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Tese

Estratégias para a pecuária leiteira: efeitos da inclusão de gordura protegida na dieta e utilização de feromonioterapia em vacas da raça Holandês

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Para minha família e amigos, com carinho e gratidão.

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“Se todo passarinho desistisse após a primeira queda, o céu seria vazio.”

Victor Machado

Resumo

ARAÚJO, Maria Carolina Narval de. **Estratégias para a pecuária leiteira: efeitos da inclusão de gordura protegida na dieta e utilização de feromonioterapia em vacas da raça Holandês.** 2025. 64f. Tese (Doutorado) - Programa de Pós-Graduação em Biotecnologia. Universidade Federal de Pelotas, Pelotas.

A utilização de gordura protegida na dieta de vacas leiteiras, bem como o uso de feromonioterapia para reduzir estresses de manejo são exemplos de ferramentas que podem ser implementadas na bovinocultura leiteira. Neste estudo, foram realizadas duas pesquisas com estratégias que podem ser utilizadas a fim de incrementar o desempenho dos animais. O objetivo do primeiro experimento foi avaliar o impacto de uma gordura hidrogenada (H) rica em ácidos graxos saturados (AGS) (C16:0 e C18:0) e duas gorduras protegidas por sais de cálcio (Ca) com perfis diferentes de AGS (C16:0 e C18:0) e ácido graxo insaturado (AGI) (cis-9 C18:1) e um grupo controle (sem suplementação de gordura) sobre o consumo, produção, composição do leite, bem como parâmetros metabólicos e zootécnicos de vacas da raça Holandês após o pico de lactação. O período experimental foi dividido em três períodos de 22 dias cada. Durante cada período, foram utilizados quatro animais por grupo, totalizando 16 animais em cada período e 48 vacas (n=48) no total ao final do experimento. Os grupos foram divididos de acordo com a composição lipídica: H-saturado (n=16), Ca-saturado (n=16), Ca-mix (n=16) e Grupo Controle (n=16). A produção diária de leite foi medida diariamente, bem como o consumo de matéria seca (CMS). Amostras de leite foram coletadas duas vezes por semana para análise dos constituintes (gordura, lactose, proteína, caseína, nitrogênio ureico e sólidos totais) e contagem de células somáticas (CCS). Amostras de sangue foram coletadas semanalmente para análise de ácidos graxos não esterificados (AGNE), beta-hidroxibutirato (BHB), albumina, triglicerídeos (TGL), ureia e glicose. As análises estatísticas foram conduzidas com o software JMP Pro 14, e o nível de significância adotado foi $P \leq 0,05$. Os grupos Ca-mix e Ca-saturado, em comparação com o grupo Controle, apresentaram menor teor de proteína no leite ($P<0,05$). A CCS foi menor nos grupos de tratamento em relação ao grupo Controle ($P<0,05$). Em relação às análises bioquímicas, o grupo H-saturado apresentou uma maior concentração de BHB em comparação ao grupo Ca-mix ($P=0,05$), sem efeito nos outros parâmetros. Não foram observados efeitos no consumo alimentar, produção de leite, peso corporal (PC) nem no escore de condição corporal (ECC). Em conclusão, ao compararmos os três grupos suplementados com gordura, a inclusão de gorduras protegidas por cálcio reduz a CCS. Além disso, o fornecimento de gordura hidrogenada não protegida resulta em maior teor de proteína e caseína, bem como em maior concentração de BHB no sangue. No segundo projeto, o objetivo foi determinar a influência do análogo sintético da substância apaziguadora bovina (SAB) sobre a produção de leite, consumo alimentar e análises sanguíneas em vacas durante o período de transição. Vinte e quatro vacas da raça Holandês multíparas (do dia 28 pré-parto ao dia 21 pós-parto) foram distribuídas aleatoriamente em dois grupos: Controle (n=12) e SAB (n=12). Cada animal recebeu 5 mL da SAB nos dias 28 e 14 pré-parto e no dia do parto da região da nuca, por via tópica. O consumo

alimentar foi avaliado por meio de alimentadores inteligentes diariamente, assim como a produção de leite, que foi determinada eletronicamente. Seis amostras de leite foram coletadas de cada animal durante o período experimental para análise da composição química e CCS. Seis amostras de sangue foram coletadas por animal para futuras análises bioquímicas (AGNE, BHB, cortisol, mieloperoxidase e paraoxonase 1). As análises estatísticas foram conduzidas com o software JMP Pro 14, e o nível de significância adotado foi $P \leq 0,05$. As vacas tratadas com SAB apresentaram maior produção de leite ($P<0,05$) do que as do grupo Controle. O CMS nos períodos pré e pós-parto foi maior no grupo Controle do que no grupo tratado ($P<0,05$). O grupo SAB apresentou redução nos níveis plasmáticos de cortisol no pós-parto ($P<0,05$). Em conclusão, as vacas tratadas com SAB apresentaram maior produção de leite, menor CMS e menores concentrações plasmáticas de cortisol em comparação ao grupo controle, demonstrando o efeito da SAB em reduzir o estresse durante o período de transição.

Palavras-chaves: Ácido Palmítico, Ácido Esteárico, Ácido Oleico, Substância Apaziguadora Bovina, Bem-Estar.

Abstract

ARAÚJO, Maria Carolina Narval de. **Strategies for Dairy Farming: Effects of Including Protected Fat in the Diet and the Use of Pheromotherapy in Holstein Cows**. 2025. 64f. Thesis (Doctorate) - Postgraduate Program in Biotechnology. Federal University of Pelotas, Pelotas.

The use of protected fat in the diet of dairy cows, as well as the use of pheromone therapy to reduce management stress, are examples of tools that can be implemented in dairy cattle farming. In this study, two experiments were conducted with strategies that can be used to enhance animal performance. The objective of the first experiment was to evaluate the impact of a hydrogenated fat (H) rich in saturated fatty acids (SFA) (C16:0 and C18:0) and two calcium-protected fats (Ca) with different SFA (C16:0 and C18:0) and unsaturated fatty acid (UFA) profiles (cis-9 C18:1) compared to a control group (no fat supplementation) on dry matter intake, milk production, milk composition, as well as metabolic and zootechnical parameters of Holstein cows after peak lactation. The experimental period was divided into three periods of 22 days each. During each period, four animals per group were used, totaling 16 animals per period and 48 cows (n=48) in total at the end of the experiment. The groups were divided based on lipid composition: H-saturated (n=16), Ca-saturated (n=16), Ca-mix (n=16), and Control Group (n=16). Daily milk production was measured, as well as dry matter intake (DMI). Milk samples were collected twice a week for analysis of milk constituents (fat, lactose, protein, casein, urea nitrogen, and total solids) and somatic cell count (SCC). Blood samples were collected weekly for analysis of non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), albumin, triglycerides (TGL), urea, and glucose. Statistical analyses were conducted using JMP Pro 14 software, and the significance level adopted was $P \leq 0.05$. The Ca-mix and Ca-saturated groups, compared to the Control group, had lower milk protein content ($P<0.05$). SCC was lower in the treatment groups compared to the Control group ($P<0.05$). Regarding biochemical analyses, the H-saturated group had a higher concentration of BHB compared to the Ca-mix group ($P=0.05$), with no effect on the other parameters. No effects were observed on feed intake, milk production, body weight (BW), or body condition score (BCS). In conclusion, when comparing the three fat-supplemented groups, the inclusion of calcium-protected fats reduces SCC. Furthermore, the provision of unprotected hydrogenated fat results in higher protein and casein content, as well as a higher concentration of BHB in the blood. In the second project, the objective was to determine the influence of a synthetic analog of bovine calming substance (BCS) on milk production, feed intake, and blood analyses in cows during the transition period. Twenty-four multiparous Holstein cows (from 28 days prepartum to 21 days postpartum) were randomly assigned to two groups: Control (n=12) and BCS (n=12). Each animal received 5 mL of the BCS on days 28 and 14 prepartum and on the day of parturition, applied topically to the nape of the neck. Feed intake was assessed daily using smart feeders, as was milk production, which was electronically determined. Six milk samples were collected from each animal during the experimental period for chemical composition and SCC analysis. Six blood samples were collected from each animal for future biochemical analyses (NEFA, BHB, cortisol, myeloperoxidase, and paraoxonase 1). Statistical analyses were conducted using JMP Pro 14 software, and the significance level adopted was $P \leq 0.05$. Cows treated with BCS showed higher milk production ($P<0.05$) than the Control group. DMI in the prepartum and postpartum periods was higher in the Control group than in the treated group ($P<0.05$). The BCS group showed reduced plasma cortisol levels postpartum ($P<0.05$). In conclusion,

cows treated with BCS had higher milk production, lower DMI, and lower plasma cortisol concentrations compared to the control group, demonstrating the effect of BCS in reducing stress during the transition period.

Keywords: Palmitic Acid, Stearic Acid, Oleic Acid, Bovine Calming Substance, Welfare.

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Lista de Abreviaturas

ACTH - Hormônio Adrenocorticotrófico

AG – Ácidos Graxos

AGI - Ácidos Graxos Insaturados

AGNE - Ácidos Graxos Não Esterificados

AGPI – Ácidos Graxos Poliinsaturados

AGS – Ácidos Graxos Saturados

BAS – Bovine Appeasing Substance

BCS - Body Condition Score

BEA – Bem-estar Animal

BHB - Beta-hidroxibutirato

BHB - Beta-Hydroxybutyrate

BW – Body Weight

C16:0 – Ácido palmítico

C18:0 – Ácido Esteárico

C18:2 – Ácido Linoleico

C18:3 – Ácido Linolênico

Ca – Cálcio

Ca – Calcium

CCS - Contagem de Células Somáticas

cis-9 C18:1 – Ácido Oleico

CMS - Consumo de Matéria Seca

CP - Crude Protein

CRH - Hormônio Liberador de Corticotrofina

DIM - Days In Milk

DMI - Dry Matter Intake

ECC – Escore de Condição Corporal

EE - Ether Extract

FA - Fatty Acids

FDA - Acid Detergent Fiber

H – Hidrogenada

H – Hydrogenated

HHA – Hipotálamo-Hipófise-Adrenal

IMS – Ingestão de Matéria Seca

NDF - Neutral Detergent Fiber

NEFA - Non-Esterified Fatty Acids

NUN - Non-Urea Nitrogen

PC - Peso Corporal

RM - Mineral Residue

SAB – Substância Apaziguadora Bovina

SCC - Somatic Cell Count

SFA - Saturated Fatty Acids

SNC – Sistema Nervoso Central

SNS – Sistema Nervoso Simpático

TGL – Triglicerídeos

TGL – Triglycerides

TMR - Total Mixed Ration

UFA - Unsaturated Fatty Acids

Sumário

1 INTRODUÇÃO GERAL

A utilização de gordura protegida na dieta de vacas leiteiras, bem como o uso de substância apaziguadora bovina (SAB) para reduzir estresses de manejo são exemplos de ferramentas que podem ser implementadas na pecuária leiteira, com o objetivo de melhorar o desempenho produtivo e o bem-estar animal (BEA).

A suplementação com ácidos graxos (AG) é comumente usada para aumentar a densidade energética das dietas para vacas leiteiras (Bales *et al*., 2024). Tendo isso em vista, a administração de uma fonte de gordura protegida na dieta dos bovinos tornou-se uma estratégia para obter resultados positivos na produção e nos constituintes do leite (Weiss & Pinos-Rodríguez, 2009; Santos Neto *et al*., 2021).

Os lipídios são compostos de AG pertencentes a dois grupos: ácidos graxos saturados (AGS) e ácidos graxos insaturados (AGI). O estado de saturação é uma importante característica nutricional. Quimicamente, os AGS são aqueles sem duplas ligações na estrutura e os AGI apresentam duplas ligações (Lehninger, 2000). Além disso, dependendo da fonte alimentar, o tipo de AG pode variar, a maioria dos lipídios vegetais, como presentes em pastagens, é rico em AGI. Em cereais e na maioria das sementes oleaginosas, como caroço de algodão e grão de soja, há predominância de ácido linoleico (C18:2), enquanto em forragens o mais comum é o linolênico (C18:3) (Carneiro *et al*., 2017). Além disso, também podem ser fornecidos na forma de gordura protegida ou inerte, assim chamados por não interferirem na atividade microbiana do rúmen (Palmquist & Jenkins, 2017). Neste caso, o objetivo é proteger as estruturas dos AG da biohidrogenação para ser aproveitado em nível intestinal.

Estudos científicos examinaram os efeitos da inclusão de AG na alimentação de vacas leiteiras e observaram que estes dependem da dieta fornecida, nível de produção dos animais e perfil lipídico do produto (Rabiee *et al*., 2012). As fontes de gordura protegida geralmente contêm altas concentrações de AG de cadeia longa, sendo os mais comuns o palmítico (C16:0), esteárico (C18:0), oleico (C18:1) e linoléico (C18:2) (Loften *et al*., 2014). Diante disso, é importante conhecer o perfil de AG predominante nos produtos, pois dependendo da composição lipídica pode haver influência no consumo alimentar, na produção, bem como nos teores de sólidos do leite.

O suplemento de gordura ideal não tem efeito sobre a fermentação ruminal ou no consumo, apresenta alta digestibilidade intestinal e aumenta a produção de leite e de seus constituintes. Entretanto, alguns produtos diminuem a digestibilidade da fibra (Palmquist & Jenkins, 1980), diminuem o consumo alimentar (Allen, 2000) ou alteram a biohidrogenação dos ácidos graxos (Beam *et al*., 2000).

Especificamente, os AGI frequentemente resultam em uma maior diminuição na ingestão em comparação com fontes de gordura saturada (Relling

& Reynolds, 2007) e estão associados à produção ruminal de AG intermediários que induzem à depressão da gordura do leite (Harvatine *et al*., 2009). Por isso, os AGS protegidos da degradação ruminal se tornam uma alternativa para suplementação. Suplementos de C16:0 inertes ao rúmen ou C18:0, influenciaram o conteúdo de gordura do leite e também modificaram seu perfil de AG (Grummer *et al*., 1995; Harvatine & Allen, 2005), além de aumentarem a produção de leite (Mosley *et al*., 2007; Warntjes *et al*., 2008).

Outra estratégia, a utilização de feromonioterapia em animais de produção, tem ganhado atenção como uma ferramenta para melhorar o BEA, reduzir o estresse e, consequentemente, aumentar a produtividade. Os feromônios são compostos químicos com um papel fundamental na comunicação entre os indivíduos de uma mesma espécie (Pickett *et al*., 2014). A SAB, o análogo da substância apaziguadora bovina, vem ganhando espaço como um modulador do estresse de bovinos. Diferentemente da inclusão de gordura protegida, a administração de SAB em bovinos ainda é limitada, embora se saiba o impacto negativo do estresse nos índices produtivos.

Esse feromônio é secretado pela vaca logo após o parto, produzido numa área específica entre as duas cadeias mamárias (Osella *et al*., 2018; Cappellozza & Cooke, 2022), sendo detectado pelo órgão vomeronasal (ou de Jacobson), localizado na extremidade rostral do palato duro dentro da cavidade nasal (DePorter, 2016). Pageat (2001) isolou pela primeira vez na glândula mamária de porcas o feromônio apaziguador suíno, um análogo sintético que mimetiza a secreção liberada pela fêmea suína lactante. Posteriormente, identificou-se efeitos positivos nos animais que receberam a substância e então começou a investigação dos feromônios apaziguadores nas demais espécies mamíferas (Pageat e Gaultier, 2003; Gunn-Moore *et al*., 2004; Tod *et al*., 2005; Hargrave, 2014; Osella *et al*., 2018).

Há alguns estudos que aplicaram a SAB em bezerros no período de desmame, descorna e castração, além do uso em bois em confinamento e em transporte para abate (Angeli *et al*., 2020; Cappellozza *et al*., 2020; Cooke *et al*., 2020; Schubach *et al*., 2020). Em todos os trabalhos, é possível observar efeitos positivos do tratamento (Osella *et al*., 2018; Cooke *et al*., 2020; Schubach *et al*., 2020; Hervet *et al*., 2021). Porém, para vacas em período de transição ainda não foi realizado nenhum estudo. Acredita-se que a administração desta substância tem potencial para atenuar os efeitos negativos deste período crítico no metabolismo das vacas de alta produção.

Portanto, essas ferramentas representam a oportunidade de aprimoramentos no sistema produtivo leiteiro, focando tanto na nutrição quanto no BEA, pilares cruciais da bovinocultura leiteira. Acredita-se que, com o uso dessas estratégias, os animais terão melhor desempenho na produção de leite e maior longevidade.

1.1 Uso de gordura protegida na dieta de vacas leiteiras

A suplementação com ácidos graxos (AG) é uma estratégia alimentar para aumentar a energia dietética para vacas leiteiras (Bales *et al*., 2024). Tendo isso em vista, a administração de uma fonte de gordura protegida tornou-se comum para obter resultados positivos no organismo animal (Weiss & Pinos-Rodríguez, 2009; Santos Neto *et al*., 2021).

Os lipídios são compostos de AG pertencentes a dois grupos: AGS e AGI. O estado de saturação é uma importante característica nutricional. Quimicamente, os AGS são aqueles sem duplas ligações na estrutura e os AGI apresentam duplas ligações (Lehninger, 2000). Além disso, dependendo da fonte alimentar, o tipo de AG pode variar, a maioria dos lipídios vegetais, como presentes em pastagens, é rico em AGI. Em cereais e na maioria das sementes oleaginosas, como caroço de algodão e grão de soja, há predominância de ácido linoleico (C18:2), enquanto em forragens o mais comum é o alfa-linolênico (C18:3) (Carneiro *et al*., 2017). Além disso, também podem ser fornecidos na forma de gordura protegida ou inerte, assim chamados por não interferirem na atividade microbiana do rúmen (Palmquist & Jenkins, 2017). Neste caso, o objetivo é proteger as estruturas dos AG da biohidrogenação para ser aproveitado em nível intestinal.

O processo de digestão dos lipídeos consiste em duas principais etapas: lipólise e biohidrogenação de AGI. O processo de lipólise consiste na quebra das ligações éster entre os AG e o glicerol, e é realizado pelas enzimas microbianas lipolíticas tendo como consequência a formação de AG livres e glicerol (Jenkins, 1993). Essa etapa é predominantemente realizada pelas bactérias ruminais, sendo geralmente alta (>85%) e pode ser influenciada por alguns fatores, como o nível de gordura na dieta, o pH ruminal e a utilização de ionóforos, que podem inibir a atividade e crescimento de bactérias (Doreau & Chilliard, 1997).

As taxas de lipólise e biohidrogenação dependerão da quantidade e do tipo de fonte lipídica (Beam *et al*., 2000) e do pH ruminal (Van Nevel & Demeyer, 1995). As bactérias do rúmen são sensíveis aos ácidos graxos poliinsaturados (AGPI) e, portanto, necessitam convertê-los em AGS por meio do processo de biohidrogenação. No entanto, o grau dessa etapa pode variar de 60 a 90%, sendo encontrado valores médios de 70% (Zinn *et al*., 2000), o que resulta na formação de intermediários. Ao final do processo de biohidrogenação, o C18:0 é o principal produto e o C16:0 é o AGS mais abundante no tecido adiposo e na gordura do leite (Palmquist *et al*., 2006; Tzompa-Sosa *et al*., 2014).

Estudos científicos examinaram os efeitos da inclusão de AG na alimentação de vacas leiteiras e observaram que estes dependem da dieta fornecida, nível de produção dos animais e perfil lipídico do produto (Rabiee *et al*., 2012). As fontes de gordura protegida geralmente contêm altas concentrações de AG de cadeia longa, sendo os mais comuns o palmítico (C16:0), esteárico (C18:0), oleico (C18:1) e linoléico (C18:2) (Bales *et al*., 2024). Diante disso, é importante conhecer o perfil de AG predominante nos produtos,

pois dependendo da composição lipídica pode haver influência no consumo alimentar, na produção, bem como nos teores de sólidos do leite.

Pesquisas demonstraram que a oferta de C16:0 aumenta a partição de energia para o leite, promovendo impacto positivo na produção de leite e gordura do leite (Souza & Lock, 2018b; Souza *et al*., 2019b). Por outro lado, as respostas positivas de produção à suplementação de C18:0 não foram consistentes, o que pode estar relacionado à diminuição da digestibilidade de AG à medida que a suplementação de C18:0 aumenta (Piantoni *et al*., 2015; Boerman *et al*., 2017)

O suplemento de gordura ideal não tem efeito sobre a fermentação ruminal ou no consumo, apresenta alta digestibilidade intestinal e aumenta a produção de leite e de seus constituintes. Entretanto, alguns produtos diminuem a digestibilidade da fibra (Palmquist & Jenkins, 1980), diminuem o consumo alimentar (Allen, 2000) ou alteram a biohidrogenação dos ácidos graxos (Beam *et al*., 2000). Entender os efeitos de diferentes fontes de AG no metabolismo animal é importante, e ultimamente tem sido dada atenção à determinação dos impactos de AG individuais específicos (Souza *et al*., 2019b).

De Souza *et al*. (2019a) e Western *et al*. (2020) ao comparar as proporções de C16:0 + cis-9 C18:1, observaram que o aumento da proporção de cis-9 C18:1 em uma mistura de AG aumentou a produção de leite de vacas de maior produção, enquanto o aumento da proporção de C16:0 aumentou a produção de leite de vacas de menor produção. Pesquisas anteriores usando semente de algodão para aumentar o conteúdo basal de AG relataram um efeito positivo na produção de leite (Santos Neto *et al*., 2021), nenhuma diferença (Rico *et al*., 2014) ou uma diminuição (Souza *et al*., 2018a). Um estudo recente no qual as vacas receberam como dieta basal alto ou baixo teor de gordura misturado ao caroço de algodão demonstrou que os suplementos de AG (contendo 80% C16:0 + 10% cis- 9 C18:1 ou 60% C16:0 + 30% cis- 9 C18:1) aumentaram as respostas de produção em comparação com nenhuma suplementação de AG, independentemente da dieta basal (Bales *et al*., 2024).

De acordo com Souza *et al*. (2021), ao aumentar a porcentagem de cis-9 C18:1 na dieta de vacas no início da lactação houve incremento na IMS e redução das perdas de PC e ECC. Para Piantoni *et al*. (2015), a suplementação com C18:0 aumentou a IMS. Já para Bales *et al*. (2024), vacas suplementadas com 80% C16:0 + 10% cis- 9 C18:1 tiveram maior CMS em comparação a vacas suplementadas com 60% C16:0 + 30% cis- 9 C18:1. Ao fornecer sabões de cálcio de óleo de palma, Santos Neto *et al*. (2021) encontraram diminuição da IMS. De fato, o efeito da gordura dietética rica em AGI pode ter impacto na secreção de hormônios intestinais e peptídeos que inibem o esvaziamento gástrico, o que pode diminuir o consumo (Holst, 1997; Benson & Reynolds, 2001; Relling & Reynolds, 2007).

Dados anteriores sobre a resposta da gordura do leite à suplementação de AGS têm sido inconsistentes. Estudos relataram tanto um aumento na concentração de gordura do leite quanto nenhum efeito (Piantoni *et al*., 2015;

Mathews *et al*., 2016; Souza & Lock, 2019a; Shepardson & Harvatine, 2021). Prom & Lock (2021) suplementaram a dieta com cis-9 C18:1 e observaram uma diminuição na concentração de gordura do leite, sem efeito na produção total de gordura. Isso é atribuído à produção de ácidos graxos intermediários que resistem à biohidrogenação ruminal (Bauman *et al*., 2011). De acordo com Bales *et al*. (2024), vacas suplementadas com 80% C16:0 + 10% cis- 9 C18:1 tenderam a diminuir o teor de gordura do leite em comparação com vacas suplementadas com 60% C16:0 + 30% cis- 9 C18:1.

No estudo de Souza *et al*. (2021), no qual os tratamentos foram dieta controle não suplementada com AG; dieta suplementada com mix de AG contendo 80% de C16:0 e 10% de cis -9 C18:1; dieta suplementada com mix de AG contendo 70% de C16:0 e 20% de cis -9 C18:1; e dieta suplementada com mix de AG contendo 60% de C16:0 e 30% de cis -9 C18:1, as vacas que receberam AG independente da concentração, tiveram maior teor de lactose do leite. Em relação aos teores de gordura e proteína do leite, não foram observadas diferenças entre os tratamentos (Souza *et al*., 2021).

Sendo assim, a suplementação de AG na dieta de vacas leiteiras tem demonstrado diversos efeitos sobre a produção e composição do leite, com resultados que variam de acordo com a fonte lipídica, tipo de AG e perfil da dieta fornecida. A compreensão do impacto específico de diferentes tipos de AG, como o cis-9 C18:1 e o C16:0, é fundamental para otimizar as estratégias nutricionais, visto que podem influenciar tanto a produção quanto a composição do leite de forma diferenciada. Além disso, a escolha adequada da fonte de gordura protegida, que não interfere na fermentação ruminal e promove maior digestibilidade intestinal, é essencial para maximizar os benefícios da suplementação lipídica.

1.2 Substância Apaziguadora bovina: as implicações do estresse nas vacas de leite e o uso da SAB como moduladora de eventos estressantes

Atualmente, muito se tem falado de BEA nos mais diferentes setores da medicina veterinária, e no setor pecuário não seria diferente. A inclusão do ensino de BEA no Brasil ganhou notoriedade, pois havia cobrança por parte da população aos órgãos de fiscalização a fim de que estes garantissem aos animais proteção e qualidade de vida (Chebel *et al*., 2016).

A ciência do BEA é uma área interdisciplinar com o objetivo de estudar, identificar e reconhecer as exigências básicas dos animais. Ainda, estabelecer o nível em que as necessidades físicas, fisiológicas, sociais, comportamentais e ambientais estão sendo atendidas (Keeling *et al*., 2011). Dessa forma, “O Modelo dos Cinco Domínios”, se fundamenta nesse princípio, consistindo numa metodologia de avaliação e gerenciamento, considerando cinco domínios: “Nutrição”, livre de sede, fome e má nutrição (Domínio 1); “Ambiente”, livre de

desconforto (Domínio 2); “Saúde”, livre de dor, ferimentos e doenças (Domínio 3); “Comportamento”, livre de medo e estresse (Domínio 4); e “Mental” (Domínio 5) (Ceballos & Sant’anna, 2018). Quando essas condições são atendidas, o BEA é otimizado, resultando em animais saudáveis, com menos estresse e maiores níveis de produtividade e qualidade de vida.

O conceito de estresse foi introduzido pela primeira vez pelo endocrinologista Hans Selye, como uma resposta inespecífica do organismo a diferentes estímulos a fim de manter sua homeostase (Sordillo, 2022). As respostas ao estresse envolvem ativação do sistema nervoso simpático (SNS), sistema nervoso central (SNC) e eixo hipotálamo-hipófise-adrenal (HHA) (Dhabhar, 2014; Deak *et al*., 2017).

Quando o eixo HHA é estimulado, o hipotálamo secreta o hormônio liberador de corticotrofina (CRH) e em resposta, a hipófise secreta o hormônio adrenocorticotrófico (ACTH). Isso, por sua vez, estimula a secreção de cortisol do córtex das glândulas adrenais (Zhang *et al*., 2021). O cortisol é um hormônio liberado em condições estressantes e quando ele entra na corrente sanguínea, ativa a resposta antiestresse e anti-inflamatória.

À medida que o estresse se torna maior em magnitude ou mais prolongado em duração, a perda da homeostase pode ser deletéria. Sendo assim, a mobilização de processos fisiológicos para garantir a recuperação do organismo frente ao evento estressor configura-se num custo de longo prazo para o animal (Deak *et al*., 2015). A perda da homeostase em decorrência do estresse tem efeitos prejudiciais no gado leiteiro, incluindo supressão imunológica, diminuição da ingestão alimentar, alteração da função hepática e liberação prejudicada de hormônios reprodutivos, afetando, por sua vez, o desempenho produtivo e reprodutivo (Earley *et al*., 2012; Sordillo & Raphael, 2013; Bishop-Williams *et al*., 2015; Curtis *et al*., 2017).

A demonstração de que mediadores inflamatórios são frequentemente produzidos em resposta a eventos estressantes, nos quais não há agentes patogênicos aparentemente envolvidos, demonstra que há ativação do sistema imunológico (Deak *et al*., 2015). Diversos mediadores inflamatórios surgiram como altamente responsivos ao estresse, por exemplo, em bovinos níveis elevados de haptoglobina são diretamente relacionados a distocia, retenção de placenta, metrite ou estresse metabólico periparturiente (Huzzey *et al*., 2009; Pohl *et al*., 2015).

Já os estressores sociais parecem aumentar a liberação de interleucina-6 (IL-6), tanto no plasma quanto no cérebro de camundongos (Hodes *et al*., 2014; Wood *et al*., 2015). Além de algumas das citocinas clássicas (como IL-1β, IL-6, fator de necrose tumoral alfa [TNF-α]), evidências sugerem que as quimiocinas também são alteradas por desafios de estresse (Wohleb *et al*., 2013). Além disso, as prostaglandinas surgiram como mediadores inflamatórios sensíveis ao estresse e, em função do papel que desempenham como mediadores da resposta febril, as prostaglandinas também são provavelmente mediadoras de

respostas febris induzidas pelo estresse (Furuyashiki *et al*., 2011). Assim, uma gama de sinalização inflamatória é mobilizada por causa de eventos estressantes e impactam significativamente na função do SNC.

Especificamente em bovinos, o estresse impacta no desempenho produtivo e reprodutivo, bem como na saúde do rebanho. Dentre as situações estressantes a que os bovinos estão expostos, o transporte, a restrição alimentar e hídrica, bem como o desmame são reconhecidos como os principais desencadeadores da ativação do eixo HHA e de proteínas de fase aguda (Carroll & Forsberg, 2007; Marques *et al*., 2012).

A SAB é um feromônio sintético análogo ao secretado pela vaca logo após o parto (Osella *et al*., 2018; Cappellozza & Cooke, 2022). Os feromônios estão presentes nos fluidos corporais dos animais, são detectados pelo órgão vomeronasal (ou de Jacobson), localizado na extremidade rostral do palato duro dentro da cavidade nasal (DePorter, 2016). Pageat (2001) isolou pela primeira vez na glândula mamária de porcas o feromônio apaziguador suíno, um análogo sintético que mimetiza a secreção liberada pela fêmea suína lactante. Alguns estudos utilizaram este feromônio em leitões desmamados e observaram efeitos positivos nos animais que receberam a substância, como diminuição da agressividade e lesões, aumento no consumo, assim como na conversão alimentar (Guy *et al*., 2009). A partir de então, começou a investigação dos feromônios apaziguadores nas demais espécies mamíferas, como cães, gatos e bovinos (Pageat & Gaultier, 2003; Gunn-Moore *et al*., 2004; Tod *et al*., 2005; Hargrave, 2014; Osella *et al*., 2018).

Há alguns estudos que aplicaram a SAB em bezerros no período de desmame, descorna e castração, além do uso em bois em confinamento e em transporte para abate (Angeli *et al*., 2020; Cappellozza *et al*., 2020; Cooke *et al*., 2020; Schubach *et al*., 2020). No estudo de Angeli et al. (2020), bezerras Gir × Holandesas receberam SAB a cada 14 dias e tiveram maior PC no desmame e tendência a ter maior PC no dia 56 em comparação a bezerras que não receberam o feromônio. Da mesma forma, o ganho médio diário de peso foi maior para o grupo SAB dos dias 42 a 56 e tendeu a ser maior dos dias 56 ao desmame quando comparado com o grupo controle. Sendo assim, neste estudo pode-se afirmar que a administração de SAB melhorou o crescimento de bezerras recém-desmamadas. A resposta tardia observada no desempenho pode estar relacionada à ocorrência de diarreia e pneumonia observadas nos animais. Pode-se especular que as ocorrências de diarreia e pneumonia podem ter limitado a ação da SAB no desempenho durante os primeiros períodos da vida dos animais, mas colaboraram para diminuir a gravidade e a quantidade de tratamentos farmacológicos necessários para recuperar a saúde das bezerras.

Recentemente, ao usar o feromônio em bezerros desmamados, Alvaréz et al. (2025) observou que o ganho médio diário de peso dos tratados com SAB foi 0,150 kg maior em comparação com bezerros tratados com placebo. É sabido que o desmame tem um efeito prejudicial no ganho de peso e que pode elevar o

cortisol salivar (Black et al., 2017), fato este não encontrado neste experimento. Entretanto, bezerros tratados com placebo tenderam a apresentar maiores níveis de cortisol salivar após o desmame completo em comparação com bezerros parcialmente desmamados, o que não foi encontrado em bezerros tratados com SAB. Isso provavelmente demonstra o efeito de atenuação do feromônio. Ao mesmo tempo, o cortisol salivar tendeu a atingir o pico, especialmente após o desmame em bezerros que receberam o placebo, reforçando a hipótese de que a SAB pode realmente reduzir a resposta endócrina ao estresse ao desmame (Alvaréz et al., 2025).

No estudo de Schubach *et al*. (2020), foram avaliados os impactos da administração da SAB a bezerros de corte no desmame no desempenho, respostas fisiológicas e comportamento durante um programa de pré-condicionamento de 42 dias. O ganho médio diário do dia 0 ao 42 não teve diferença, mas foi maior em bezerros SAB do dia 0 ao 28. Já as concentrações de cortisol capilar foram maiores em bezerros controle no dia 14. Neste experimento concluiu-se que a administração de SAB a bezerros aliviou as reações fisiológicas induzidas pelo estresse, além de aumentar a imunidade humoral adquirida pela vacinação contra doenças respiratórias.

Nos demais trabalhos, é possível observar efeitos positivos do tratamento, como maior ganho médio diário de peso e peso final em bezerros (Cooke *et al*., 2020), bem como na resposta imune de bois diagnosticados com doenças respiratórias no período de engorda (Hervet *et al*., 2021). No estudo de Hervet *et al*. (2021), foram utilizados 265 bois de engorda da raça Charolês e foi administrada a SAB no início do período de engorda a fim de avaliar parâmetros zootécnicos e de saúde ao longo de algumas semanas. Sabe-se que o período de engorda em bovinos de corte é uma das fases mais complexas dentro do ciclo de produção. O estresse nessa situação tem um papel crucial no desencadeamento de infecções respiratórias por meio de seus efeitos deletérios nas defesas imunológicas (Hervet *et al*., 2021). Nos animais tratados com o feromônio, houve aumento dos sinais clínicos no dia 8 e redução dos sinais clínicos no dia 30 em bois que receberam o feromônio e uma maior expressão de transcritos de interleucina-8 neste grupo do que no grupo controle no dia 8. Os autores concluíram que há um potencial dos feromônios apaziguadores como uma abordagem complementar no manejo de bois jovens em unidades de engorda a fim de melhorar o status imunológico.

Em vacas de leite, Osella *et al*. (2018) investigou os efeitos da SAB em vacas leiteiras em troca de sistema de alojamento. A produção diária de leite foi maior no grupo SAB do que no grupo controle e a CCS foi maior nas vacas que não receberam o feromônio, sem impacto na composição química do leite. Os autores concluíram que o uso de SAB parece modular a adaptação de formas que podem incrementar o desempenho das vacas quando há mudanças nas rotinas de manejo. Porém, para vacas em período de transição ainda não foi realizado nenhum estudo. Acredita-se que a administração desta substância tem

potencial para atenuar os efeitos deletérios deste período crítico no metabolismo das vacas de alta produção, visto que a SAB apresentou efeitos positivos no metabolismo energético e sistema imune em estudos já publicados com outros delineamentos experimentais (Osella *et al*., 2018; Angeli *et al*., 2020; Hervet *et al*., 2021).

O periparto é extremamente complexo, no qual as vacas leiteiras enfrentam diversos desafios desde o final da gestação até o início da lactação. Dentre esses obstáculos estão a regulação endócrina coordenada para o crescimental fetal, o processo de renovação da glândula mamária e a preparação para o parto. Esses processos fisiológicos alteram a partição de nutrientes entre as diferentes funções corporais na vaca periparturiente (Horst *et al*., 2021; Trevisi *et al*., 2025). Atrelado a isso, os animais apresentam redução no consumo alimentar (Drackley *et al*., 2005; Schoenberg *et al*., 2012; Marett *et al*., 2015) e a mobilização das reservas corporais é a consequência para este período (Drackley, 1999). Além disso, os animais passam por uma disfunção imunológica (Bradford & Swartz, 2020) que pode prejudicar a saúde da vaca no período pós-parto (Caixeta *et al*., 2017), bem como interferir na saúde do neonato e na produção de leite (Abuelo, 2020).

No entanto, por mais que a mobilização das reservas lipídicas seja necessária, o aumento da lipólise periparto afeta o sistema imunológico (Trevisi *et al*., 2025). Dessa forma, as vacas leiteiras são as que mais apresentam episódios de doenças infecciosas e distúrbios metabólicos durante essa fase (Sepúlveda-Varas et al., 2015). Aliado a isso, o estresse presente nos manejos dos animais diariamente, assim como o estresse oxidativo advindo desse período periparturiente (Trevisi *et al*., 2025), podem influenciar negativamente neste período (Sordillo, 2022). Sendo assim, é necessário atenuar os efeitos negativos que podem estar associados, como por exemplo, o uso de tecnologias que promovam o bem-estar das vacas leiteiras, uma vez que as vacas são mais suscetíveis a doenças durante o período de transição (Ceciliani *et al*., 2018).

A avaliação dos níveis de estresse em bovinos lactantes é uma ferramenta importante para monitorar o bem-estar, identificar potenciais fontes de estresse e desenvolver estratégias de manejo para mitigar os efeitos negativos do estresse (De Passillé *et al*., 2005). Sendo assim, a SAB configura-se como um recurso para atenuar os efeitos negativos do estresse nos bovinos.

2 HIPÓTESE E OBJETIVOS

2.1 Efeito de gorduras protegidas e gordura hidrogenada no consumo, produção, composição do leite e parâmetros zootécnicos e metabólicos de vacas da raça Holandês

Hipótese

A suplementação de uma fonte de gordura protegida a base de ácido esteárico e palmítico superará a produção de leite quando comparado com outras fontes de gorduras, tanto na forma de sais de cálcio, quanto na forma hidrolisada.

Objetivos

O objetivo do presente estudo foi avaliar o impacto da suplementação de três fontes de gordura protegida no consumo, produção e componentes do leite de vacas da raça Holandês após o pico de lactação.

2.2 Efeito da substância apaziguadora bovina em vacas multíparas da raça Holandês durante o período de transição

Hipótese

Os animais que receberem o tratamento durante o período de transição, terão menores níveis de cortisol e consequentemente, terão melhor desempenho pós-parto, o que repercute positivamente na saúde e na produtividade.

Objetivos

O objetivo do presente trabalho foi avaliar os efeitos da substância apaziguadora bovina (SAB) no desempenho, metabolismo energético, estresse e inflamação de novilhas e vacas leiteiras durante o período de transição.

Quanto aos objetivos específicos, estes foram avaliar os efeitos da SAB:

- No consumo e comportamento alimentar;
- Na produção e composição do leite;
- Em biomarcadores sanguíneos do metabolismo energético e relacionados aos mecanismos inflamatórios;
- Além do biomarcador de estresse e bem-estar animal.

3 MANUSCRITOS

4.1 Manuscrito 1 – Effect of protected and hydrogenated fats on feed consumption, milk production, composition, zootechnical and metabolic parameters in Holstein cows

Manuscrito submetido à revista *Acta Veterinaria Brasilica*

Effect of protected and hydrogenated fats on feed consumption, milk production, composition, zootechnical and metabolic parameters in Holstein cows

ABSTRACT- The study evaluated the effects of a hydrogenated fat rich in saturated fatty acids (SFA) (C16:0 and C18:0) and two calcium salts-protected fats with varying SFA (C16:0 and C18:0) and unsaturated fatty acid (UFA) (cis-9 C18:1) profiles on Holstein cows post-peak lactation. Forty-eight cows were assigned to four groups: (I) Control without fat supplementation (n=12); (II) calcium salts-protected fats with palmitic and oleic acids (Ca-mix, n=12); (III) calcium salts-protected fats rich in SFA (Ca-saturated, n=12); and (IV) hydrogenated fat rich in SFA (H-saturated, n=12). Diets were fed for 22 days, with fats included at 1.5% of dry matter (DM). Milk production and DM intake (DMI) were measured, and milk samples were analyzed for constituents (fat, protein, casein, lactose, urea nitrogen, total solids) and somatic cell count (SCC). Blood samples were analyzed weekly for non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), albumin, triglycerides, urea, and glucose. Milk protein content was lower in Ca-mix and Ca-saturated groups compared to Control ($P<0.05$). SCC decreased in all fat-supplemented groups ($P<0.05$). The H-saturated group showed higher BHB levels than the Ca-mix group ($P=0.05$), with no significant differences in other biochemical parameters. No effects were observed on feed intake, milk yield, body weight, or body condition score. In conclusion, calcium-protected fats reduce SCC, while hydrogenated fat enhances milk protein, casein, and BHB levels.

Keywords: SCC; milk protein; palmitic acid; stearic acid; oleic acid.

INTRODUCTION

Supplementation of protected fatty acids (FA) has become a alternative to vegetable fats commonly used in diet formulations for dairy cows. These fats contribute energetically and have structural, metabolic, and physiological effects on the animals (Palmquist & Jenkins, 2017). Including FA in the diet of dairy cows increases its energy density and milk production, while also impacting milk composition (Rabiee *et al.*, 2012; Western *et al.*, 2020). However, the effects of FA supplementation depend on the overall diet, FA profile of the product employed, and milk productivity (Mathews *et al.*, 2016; Western *et al.*, 2020).

Palmitic (C16:0), stearic (C18:0), and oleic (cis -9 C18:1) acids are the main FAs in milk, adipose tissue, and most commercially available supplements (Palmquist *et al.*, 2006). Commercial saturated fatty acids (SFA) products may differ in the concentration of C16:0 and C18:0, which may affect intake, milk production, and digestibility (Mathews *et al.*, 2016). However, compared to the unsaturated FAs (UFA) commonly found in vegetable fats, supplementation with SFAs is less likely to change the rumen environment and reduce feed intake (Allen, 2000). Moreover, SFA supplementation can increasing milk production, as well as milk fat and protein (Hu *et al.*, 2017). Nonetheless, the impact on these parameters and that on dry matter intake (DMI) and metabolism is variable (Lock *et al.*, 2013).

A wide variety of lipid supplements are protected from ruminal degradation. The main advantage of this is reducing the deleterious effect of SFA on ruminal microorganisms (Jenkins, 1993) and increasing nutrient utilization. Protected fat sources usually contain high concentrations of long-chain FAs, most commonly C16:0, C18:0, C18:1, and linoleic acid (C18:2) (Loften *et al.*, 2014).

The aim of this study was to evaluate the impact of the supplementation of a hydrogenated fat (H) with different SFA and two fats protected with calcium salts (Ca) with different SFA and UFA on feed consumption, milk production and composition, zootechnical and metabolic parameters of Holstein cows after peak lactation.

MATERIALS AND METHODS

All animal procedures were approved by the Animal Ethics and Experimentation Committee of University XXX under code 030768/2021-07.

Location and Facilities

The experiment was conducted on a commercial property located in the south of Brazil. Cows were housed in a confined compost barn system, receiving total mixed ration (TMR) in automatic feeders twice a day.

Group separation and experimental design

The animals (n=48) were randomly distributed into four groups: Control with no supplemental fat (n=12), fed with the basal diet, and 3 fat supplement treatments fed at 1.5% of diet dry matter (DM) of a product commercial mix of palmitic acid and oleic acid (Ca-mix (n=12), with 33.60% C16:0, 26.48% cis-9 C18:1); a product commercial high level of satured fatty acids (Ca-satured (n=12), with 49.37% C16:0, 40.14% C18:0), and a product commercial high level of satured fatty acids (H-satured (n=12), with 41.76% C16:0, 45.86 C18:1). The Ca-mix and Ca-saturated groups received a supplement of calcium soap-protected fat, while the H-saturated group received a supplement of unprotected hydrogenated fat. The cows were

selected according to the days in milk (DIM) (90–150 days, mean DIM 114, 100, 106, and 114, for the Ca-mix, Ca-saturated, H-saturated, and Control groups, respectively), milk yield in the two weeks prior to the experiment (≥ 30 kg/day), lactation order (2^{nd}– 4^{th} parturition), and body condition score (BCS 2.75–3.75).

The cows had received the TMR without fat for seven days before the start of supplementation. After this period, the supplements were administered mixed with the diet directly in the feeders of each treatment group at the time when the diet was provided to the animals. Fat supplements were provided twice a day, in the morning and in the afternoon. The following amounts were used for each supplement: 300 g/day/animal of Ca-mix, 250 g/day/animal of Ca-saturated, and 250 g/day/animal of H-saturated. Fat was added to the base TMR rather than replacing a single ingredient. The analyses of the fatty acid profile of commercial products was carried out in a private laboratory. The FA profile of each product and diet composition are shown in table 1 and 2, respectively.

Table 1 - Fatty acid profile of the three fat supplements provided to Holstein cows at their lactation peak over 22 days

FA (g/100 g)	**Supplements**		
	Ca-mix	**Ca-saturated**	**H-saturated**
C16:0	33,60	49,37	41,76
C18:0	03,60	40,14	45,86
cis-9 C18:1	26,48	0,85	00,23
Other SFAs	04,50	05,49	02,08
Other UFAs	05,13	02,80	0,16
Total SFA	41,70	95,00	89,70
Total UFA	31,61	03,65	0,39
Total FAs	73,31	98,65	90,09

Abbreviations: FA, fatty acids; C16:0, palmitic acid; C18:0: stearic acid; *cis-9* C18:1: oleic acid; SFA, saturated fatty acid; UFA, unsaturated fatty acid.

Table 2 - Bromatological composition of the TMR and of silage and haylage provided to Holstein cows after the lactation peak over 22 days

	Ingredients		
Composition	***TMR*[1]**	***Silage***	***Haylage***
Final dry matter	92,93	93,16	88,93
Mineral matter	07,07	05,23	11,13
Organic matter	92,93	94,76	88,88
Neutral detergent fiber	40,99	45,84	52,10
Acid detergent fiber	23,59	31,58	38,81
Total protein	16,04	08,93	13,16
Ether extract	04,73	08,28	01,70

[1]TMR: total mixed ration.

Feed sample collection and bromatological analyses

In the course of the experimental period, corn silage and pre-dried ryegrass samples were collected two days a week to form a weekly pool. Apart from this, the TMR samples from the experimental batches were also gathered every day of the week. The samples were maintained in a frozen state for bromatological analyses to be performed at a later date (dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (FDA), ether extract (EE), mineral residue (RM)).

The food samples were analyzed at an animal nutrition laboratory. Initially, the foods underwent a 72-hour pre-drying process in a forced-air circulation oven at 55 °C. Subsequently, the samples were ground in a wiley knife mill through a 1mm sieve. For the definitive analysis of dry matter, the ground samples were placed in an

oven at 105°C for a minimum of 8 hours (Easley *et al.*, 1965). For mineral analysis, 1g of ground sample was weighed in triplicate and placed in a muffle furnace at 600 °C, where it remained for 3 hours to burn off the organic residue (Cunniff, 1995). Subsequently, the obtained value was subtracted, and crude mineral matter was defined.

In order to determine the crude protein, an analysis was done adopting the method Kjeldhal (Cunniff, 1995) for nitrogen determination, but modified by the addition of 4% (w/v) boric acid solution as a free ammonia receptor during distillation, a solution of 0.2% (w/v) bromocresol green, 0.1% (w/v) and methyl red as the indicator and a standard sulfuric acid solution for titration, as explained by Kozloski *et al.* (2003). Next, the Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) analyses were performed according to Van Soest & Robertson (1985). The average chemical composition of the diets provided to the animals during the experimental phase is clear from table 2.

Feed consumption

Feed consumption data were obtained daily, individually, and automatically, using smart feeders. A sample of the offered TMR was collected daily and dried using an Air Fryer to convert the intake of as-fed material obtained by the smart feeders into dry matter intake (DMI). For this, we weighed 100 g of the sample and dried it at 105 °C for 30 minutes. The dried sample was weighed, and the value was recorded. Subsequently, the sample was dried for an additional 5 minutes and weighed again. If both weights were equal for the dried sample, this was considered the dry matter value. Otherwise, additional drying cycles of 5 minutes each were performed until at least two equal weight values were obtained (Nino *et al.*, 2021). The diet composition and nutritional levels were in accordance with the National Research Council (NRC, 2001).

Milk production and composition analysis

Cows were milked three times a day. Milk production was measured electronically using the DelPro™ software (DeLaval®, Tumba, Botkyrka, Sweden), and all milkings were monitored in person.

Milk samples were retrieved on two days per week from three consecutive milkings on each day, totaling six samples/cow. The samples were stored at 4°C then sent to a laboratory for the analysis of milk quality. Milk composition (fat, lactose, protein, non-urea nitrogen (NUN), total solids; spectrophotometric analysis) and somatic cell count (SCC; flow cytometry) were analyzed in this laboratory.

Blood analysis

Blood samples were obtained weekly via puncture of the coccygeal arteriovenous complex, totaling four samples/cow. Serum and plasma samples were obtained for analysis of non-esterified fatty acids (NEFA), beta hydroxybutyrate (BHB), albumin, triglycerides (TGL), urea, and glucose using commercial kits. Analyses were performed using an automatic biochemical analyzer.

Clinical and zootechnical evaluations

Clinical examinations were performed on all animals weekly to assess overall health, body weight (BW), and BCS. The BCS was determined by a trained evaluator using a 1–5 scale in 0.25-point increments (Edmonson *et al.*, 1989). Weight was assessed using a bovine weighing tape positioned posterior to the scapulohumeral joint to determine the thoracic perimeter.

Statistical analysis

Data were analyzed using the Mixed Model procedure for repeated measures in the statistical program JMP® Pro 14. Treatment, time (days), and their interactions were considered fixed effects, and group was considered a random effect. Differences were considered significant when $P \leq 0.05$.

RESULTS

The protein levels were lower (with the exception of the H-saturated group, which did not differ) for the treatments compared to the Control group. Regarding the treatments, the Ca-saturated group had a lower protein and casein content than the H-saturated ($P<0.05$). The treatment groups showed lower SCC compared to the Control group ($P<0.05$). NUN and total solids did not differ between groups ($P>0.05$). Milk yield, DMI, body weight, and BCS did not differ between groups ($P>0.05$). These results are shown in table 3.

Table 3 - Milk production and composition (fact, lactose, protein, casein, urea nitrogen, total solids), somatic cell count (SCC), dry matter intake (DMI), body weight and BCS of Holstein cows after the lactation leak over 22 days.

	Group				P value		
	Ca-mix	**Ca-saturated**	**H-saturated**	**Control**	**Group**	**Day**	**G*D**
[1]Milk yield	45,75±0,41	45,92±0,42	46,52±0,41	46,00±0,42	0,58	<0,01	1,00
[2]Fat	3,94±0,06	3,94±0,07	4,11±0,06	4,00±0,07	0,22	<0,01	0,89
[2]Lactose	4,48±0,02	4,54±0,02	4,52±0,02	4,50±0,02	0,24	0,20	1,00
[2]Protein	3,01±0,02^{bc*}	2,97±0,02^{c}	3,06±0,02^{ab}	3,10±0,02^{a}	<0,01	<0,01	1,00
[2]Casein	2,41±0,02^{ab}	2,37±0,02^{b}	2,46±0,02^{a}	2,42±0,02^{a}	<0,01	<0,01	1,00
[3]NUN	13,43±0,36	13,78±0,39	13,50±0,35	13,17±0,38	0,74	<0,01	0,94
[2]Total solids	12,42±0,08	12,42±0,08	12,67±0,08	12,54±0,09	0,10	0,12	0,99
[4]SCC	2,98±0,05^{a}	2,98±0,05^{a}	3,02±0,05^{a}	3,19±0,05^{b}	<0,01	0,15	1,00
[1]DMI	25,88±0,25	25,80±0,27	26,27±0,25	25,71±0,27	0,44	0,42	0,99
[5]Body weight	668,39±8,45	655,58±8,83	664,63±9,26	681,53±8,70	0,22	0,96	0,99
BCS	3,36±0,05	3,21±0,05	3,26±0,06	3,18±0,05	0,09	0,82	0,98

[1]Kg/day; [2]g/100g; [3]mg/dL; [4]log10/mL; [5]Kg.

Abbreviations: NUN, non-urea nitrogen; SCC, somatic cell count; DMI, dry matter intake; BCS, body condition score; G*D, group*day.

*Different letters indicate a significant difference between groups.

With respect to biochemical analysis, the concentration of BHB in the treatments did not differ from the control group. However, when comparing the treatment groups,

the H-saturated group exhibited higher BHB levels than the Ca-mix group (P=0.05). The concentrations of glucose, NEFA, TGL, albumin and urea did not differ between groups (P>0.05). These results are shown in table 4.

Table 4 - Biochemical analyses of blood samples from Holstein cows after the lactation peak over 22 days

	Group				**P value**		
	Ca-mix	**Ca-saturated**	**H-saturated**	**Control**	**Group**	**Day**	**G*D**
[1]Glucose	54,84±0,72	56,56±0,70	56,63±0,73	56,82±0,73	0,18	<0,01	0,47
[2]NEFA	0,41±0,03	0,41±0,03	0,34±0,03	0,38±0,03	0,24	<0,01	0,59
[2]BHB	0,43±0,02[b*]	0,44±0,02[ab]	0,50±0,02[a]	0,46±0,02[ab]	0,05	0,88	0,76
[1]TGL	16,80±1,52	15,57±1,45	14,18±1,50	14,99±1,50	0,66	<0,01	0,18
[3]Albumin	3,07±0,04	2,94±0,04	2,97±0,04	2,96±0,04	0,08	<0,01	0,95
[1]Urea	37,05±1,37	36,36±1,41	36,05±1,39	37,64±1,37	0,85	<0,01	0,99

[1]mg/dL, [2]mmol/L, [3]g/dL

Abbreviations: NEFA, non-esterified fatty acids; TGL, triglycerides; BHB, betahydroxybutyrate; G*D, group*day.

* Different letters indicate a significant difference between groups.

DISCUSSION

In this study, the effect of supplementing two calcium soap-protected fat products and one unprotected hydrogenated fat product, each with different fatty acid profiles, administered in the diet of Holstein cows after peak lactation, was evaluated.

The supplements of saturated fatty acids may differ in the concentration of C16:0 and C18:0, which can modify responses in DMI, production and metabolic parameters. Additionally, the currently commercially available supplements can vary from a nearly equal mixture of C16:0 and C18:0 to moderately (~85%) or highly (>95%) enriched in these fatty acids. Many of these saturated fatty acid supplements contain varying levels of cis-9 C18:1, which can affect the responses (Shepardson & Harvatine, 2021). Furthermore, inconsistency in responses to supplemental fat has been observed, this may be associated with the FA profile of supplemental fat and timing when supplementation starts (Piantoni *et al.*, 2015).

According Harvatine & Allen (2005), dietary FA saturation appears to be an important factor affecting milk protein response to FA treatment. Possible mechanisms include inhibition of microbial protein production, modification of insulin signaling, and changes in the somatotropic axis. In this study, the Ca-saturated group exhibited a lower protein and casein concentration compared to the H-saturated and Control groups. These results are consistent with Lock *et al.* (2013) and Rico *et al.* (2014), who found a lower concentration of this component in cows that received a supplement rich in SFA (90%). There are studies that mention that fat supplementation can lead to a reduction in this component, either due to the dilution effect resulting from increased milk production – which was not found in this study – or as a result of the lipid profile of the product affecting microbial protein synthesis (Jenkins, 1993). However, according to the meta-analysis by Hu *et*

al. (2017), the milk protein concentration generally is not influenced by saturated fatty acid supplements.

The metabolism of FA begins in the rumen through lipolysis, followed by biohydrogenation, which is a defense mechanism of ruminal microorganisms against UFA - known to be toxic to certain bacterial populations - and culminates in the formation of SFA (Jenkins, 1993). In this study, the supplement that decreased the milk protein concentration was rich in SFA (89.51%), so it would unlikely affect the population of bacteria responsible for microbial protein synthesis. Moreover, the dilution effect is also ruled out, as milk production did not differ among the groups. Shpirer *et al.* (2023) attribute this effect to the form of the product, but in this study, the supplements were in powder and granule forms. Therefore, we believe that this does not apply to this study.

Previous data on milk fat response to SFA supplementation has been inconsistent. Studies have reported both an increase in milk fat concentration and no effect (Piantoni *et al.*, 2015; Mathews *et al.*, 2016; Souza & Lock, 2019b; Shepardson & Harvatine, 2021). Prom & Lock (2021) supplemented the diet with cis-9 C18:1 and observed a decrease in milk fat concentration, with no effect on total fat production. This is attributed to the production of intermediate FA that resist ruminal biohydrogenation (Bauman *et al.*, 2011). In this study, there was no effect of treatments on milk fat content.

FAs found in milk have two origins: de novo synthesis in the mammary gland and extraction from plasma. Additionally, there are mixed-origin FAs (C16:0 and cis-9 C18:1), originating from both de novo synthesis in the gland and plasma extraction (Western *et al.*, 2020). Previous research has noted an increase in the concentration of this component compared to a control group, likely due to the incorporation of dietary fat into the milk (Mathews *et al.*, 2016; Souza & Lock, 2018; Shepardson & Harvatine, 2021).

Regarding SCC, the Control group showed a higher count compared to the others ($P<0.01$). The nutraceutical effect of linoleic and linolenic essential UFA is well established (Hess *et al.*, 2008). However, this effect has not been described for cis-9 C18:1. In the literature, no study discusses the effect of lipid supplementation on SCC, perhaps in this study, the supplemented FAs likely improved the immunological status of the treated animals. As for other milk constituents, total solids and NUN content did not differ between groups.

Supplementing in cows after peak lactation with FA can influence their metabolic status (Pires *et al.*, 2007). In this study, animals from all groups exhibited biochemical parameters within the physiological range (González *et al.*, 2022). However, the cows supplemented with hydrogenated UFAs showed a higher concentration of BHB than cows in the other groups, with no observed effect on NEFA levels. These data corroborate those of Yanting *et al.* (2019), who found an increased BHB concentration in C18:0-supplemented cows and no effect on NEFA levels.

Furthermore, there are studies that find an increase in milk production; however, productive responses can vary according to the FA profile of the product and the form of the provided fat (Lock *et al.*, 2013). In this study, milk production did not differ between groups. Although there was no effect on production, it is important to note that the FA profile of the product alone does not influence the effect on milk synthesis. Additionally, there are other factors related to the animal and the offered diet that interfere with the response (Mathews *et al.*, 2016). Similar milk production among groups may be partially explained by the lactation stage of the animals, which were after the peak of lactation, where energy requirements are not as intense. Furthermore, according to Mathews *et al.* (2016), discrepancies in production regarding the supplemented FAs observed between studies are likely due to differences in experimental design, quantity and concentration of the supplement,

lactation stage, supplementation duration, and energy level of the experimental control diet.

The supplements used in this experiment varied in the proportion of FA; however, feed intake did not differ between groups, aligning with other authors using different SFA and UFA supplements (Prom & Lock, 2021; Souza *et al.*, 2018). The effect on intake is generally related to the FA profile of the product (Allen, 2000; Rabiee *et al.*, 2012), with DMI typically decreasing as the degree of unsaturation increases due to increased digestibility of UFA (Souza *et al.*, 2018). Therefore, commercially available products enriched in SFA, UFA, or a combination of both have variable effects on intake (Loften *et al.*, 2014; Hu *et al.*, 2017; Souza and Lock, 2018). Within commonly fed FA supplements, calcium salts of palm FA linearly decreased DMI with increasing dietary concentration whereas hydrogenated FA had no effect on DMI (Allen, 2000).

In this study, although the supplements had different FA proportions, there was no change in DMI. This effect may be partially explained by the inclusion of the product at 1.5% of the diet, which may not have been sufficient to affect the intake of the supplemented groups compared to the control group. Literature mentions that the inclusion more than 6% rumen-available fat (especially unsaturated forms) have long been known to inhibit fiber digestion by ruminal microbes, and in some cases, decrease feed intake (Palmquist & Jenkins, 1980). Additionally, a 1.5% supplementation may not have been enough to alter palatability or cause a significant change in intake. Furthermore, the increase in milk production was not significant enough to demand higher feed consumption to sustain the increment.

Treatments did not affect BW gain or BCS changes. Experimental periods were only 22 days in length, reducing our ability to detect body tissue changes. The lack of effects of treatment on BW, BCS, or plasma NEFA and BHB concentration indicates

that cows did not change energy balance or that experimental periods were too short to observe differences.

To reiterate, supplementation using hydrogenated fats and calcium soap-protected fats with varied profiles of SFA and UFA influenced protein concentration, casein, SCC, and BHB in cows after peak lactation. However, no effect was observed on milk production, DMI, and other metabolic parameters, justifying the need for further research to assess the inclusion of protected fats in the diet of dairy cattle.

CONCLUSION

In conclusion, the inclusion of calcium-protected fats reduces SCC. Additionally, providing unprotected hydrogenated fat results in higher protein and casein content, as well as a higher concentration of BHB in the milk.

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3.2 Manuscrito 2 – Effect of bovine appeasing substance on multiparous Holstein cows during the transition period

Manuscrito submetido à revista *Journal of Dairy Research*

Effect of bovine appeasing substance on multiparous Holstein cows during the transition period

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ABSTRACT
The aim of experiment reported in this research paper was to determine the influence of the bovine appeasing substance (BAS) on milk yield, energy metabolism, inflammation, and stress in cows during the transition period. Twenty-four multiparous Holstein cows, (day 28 pre-partum to 21 post-partum) were distributed randomly into two groups: control (n=12) and BAS (Secure Cattle®; n=12). Each animal was administered 5 mL of the product on days 28 and 14 pre-partum and on the calving day. The feed intake was assessed using smart feeders and the milk yield was determined electronically. Six milk samples were obtained from each animal, which were analyzed for chemical composition and Somatic Cell Count. Six blood samples were obtained per animal for future biochemical analyses (Free Fatty Acids, betahydroxybutyrate, cortisol, myeloperoxidase and paraoxonase 1). The statistical analyses were conducted with the JMP Pro 14 software, the accepted level of significance was P≤0.05.BAS-treated cows showed higher milk yield (P<0.05) than controls. Dry Matter Intake (DMI) during the pre- and post-partum periods was greater for the control than treated group (P<0.05). The BAS group exhibited reduced plasma cortisol postpartum (P<0.05). In conclusion, cows treated with BAS showed higher milk yield, lower DMI, and lower plasma cortisol concentrations than controls.

Keywords: pheromone; cortisol; milk yield; dry matter intake

Introduction
The term 'well-being' pertains to physical and psychological status relative to an individual's efforts to adapt to the environment (Broom and Johnson, 2019). According to John Webster a fitting breeding environment must favor five freedoms: (1) freedom from thirst, hunger, and malnutrition; (2) freedom from pain, injury, and disease; (3) freedom from discomfort; (4)

freedom to express their natural behavior; and (5) freedom from fear and stress (Pacheco, Saad and Trevizan, 2012). These guidelines facilitate the qualitative assessment of physical, mental, and natural factors of well-being and of the likely discomforts in each of these aspects.

Today, technologies that encourage animal welfare include the use of pheromones, the semi-chemicals that can influence members within a species (Falewee *et al.*, 2006; Taylor and Mills, 2007; Temple *et al.*, 2016**)**. These pheromones can be recognized by the vomeronasal (or Jacobson's) organ (Crowell-Davis and Houpt, 1985; Kekan *et al.*, 2017). The porcine appeasing pheromone was first isolated from sow mammary glands (Pageat, 2001). Thereafter, these appeasing pheromones were investigated in other mammalian species, such as dogs, cats, and cattle (Pageat and Gaultier, 2003; Gunn-Moore and Cameron, 2004; Tod, Brander and Waran, 2005; Hargrave, 2014; Osella *et al.,* 2018).

Bovine appeasing substance (BAS) is a synthetic pheromone similar to the natural hormone the cow secretes just after calving. It is composed of a blend of fatty acids that reproduces the original substance (Osella *et al.,* 2018; Cappellozza and Cooke, 2022). When BAS was used in cattle, it increased daily weight gain and the final weight of calves (Cooke *et al.,* 2020); it also lowered hair cortisol and haptoglobin levels in beef steers (Schubach *et al.,* 2020). Furthermore, it improved the immune response of animals with respiratory diseases (Hervet *et al.,* 2021) and supported increased milk production and decreased somatic cell count (SCC) in dairy cows (Osella *et al.,* 2018).

The greatest challenge for dairy cattle welfare is peripartum, during which homeorhetic adaptations are observed to support the rise in colostrum and milk production (Marett *et al.,* 2015). These metabolic adjustments increase susceptibility to illnesses and immunological dysfunctions, with >70 % of illnesses occurring during this stage (Ortolani, 2009). These conditions warrant the development of technologies that promote animal welfare (Falewee *et al.,* 2006; Taylor and Mills, 2007; Carroll and Forsberg, 2007; Temple *et al.*, 2016; Hervet *et al.,* 2021; Cappellozza and Cooke, 2022).

BAS improved milk production and quality, energy metabolism, and the immune system of cattle (Osella *et al.,* 2018, Angeli *et al.*, 2020; Hervet *et al.,* 2021; Cappellozza and Cooke, 2022). Moreover, BAS can reduce the negative effects of the transition period on the metabolism and performance of highly productive dairy cows.

Therefore, we assessed the effect of BAS on feed intake, milk output, blood parameters, energy metabolism, stress, and inflammation in dairy cows during the transition period.

Materials and Methods

This study was conducted on a commercial property in the southern region of Rio Grande do Sul state (32°, 16'S, 52 67° 32' E), with 400 lactants cows (12,000 liters/day). Cows were housed in a compost barn-type confinement system, fed total mixed ration (TMR) using automatic feeders (AF 1000 electronic trough, Intergado®, Betim, Minas Gerais, Brazil) twice daily. The animals received water ad libitum. Twice daily, the animals were milked in the morning (8:00) and in the evening (20:00) (DeLaval®, Tumba, Botkyrka, Sweden). All animal procedures were approved by the Animal Ethics and Experimentation Committee of the Federal University of Pelotas (project number 044683).

To determine sample size, blood cortisol levels were evaluated starting three weeks prior to calving up to two weeks post-calving. The calculation was performed using the OpenEpi epidemiological calculator, generated by the formula available at (https://www.openepi.com/SampleSize/SSMean.htm). Therefore, for the sample size calculation of each cycle, a total sample size of 24 animals was determined.

Separation of animals and experimental design

Twenty-four multiparous cows were selected and monitored from day 28 pre-partum to day 21 post-partum. As the completely randomized experimental design was adopted, cows were

separated into two experimental groups, namely the control (n=12) and BAS groups (SecureCattle®, Nutricorp©, Araras, São Paulo, Brazil; n=12). The inclusion criterion was cows entering their 2nd through 5th lactation (the average number of lactation for both groups was 3). All animals were kept in a single compost barn type shed, which was subdivided by electric fencing to maintain a minimum distance of 50 meters between groups due to the volatile nature of the product. A 5 mL/animal dose of the product was administered topically in the nape at -28 and -14 days in relation to the expected birth as well as on the calving day (D0). No management was performed to simulate the application of the product in the Control group (placebo), as the application is topical and did not induce stress. Throughout the experiment, at least 50 m distance was maintained between the groups. The experiment took place from January to August 2022.

Feed samples and bromatological analyses

Corn silage and pre-dried ryegrass samples were obtained twice weekly to form a weekly pool. TMR samples from the experimental batches were also obtained daily for dry matter analysis and a weekly pool was made for the other analyses. The samples were frozen for bromatological analyses (dry matter [DM], crude protein [CP], neutral detergent fiber [NDF], acid detergent fiber [ADF], ether extract [EE], non-fibrous carbohydrates [NFC], mineral residue [MR]).

The feed samples were analyzed at the Animal Nutrition Laboratory, in the NUPEEC Hub, at the Federal University of Pelotas. First, samples were subjected to a 72-hour pre-drying process in a forced circulation oven, set to 55° C. Next, the samples were ground in a knife mill. To accomplish the definitive DM analysis, the ground samples were placed in an oven set to 105° C for at least 8 h (Easley *et al.,* 1965). For mineral matter analysis, 1 g of ground sample was weighed in triplicate and added to the muffle furnace at 600° C for 3 h to burn the organic residue (AOAC, 1995). Subsequently, the value obtained was subtracted and the gross mineral matter was defined.

To determine the crude protein, the method cited by Kjeldhal (AOAC, 1995) for nitrogen determination was used including 4 % (w/v) boric acid solution as a free ammonia receptor during distillation, a solution of 0.2% (w/v) bromocresol green, 0.1% (w/v) methyl red as the indicator, and a standard sulfuric acid solution for titration (Kozloski *et al.,* 2003). Next, the NDF and ADF analyses were performed as previously described (Van Soest and Robertson, 1985). NFC were estimated using the formula NFC = 100-CP-MR-EE-NDF (Sniffen *et al.,* 1992). The average chemical composition of the diets provided to the animals during the experimental phase is shown in Table 1.

Feed consumption

Feed intake was recorded 24 hours per day, automatically and individually using intelligent feeders (Electronic Trough AF 1000, Intergado®, Betim, Minas Gerais, Brazil). The control group was housed on one side of the barn and given access to eight feeders, while the BAS group was restricted to the other side, with access to eight different feeders. According to the manufacturer's recommendations, there were at most 1.6 animals per feeder.

Animals were limited to a space in the compost barn that allowed sole access to the feeders via a button attached to their ears. One TMR sample was obtained was daily from the output and dried in an air fryer to ascertain the concentration of DM (Nino *et al.,* 2021). The TMR was made available twice a day at 11:00 a.m. and 4:00 p.m.

Milk production and analysis of milk constituents

Using the DelPro™ software, milk production was assessed electronically (DeLaval®, Tumba, Botkyrka, Sweden), and in-person monitoring was performed during the two daily milkings. The milk from two sequential milkings was collected with twice a week for each animal, totaling six samples per cow. The samples were preserved with bronopol and kept at room temperature, being analyzed within 7 days and send to the Centralized Milk Quality Analysis Laboratory (LCAQL) (APCBRH, Curitiba, Paraná, Brazil) to analyze the milk constituents (fat, lactose,

protein, nitrogen urea, total solids) via spectrophotometry, as well as the somatic cell count (SCC) via flow cytometry (NexGen, Bentley Instruments®, Chaska, United States).

Blood analyses

Six blood samples (4 mL) were retrieved per cow by puncturing the coccygeal arteriovenous complex on days -28 and -14 to expected parturition, on the parturition day (D0), and on days 7, 14, and 21 post-partum. These collections were performed prior to the provision of the morning diet. The samples were kept at room temperature for a maximum of 30 min before processing and centrifuged at 1,800 × g for 15 min. Serum samples were drawn and analyzed for free fatty acids (FFA), beta-hydroxybutyrate (BHB), myeloperoxidase (MPO), paraoxonase (PON-1) and blood cortisol. Plasma (KF-EDTA) was used for glucose analysis. The FFA, BHB and glucose were analyzed using an automatic biochemical analyzer (Labmax Plenno®, LabTest, Lagoa Santa, Minas Gerais, Brazil) with commercial kits (DiaSys Diagnostic Systems kits). Myeloperoxidase were investigated employing the enzyme-linked immunosorbent assay (ELISA; ELK Biotechnology CO., LTD, Wuhan, Hubei, China) using an ELISA plate reader with a wave length of 450 nm (Thermoplate, Palm City, FL, USA). PON-1 activity was assessed using a commercial kit (ZeptoMetrix Corporation®, USA) via kinetic spectrophotometry (T80 UV/VIS, PG Instruments, England) in duplicate. Blood cortisol was analyzed using an immunoassay analyzer (Access 2, Beckman Coulter®, Brea, California, United States) with a commercial kit (Access Cortisol kit). The Metabolism Laboratory, NUPEEC Hub (Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil) supplied all the equipment utilized for these tests. Intra- and inter-assay precision was determined by analyzing six samples obtained on the same or on different days, and the variation coefficient remained below 5%.

Clinical and zootechnical evaluations

Clinical examinations of all the animals were performed weekly, and their weight and body condition score (BCS) were recorded. A bovine weighing tape was used to record the weights, by positioning it posterior to the scapulohumeral joint to determine the circumference of the thoracic perimeter. Heart rate, respiratory rate, rumen movements, rectal temperature, color of the ocular and vaginal mucous membranes, and capillary refill time were evaluated (Feitosa**,** 2020).

Feed efficiency was calculated through total milk production divided by DMI (Blake and Custodio, 1984). A trained evaluator performed the BCS on a scale of 1 to 5, using 0.25 subdivision points (Edmonson *et al*., 1989).

Energy balance (EB) was calculated for each cow using previously described equations [31]. Net energy intake (NEI) was determined using daily DMI multiplied by the laboratory-calculated NEL density of the diet. Net energy for maintenance was calculated as NEM = 0.10 × BW kg0.75. Requirements of NEL for milk production were calculated as NEMILK = 9.29 × kg fat/kg milk + 5.5 × kg crude protein/kg milk + 3.95 × kg lactose/kg milk. NEL available (Mcal/kg loss) was calculated as NEL available = 5.6 Mcal NEL/kg BW loss. The equation used to calculate post-partum EB was EBPOST (Mcal/d) = NEI – (NEM + NEMILK + NEL available). The data used in the equations were collected over 21 days postpartum. Milk production and dry matter intake (DMI) data were obtained daily during the 21 postpartum days. Weight data were obtained from the two postpartum weights and used as an average for the 21 days. The milk component data were from the six postpartum samples and used as an average for the 21 days.

Statistical analysis

The statistical analyses were all done using the JMP Pro 14 software. Analysis of the dependent variables was performed using a mixed analysis of variance by repeated measures (ANOVA) model, adopting the PROC MIXED procedure. The model was constructed with the variables of treatment and time (days), and interactions between variables were treated as the fixed effects, while animals were regarded as a random effect. In the randomization process, both the number

of lactations and the production in the previous lactation were taken into consideration. The Tukey test was used to compare the means between the groups. The covariance structure used was Variance Components, chosen due to having the lowest value in the Bayesian Information Criterion (BIC). Data were analyzed for normality and homogeneity of variance through a histogram analysis and the Shapiro-Wilk method, with a value above 0.80 considered acceptable. The accepted level of significance was P≤0.05. Trends were recognized if P>0.05 and P≤0.10.

Results

The BAS group produced 29.04±0.53 Kg milk/day, while the control group produced 26.12±0.53 Kg milk/day, showing a rise of 2.92 Kg of milk/day in BAS-treated cows (P<0.05) as shown in Figure 1.

Cows in the control group exhibited a DMI of 14.31±0.40 kg/day pre-partum, compared to 10.34±0.34 Kg/day for the cows in the BAS group (P<0.05) (Figure 2A). Moreover, the control group exhibited a DMI of 16.24±0.44 Kg/day post-partum, whilethe BAS group consumed 12.91±0.41 Kg/day (P<0.05) (Figure 2B). This indicates that cows in the BAS group produced milk more efficiently despite lower feed consumption (Feed efficiency: BAS = 2.21; control = 1.38; P<0.01).

The blood analyses results for the control and BAS groups are shown in Table 2. Pre-partum, all parameters for both groups were within the physiological range. Post-partum, the plasma cortisol level reduced in the BAS group (P<0.05). Cows in the BAS group had an energy balance of -10.69 ± 0.92 Mcal/day, whereas cows in the control group had 3.01 ± 0.92 Mcal/day (P<0.01).

The results for body weight and BCS are presented in Table 3 and values did not differ between groups. (P>0.05). Milk composition results for multiparous cows in both groups are shown in Table 4. The BAS group had slightly higher lactose levels and total solids than the control group (P=0.10 and P=0.08, respectively).

Discussion

In the present study, the milk yield increased by 2.92 Kg/day in cows that received BAS during the transition period. This is consistent with previous findings showing an increase of 1.65 Kg milk/day in cows receiving BAS during the transition between a confined and a semi-confined system (Osella *et al.,* 2018). The authors attributed this result to the reduced levels of environmental change-related stress. Cortisol can inhibit the production of oxytocin, which controls milk secretion. High output of cortisol can negatively affect prolactin levels which control milk production. Therefore, animals that experience stressful periods show decreased milk secretion caused by the negative effect that stress exerts on oxytocin and prolactin (Edmonson *et al.,* 1989).

BAS activity can reduce reactivity to neuroendocrine stress (Cappellozza and Cooke, 2022). This is consistent with our results, which demonstrated a clear BAS-enabled reduction in plasma cortisol levels. A drop in blood cortisol upon BAS treatment was previously described in Angus steers in a feedlot system (NASEM, 2021). The pheromone used in this study likely decreased the response of the pituitary-pituitary-adrenal axis (Bobić *et al*., 2011; Osella *et al.,* 2018, Cooke *et al.,* 2020; Schubach *et al.*, 2020). By contrast, BAS did not lower cortisol concentrations calves (¾ Angus x ¼ Bos indicus) at the time of weaning (Cooke *et al.,* 2020; Schubach *et al*., 2020) work with. Cortisol levels have been extensively applied to assess the adrenocortical responses in cattle (Carroll *et al.,* 2007); however, the outcomes may be affected by stress during sample (Cooke, 2017; Colombo *et al.,* 2020). Therefore, hair retrieval is suggested to determine hair cortisol, which provides a long-term response (Cooke, 2017). However, BAS had no effect in hair cortisol, suggesting that this substance likely affects metabolism through alternate metabolic pathways (Cooke *et al.,* 2020).

In this study, the influence exerted by the pheromone on the hypothalamic-pituitary-adrenal pathway partially explains the increased milk production in treated cows. Nonetheless, the

galactopoietic hormones, serotonin and prolactin, can also affect milk production. BAS is a well-known promoter of comfort and well-being with the ability to lower blood cortisol concentrations. Furthermore, serotonin plays fundamental roles in animal metabolism and is also an important hormone produced in the mammary gland to assist in coordinating lactation (Forslund, Ljungvall and Jones, 2010). Recently, studies have demonstrated the influence of serotonin on calcium homeostasis during lactation in rodents and dairy cows (Schubach *et al.*, 2017; Connelly *et al.*, 2021) and several studies have demonstrated the relationship between serotonin and prolactin in humans and rodents (Noel *et al.*, 1974; Panerai *et al.*, 1985; Laporta *et al.*, 2015; Slater *et al.*, 2018). Therefore, we hypothesized that BAS increases serotonin concentrations by reducing cortisol levels, ultimately increasing prolactin secretion and milk production. Nonetheless, prolactin has been scarcely evaluated in studies with cattle.

Cows receiving BAS produced milk more efficiently, with an intake of 3.33 Kg of DM/day, which is less than that of cows in the control group. These data are consistent with the mechanism of BAS which includes improved nutrient utilization resulting in in greater feed efficiency (Apfelbaum, 1987). Reduced DM intake is a common feature of peripartum cows (**Coiro** *et al.*,1987; Marett *et al.*, 2015) [43, 44] and generally exerts harmful effects on animal metabolism.

The negative energy balance (NEB) during early lactation results from the gap between the energy needed for maintenance and milk secretion, and the energy supply provided by feed intake (Drackley *et al.*, 2005). To sustain increasing milk yields during the first weeks of lactation, dairy cows must mobilize body reserves, mainly fat. The release of fatty acids (FA) from the adipose depots is reflected in increased circulating FA concentrations (Schoenberg, Ehrhardt and Overton, 2012). In the present study, reduced feed intake and higher milk production for cows treated with BAS resulted in a NEB. Despite this, FFA levels remained within physiological ranges, with no significant differences between groups. Additionally, the body weight of the animals did not differ pre- and post-partum, and the BCS tended to be higher in the control than in the BAS group pre-partum, which did not occur postpartum.

Greater clarity regarding the relationship between prolactin, serotonin, cortisol and peripartum in dairy cows will significantly improve understanding on how well-being improves milk production.

Conclusions

In conclusion, BAS treatment during the transition phase decreased plasma cortisol, increased milk output, reduced DMI, and improved better feed efficiency in multiparous Holstein cows.

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Table 1: Average chemical composition and ingredients of pre- and post-partum diets during the experimental period.

	Pre-partum	Post-partum
Nutrients		
Dry matter (%)	43.14	42.29
Non-fibrous carbohydrates (% DM)	22.71	26.79
Ether Extract (% DM)	3.09	4.90
Acid Detergent Fiber (% DM)	37.70	31.33
Neutral Detergent Fiber (% DM)	51.64	46.13
Crude Protein (% DM)	12.29	14.67
Mineral Residue (% DM)	9.47	8.20
Chlorine (g/kg DM)	6.20	*
Boron (mg/kg DM)	10.71	*
Calcium (% DM)	0.60	*
Copper (mg/kg DM)	22.42	*
Sulfur (% DM)	0.27	*
Iron (mg/kg DM)	506.02	*
Phosphorous (% DM)	0.43	*
Ingredients		
Concentrate pre-partum (kg)	5,00	-
Concentrate lactation (kg)	-	11,90
Corn silage (kg)	12,00	35,80
Oat hay (kg)	2,50	-
Pre-drying of ryegrass and clover (kg)	-	6,00

* Nutrient was not analyzed in the diet.

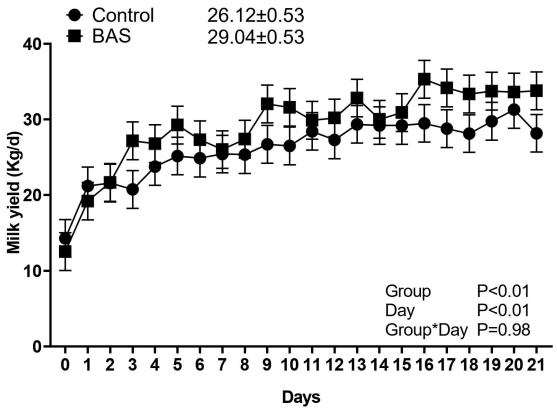

Figure 1: Milk yield of multiparous cows in the control and BAS groups over 21 days postpartum (Data are shown as mean ± standard error).

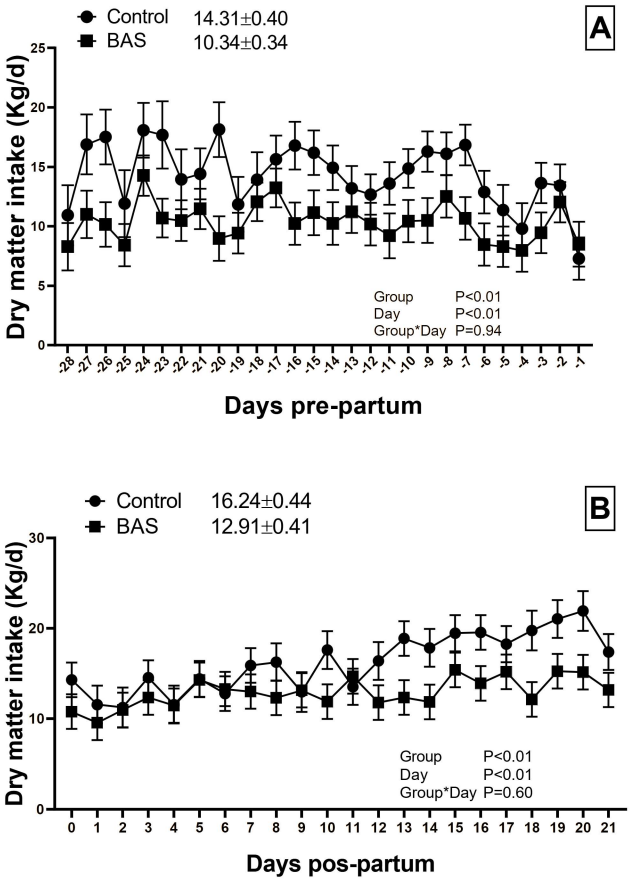

Figure 2: (A) DMI of multiparous cows in the control and BAS groups over 28 days pre-partum. (B) DMI of multiparous cows in the control and BAS groups over 21 days postpartum excluding calving day. All data are shown as mean ± standard error.

Table 2: Energy balance and blood metabolites analyses of multiparous cows in the control and BAS groups.

	Groups		P values		
Variable	Control	BAS1	Group	Day	Group*Day
Pre-partum					
Free Fatty Acids (mmol/L)	0.27±0.03	0.29±0.03	0.70	0.68	0.60
Beta-hydroxybutyrate (mmol/L)	0.46±0.04	0.42±0.03	0.36	0.22	0.76
Glucose (mg/dL)	59.13±1.63	62.57±1.46	0.12	<0.01	0.98
Cortisol (ng/mL)	20.94±2.86	17.98±3.30	0.51	0.37	0.72
Myeloperoxidase (ng/mL)	03.11±0.14	3.37±0.16	0.26		
Post-partum					
Free Fatty Acids (mmol/L)	0.52±0.04	0.42±0.03	0.46	<0.01	0.90
Betahydroxybutyrate (mmol/L)	0.57±0.05	0.57±0.04	0.92	0.11	0.90
Glucose (mg/dL)	54.49±2.42	54.12±2.34	0.91	<0.01	0.96
EB2 (Mcal/d)	-0.09±0,92	-13.87±0,92	<0.01	0.76	0.29
Cortisol (ng/mL)	15.70±1.07	11.58±0.92	<0.01	<0.01	0.98
Paraoxonase (U/L)	56.01±3.09	59.48±3.25	0.44	0.19	0.99
Myeloperoxidase (ng/mL)	3.05±0.08	3.02±0.08	0.75	0.92	0.42

1BAS: Bovine Appeasing Substance; 2EB: energy balance; The metabolites that have * do not present the P values on the day and group*day because there was only one analysis of this parameter in the pre-partum period. Data are shown as mean ± standard error.

Table 3: Body weight and body condition score of multiparous cows from the control and BAS groups.

	Groups		P values		
	Control	BAS1	Group	Week	Group*Week
Pre-partum					
Body weight	734±11	735±11	0.92	0.33	0.77
2BCS	4.06±0.12	3.75±0.12	0.07	0.40	0.40
Post-partum					
Body weight	705±11	728±11	0.15	0.41	0.92
2BCS	3.56±0.12	3.45±0.12	0.52	0.17	0.74

1BAS: Bovine Appeasing Substance; 2BCS: Body Condition Score
Data are shown as mean ± standard error.

Table 4: Milk composition and somatic cell count (SCC) of multiparous cows in the control and BAS groups.

Component	Groups		P values		
	Control	BAS1	Group	Days	Group*Day
Fat	3.95±0.22	4.43±0.23	0.13	0.77	0.58
Lactose	4.08±0.06	4.24±0.07	0.10	0.10	0.92
Protein	3.34±0.05	3.41±0.07	0.42	<0.01	0.98
Total solids	11.79±0.29	12.63±0.38	0.08	<0.01	0.98
2SCC	2.87±0.10	2.87±0.13	0.99	0.54	0.96

1BAS: Bovine Appeasing Substance; 2Somatic Cell Count (SCC): log10/mL; Fat, lactose, protein and total solids: g/100g.
Data are shown as mean ± standard error.

5 CONCLUSÃO GERAL

Adotar estratégias versáteis na pecuária de leite é fundamental para otimizar a produtividade, a saúde e o bem-estar. Tecnologias e práticas como a inclusão de aditivos alimentares – como a gordura -, além da implementação de uma ferramenta como a feromonioterapia, têm o potencial de melhorar o desempenho produtivo de forma sustentável.

Neste estudo, a inclusão de gorduras protegidas de sabões de Ca teve impacto positivo na redução da CCS. Além disso, a oferta de gordura hidrogenada não protegida resultou em maior teor de proteína e caseína, bem como uma concentração mais alta de BHB no sangue. No que diz respeito ao experimento com a SAB, as vacas tratadas apresentaram menor concentração de cortisol plasmático, maior produção de leite, menor CMS e, consequentemente, melhor eficiência alimentar.

Essas inovações não apenas podem aumentar a eficiência da produção, mas também garantem um ambiente mais saudável para os animais. Ao focar no bem-estar animal e em aditivos alimentares, é possível alcançar resultados mais consistentes, tanto em termos de quantidade quanto de qualidade do leite, promovendo a sustentabilidade e a rentabilidade no longo prazo.

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7 ANEXOS

Anexo A – Comitê de ética projeto Efeito de gorduras protegidas e gordura hidrogenada no consumo, produção, composição do leite e parâmetros zootécnicos e metabólicos de vacas da raça Holandês

PARECER Nº **81/2023/CEUA/REITORIA**
PROCESSO Nº 23110.030768/2021-07

Certificado

Certificamos que a proposta intitulada **"IMPACTO DA SUPLEMENTAÇÃO DE FONTES DE GORDURA PROTEGIDA NO CONSUMO, PRODUÇÃO E COMPONENTES DO LEITE DE VACAS DA RAÇA HOLANDÊS APÓS O PICO DE LACTAÇÃO"**, registrada com o nº **23110.030768/2021-07,** sob a responsabilidade de **Marcio Nunes Corrêa** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética no Uso de Animais da Universidade Federal de Pelotas.

Finalidade	(x) Pesquisa ( ) Ensino
Vigência da autorização	Início: 15/05/2023 Término: 05/08/2023
Espécie/linhagem/raça	Bovina /Holandês
Nº de animais	**48**
Idade	02 a 07 anos
Sexo	Fêmeas
Origem	Fazenda comercial de sistema intensivo de produção de leite - BR 471 KM 501 4º Distrito - Taim, Rio Grande – RS.

Código para cadastro nº **CEUA 030768/2021-07**

Priscila Marques Moura de Leon

Coordenadora da CEUA

Documento assinado eletronicamente por **PRISCILA MARQUES MOURA DE LEON, Professor do Magistério Superior**, em 17/05/2023, às 23:59, conforme horário oficial de Brasília, com fundamento no art. 4º, § 3º, do Decreto nº 10.543, de 13 de novembro de 2020.

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Anexo B – Comitê de ética projeto Efeito da substância apaziguadora bovina em vacas multíparas da raça Holandês durante o período de transição

PARECER Nº **5/2023/CEUA/REITORIA**
PROCESSO Nº 23110.044683/2022-89

Certificado

Certificamos que a proposta intitulada **"Efeito da substância apaziguadora bovina (SAB) no desempenho, metabolismo energético, estresse e inflamação de novilhas e vacas leiteiras durante o período de transição"**, registrada com o nº **23110.044683/2022-89**, sob a responsabilidade de **Marcio Nunes Corrêa** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética no Uso de Animais da Universidade Federal de Pelotas.

Finalidade	(x) Pesquisa ( ) Ensino
Vigência da autorização	Início: 15/02/2023 Término: 15/08/2023
Espécie/linhagem/raça	*Bos taurus* / Holandês
Nº de animais	**48**
Idade	2 a 7 anos
Sexo	Fêmeas
Origem	Fazenda localizada na BR 471 KM 501 4º Distrito - Taim, Rio Grande – RS.

Código para cadastro nº **CEUA 044683/2022-89**

Priscila Marques Moura de Leon

Coordenadora da CEUA

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