	6.24
P-value	<0.001	<0.05		<0.001				*P*-value					0.94	0.001
Model 10														
Estimate	23.00	-3.46		0.050			-2.289	Value	266	1504	0.93	2.06	-0.019	0.093
SE	1.119	0.938		0.013			0.462	%				11.2	0.009	5.62
P-value								*P*-value					0.88	0.001

[1] Models were adjusted for random effect of study. [3] AIC = Akaike's information criterion; CCC = concordance correlation coefficient; RMSE = root mean square error.

DMI is a critical factor determining the productive and reproductive performance of ruminants by directly influencing the quantity and quality of nutrients available to animals. This consumption is influenced by physical, physiological, and environmental factors, as well as the intrinsic characteristics of the food (Mertens, 1994; Detmann et al., 2014; Oliveira et al., 2020). Various studies have indicated that fat supplementation in the diet of dairy cows leads to a decrease in consumption (Allen, 2000; Bauman and Griinari, 2003). However, this pattern is not replicated in the same way in small ruminants, as highlighted in a review by Nawas et al. (2016). Our findings support this observation, showing no discernible impact on DMI in both dairy sheep and goats with fat supplementation.

One of the key findings of this study is related to the production and proportion of milk fat. Based on previously documented responses in dairy cows, we anticipated variations in milk fat concentrations (Rabiee et al., 2012; Gallardo and Teixeira, 2023b). However, the lack of effects on milk production and milk fat percentage in goats and sheep supplemented with various lipid sources could be explained by the dynamic balance between increasing and decreasing milk FA. Our study revealed this phenomenon, particularly in the FA profile (Table 4).

Various studies have shown that lipid supplementation in the diet leads to a decrease in protein concentration, but the basis for these results is not completely clear. Explanations range from the dilution effect and reduced amino acid availability to insulin resistance, as well as endocrine and transcriptional factors (DePeters and Cant, 1992; Burgos et al., 2010; Osorio et al., 2016; Pan et al., 2023). However, these results do not seem to be linked to all supplemented lipid sources. In our findings, the protein concentration decreased only in dairy sheep supplemented with sources rich in oleic acid.

An increase in lactose concentration in dairy goats has been documented by several authors (Bernard et al., 2016; Gómez-Cortés et al., 2018; Kholif et al., 2020). Our results, in agreement with these reports, revealed an increase in lactose concentration in the milk of goats supplemented with the sources of group 1(rich in oleic acid). This increase is associated with higher levels of propionic acid, the main

precursor of lactose. However, given the bioactive function of FA, additional studies are suggested to further explore this result.

Given the growing interest of manufacturers and consumers in recent years in the FA present in milk, our study has revealed a differentiated effect of lipid supplementation on the FA profile of milk from sheep and goats. The reduction of capric, lauric, myristic, and palmitic acids in milk, in both goats and sheep supplemented with lipid sources rich in oleic acid (group 1), aligns with previous reports by Levesque et al. (2022), Hervas et al. (2021) and Thanh et al. (2023). This decrease in FA is consistent with a lower synthesis of *de novo* and mixed FA, as observed in our study. The adverse effect on these FA in milk could be linked to the biohydrogenation process of oleic acid in the rumen, resulting in the formation of C18:1 trans 10, a FA known to inhibit the synthesis of short and medium chain FA in the mammary gland.

In contrast, the same sources of supplements (Group 1) rich in stearic, oleic, and vaccenic FA in the milk of dairy goats and sheep. This was proportionally similar to the decrease in other FA, resulting in no net change in the milk fat. These results are supported by the evaluation of the behavior of preformed FA, with similar findings reported by various authors (Hervás et al., 2021; Lévesque et al., 2022; Thanh et al., 2023).

The effect of lipid supplementation on odd-chain FA was addressed by Abdoul-Aziz et al. (2021), who pointed out the potential impact of lipid supplements on these FA. Our findings indicate that only lipid supplements rich in oleic acid caused a decrease in odd-chain FA (C15:0 and C17:0). Bauman et al. (2016) also reported a decrease in these FA when soybean oil was infused in the rumen. However, in the same experiment, an increase in C17:0 was observed in milk when SFA sources were infused. The disparity in results highlights the importance of classifying FA according to their profiles to obtain more reliable results.

Finally, we analyzed the LCFA in milk, given the growing interest in improving these FA due to their association with human health benefits (Savoini et al., 2019; Nudda et al., 2020; Roque-Jiménez et al., 2021). Our results are particularly interesting for sheep, where we observed significant increases in FA C20:5, C22:5, and C22:6 when supplemented with sources rich in oleic acid. This increase was also documented

by Zisis et al. (2022); however, their study evaluated algal sources as a supplement. Although the mechanisms underlying these responses are not yet fully understood (Roque-Jiménez et al., 2021), further studies are necessary to accurately analyze the true impact of lipid supplementation on these FA in the milk of dairy small ruminants, with special attention to dairy sheep.

Meta-Regression

To the best of our knowledge, no studies have developed prediction models for milk fat production and FA profiles in small ruminants, especially in a comprehensive database that includes both sheep and goats. Milk fat is an important milk component, and understanding the mechanisms that regulate its synthesis to modulate production is an ongoing objective (Kliem and Shingfield, 2016; Bernard et al., 2018). However, this task is complex due to the influence of various nutritional and non-nutritional factors on milk fat synthesis (Palmquist et al., 1993; Bauman et al., 2011). Recently, some milk fat prediction models have been developed, but most have focused on dairy cows (Maxin et al., 2011; Daley et al., 2022). In this context, Padilha et al., (2023) developed models to predict milk fat variation in goats and sheep, using milk FA profiles as a reference. However, we consider that milk fat prediction could be achieved more accurately through highly influential factors, such as diet and source of lipid supplements. Recently, our research group conducted a study using a meta-analytical approach (Gallardo and Teixeira, 2023) to predict fat production and the FA profile of milk based on the FA profile of the diet in dairy cows.

The inclusion of ruminant species as a predictor variable for milk fat production and proportion (models 1 – 3; Table 5) is relevant, as this milk component exhibits significant differences between both species. These differences could be explained by physiological differences, either in terms of feeding type (Hofmann, 1989) or specific physiological characteristics of each species. The inclusion of milk production as a predictor variable was expected, as higher milk production is expected to result in higher milk fat production (Gallardo and Teixeira, 2023). However, it is novel that the models included C18:2 as a predictor variable. This variable has a negative relationship in models for dairy cows (Daley et al., 2022; Gallardo and Teixeira, 2023);

nevertheless, in our models, it is positively related. This could explain why no effects of lipid supplementation on milk fat production and proportion were observed in the effect size meta-analysis of the first study, indicating that the effect of lipid supplementation in the diet is different among ruminant species. Palmitic acid was associated with an increase in milk fat production and proportion (Hervás et al., 2021), and our study corroborates this result since C16:0 was included as a predictor variable in models 2 and 3.

The FA present in milk have two main sources: *de novo* synthesis in the mammary gland and blood absorption originating from the diet and/or body reserves. Developing prediction models based on the origin of FA would be of great interest, as each source encompasses a variety of FA. From a practical perspective, this could facilitate their application. However, in our data, the reporting of FA by their origin was limited, so we chose to generate predictive models for individual FA.

The inclusion of total FA in diet with a negative effect on the prediction of myristic and palmitic acids (Table 6) seems appropriate. It is well known that when fat levels in ruminant diets exceed certain limits (6 - 7% of DM), this can affect ruminal health and, consequently, milk fat production and FA profile. Similar results were reported by Hermansen (1995); however, their research was conducted on dairy cows.

The inclusion of DMI as a predictor variable for milk myristic acid (models 4 and 5) was related to the increased availability of acetate and β-hydroxybutyrate, precursors for the synthesis of short- and medium-chain FA in the mammary gland. Given its positive effect, it is inferred that an increase in DMI enhances myristic acid in the milk. On the other hand, the inclusion of C18:2 from the supplement as a predictor variable for milk myristic acid (model 5) with a negative effect aligns with the theory that long-chain FA inhibit the synthesis of *de novo* FA (<16 carbons) in the mammary gland.

The inclusion of palmitic and stearic FA from the supplement as predictor variables for palmitic acid in milk (model 6) makes sense, given that 16-carbon FA has a mixed origin (*de novo* and preformed). The incorporation of these two FA is consistent with our expectations. Additionally, previous research conducted on cows may support the inclusion of these two variables. For example, Hermansen (1995) included dietary

palmitic acid as a predictor variable for palmitic acid in milk. Similarly, de Souza et al. (2018) reported an association between dietary palmitic and stearic acid and their positive effect on milk fat synthesis.

The inclusion of oleic acid and PUFA from the supplement as predictor variables with a positive effect on the prediction of stearic acid in milk (models 7 and 8) aligns with the biohydrogenation theory (Bauman and Griinari, 2001), where UFA are hydrogenated and converted into stearic acid. Thus, a higher amount of UFA results in higher stearic acid in milk. Finally, the inclusion of ruminant species as predictor variables with a negative effect on the prediction of oleic acid in milk (models 9 and 10) reinforces the idea that the digestive physiology differs between the two species. The inclusion of linoleic acid in the supplement with a positive effect was somewhat expected, as a higher amount of PUFA in the diet increases intestinal availability, which is then absorbed into the bloodstream and uptake in the mammary gland for preformed FA synthesis.

We encourage the creation of predictive models according to the origin of milk FA (*de novo*, mixed, and preformed), which would enhance our understanding by minimizing the variables to be evaluated. As we explained above, in our study, we were unable to develop models based on these FA given the low number of observations for these variables in our database.

CONCLUSIONS

Lipid supplementation does not alter fat production and proportion in the milk of small ruminants. Classifying lipid supplements according to their FA profiles is crucial for understanding their impact on milk composition and FA profiles. Specifically, lipid supplements rich in oleic acid are key factors in the variation in response to lipid supplementation in the diet of small dairy ruminants. Additionally, our research revealed that through the FA profile of the supplement and the specific characteristics of small ruminant species, predictive models can be developed that fit with accuracy and precision. These models are valuable for predicting milk fat production, milk fat proportion, and FA profiles in the milk of goats and sheep, providing valuable information for both nutritionists and producers.

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CHAPTER 4 – Implications

Understanding the impact of dietary FA profiles and lipid supplements on fat synthesis and FA profiles in dairy ruminants is essential for diet formulation and the regulation of milk component production. In this thesis, we conducted two studies using a meta-analytical approach. In the first study, we evaluated the influence of dietary FA profiles and their effect on milk fat production and FA profiles in dairy cows. In the second study, we assessed the specific effects of FA profiles of lipid supplements on milk fat production and FA profiles in the milk of goats and sheep. Unlike the first study, in the second one, we focused exclusively on analyzing the FA profile of the lipid supplement source. In both studies, prediction models were developed to predict milk fat production and milk FA. Our results indicate the following:

The effect of lipid supplementation on the diet differs among cows, goats, and sheep.

The milk fat production of dairy cows varies depending on the FA profile of the diet. Diets with high levels of palmitic acid increased the milk fat percentage, whereas diets with moderate or high levels of monounsaturated and polyunsaturated FA, reduced the fat proportion.

Diets supplemented with lipids, irrespective of the FA profile, reduced the *de novo* FA in the milk. However, diets rich with palmitic acid lead to an increase in the mixed FA in the milk. This was the main factor contributing to the observed increase in the milk fat proportion of dairy cows.

It is feasible to predict milk fat production and FA profile in the milk of dairy cows based on the characteristics of the animal, diet, and diet FA profile. This finding is particularly important to nutritionists and producers, providing them with a tool to predict milk fat production, modulate the FA profile in milk, and improve the nutritional management of dairy cows.

Lipid supplementation did not cause changes in the production or proportion of milk fat of goats and sheep.

Lipid supplements with high levels of oleic acid play a role in the milk FA composition of sheep and goats. These sources were decisive in altering the FA profile of milk in both species. Significant increases in long chain FA were observed, especially in dairy sheep, suggesting a viable strategy for enriching milk intended for certain consumer types.

Similar to the study with dairy cows, it is feasible to predict milk fat production and FA profiles with high precision and accuracy. We propose that models to predict milk FA should focus on the FA profile instead of their origin. This approach would enable more effective monitoring of the flow of FA destined for milk fat synthesis, facilitating the design of more precise nutritional management strategies.