



**AMIDO E SAIS DE CÁLCIO DE ÁCIDOS GRAXOS DE ÓLEO DA
PALMA NA DIETA PARA CORDEIROS**

Joyanne Mirelle de Sousa Ferreira

**UNIVERSIDADE ESTADUAL DO SUDOESTE DA BAHIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA**

**AMIDO E SAIS DE CÁLCIO DE ÁCIDOS GRAXOS DE ÓLEO DA
PALMA NA DIETA PARA CORDEIROS**

Autora: Joyanne Mirelle de Sousa Ferreira
Orientador: Prof. D. Sc. José Augusto Gomes Azevêdo

ITAPETINGA
BAHIA – BRASIL
MARÇO DE 2026

JOYANNE MIRELLE DE SOUSA FERREIRA

**AMIDO E SAIS DE CÁLCIO DE ÁCIDOS GRAXOS DE ÓLEO DA
PALMA NA DIETA PARA CORDEIROS**

Tese apresentada, como parte das exigências para obtenção do título de DOUTORA EM ZOOTECNIA, no Programa de Pós-Graduação em Zootecnia da Universidade Estadual do Sudoeste da Bahia.

Orientador: Prof. D. Sc. José Augusto Gomes Azevêdo

Co-orientador: Prof. D. Sc. Henry Daniel Ruiz Alba

Co-orientadora: Prof.^a D. Sc. Lígia Lins Souza

ITAPETINGA
BAHIA – BRASIL
MARÇO DE 2026

Ficha Catalográfica Preparada pela Biblioteca da UESB, Campus de Itapetinga

636.085 Ferreira, Joyanne Mirelle de Sousa.
F441a Amido e sais de cálcio de ácidos graxos de óleo da palma na dieta para cordeiros. / Joyanne Mirelle de Sousa Ferreira. – Itapetinga-BA: UESB, 2026.

92p.

Tese apresentada, como parte das exigências para obtenção do título de DOUTORA EM ZOOTECNIA, no Programa de Pós-Graduação em Zootecnia da Universidade Estadual do Sudoeste da Bahia. Sob a orientação do Prof. D. Sc. José Augusto Gomes Azevêdo e coorientação de Prof. D. Sc. Henry Daniel Ruiz Alba e Prof.^a D. Sc. Lígia Lins Souza.

1. Cordeiro – Amido dietético – Óleo de palma – Suplementação. 2. Cordeiros – Dietas – Desempenho produtivo. 3. Cordeiros – Dietas – Metabolismo de nutrientes – Características de carcaça. I. Universidade Estadual do Sudoeste da Bahia - Programa de Pós-Graduação de Doutorado em Zootecnia, *Campus* de Itapetinga. II. Azevêdo, José Augusto Gomes. III. Alba, Henry Daniel Ruiz. IV. Souza, Lígia Lins. V. Título.

CDD (21): 636.085

Catálogo na Fonte:

Adalice Gustavo da Silva – CRB 535-5ª Região
Bibliotecária – UESB – Campus de Itapetinga-BA

Índice Sistemático para desdobramentos por Assunto:

1. Cordeiros – Nutrição animal – Palma forrageira
2. Cordeiros confinados – Dietas – Palma forrageira



Governo do
Estado da Bahia

Universidade Estadual do Sudoeste da Bahia – UESB
Recredenciada pelo Decreto Estadual
Nº 16.825, de 04.07.2016

DECLARAÇÃO DE APROVAÇÃO

Título: *“Amido e sais de cálcio de ácidos graxos de óleo da palma na dieta para cordeiros”*

Autora: Joyanne Mirelle de Sousa Ferreira

Orientador: Prof. Dr. José Augusto Gomes Azevêdo

Coorientadores: Prof. Dr. Henry Daniel Ruiz Alba

Profa. Dra. Lígia Lins Souza

Aprovada como parte das exigências para obtenção do Título de DOUTORA EM ZOOTECNIA, ÁREA DE CONCENTRAÇÃO PRODUÇÃO DE RUMINANTES, pela Banca Examinadora:



Documento assinado digitalmente
JOSE AUGUSTO GOMES AZEVEDO
Data: 03/03/2026 13:53:02-0300
Verifique em <https://validar.it.gov.br>

Prof. Dr. José Augusto Gomes Azevêdo (Orientador) – UESC/UESB



Documento assinado digitalmente
GLEIDSON GIORDANO PINTO DE CARVALHO
Data: 03/03/2026 15:43:46-0300
Verifique em <https://validar.it.gov.br>

Prof. Dr. Gleidson Giordano Pinto de Carvalho – UFBA



Documento assinado digitalmente
STEFANE ALVARENGA SANTOS
Data: 04/03/2026 19:04:13-0300
Verifique em <https://validar.it.gov.br>

Profa. Dra. Stefanie Alvarenga Santos – UFBA



Documento assinado digitalmente
PAULO SERGIO DE AZEVEDO
Data: 04/03/2026 08:59:31-0300
Verifique em <https://validar.it.gov.br>

Prof. Dr. Paulo Sérgio de Azevedo – UFPB



Documento assinado digitalmente
HENRY DANIEL RUIZ ALBA
Data: 04/03/2026 14:42:23-0300
Verifique em <https://validar.it.gov.br>

Prof. Dr. Henry Daniel Ruiz Alba (Coorientador) – UFOPA

Data de realização: 03 de março de 2026.

Campus de Itapetinga

(77) 3261-8628 | ppz@uesb.edu.br

Campus de Itapetinga
Praça da Primavera, 40
Bairro Primavera
CEP 45.700-000
PABX: (77) 3261 - 8600

Campus de Jequié
Rua José Moreira Sobrinho, s/n
Bairro Jequezinho
CEP 45.200 - 000
PABX: (73) 3528 - 9600

Campus de Vitória da Conquista
Estrada do Bem Querer, km 4
Bairro Universitário
CEP: 45031 - 300
PABX: (77) 3424 - 8600

AGRADECIMENTOS

Agradeço primeiramente aos meus sobrinhos **Mariana e Artur**, pela compreensão diante da distância, e ao meu irmão **Abdon Werner**, pelo incentivo constante.

Expresso minha gratidão especial à minha mãe, **Marli**, pelos ensinamentos. Aos colegas do PPZ, turmas de mestrado e doutorado 21.1, 21.2 e 22.1, pela convivência enriquecedora, e em especial a Vanessa Vieira e Mateus Lacerda.

Ao meu companheiro de jornada Herick, pela ajuda e apoio em cada dificuldade enfrentada (não foram poucas).

Aos estagiários Ricardo, Marcela, Mateus, Guilherme, Laís e Artur, pela dedicação e colaboração.

Ao meu orientador Prof. José Augusto Gomes Azevêdo, pela paciência, aprendizado, confiança e apoio.

Aos co-orientadores Prof.^a Lígia Lins Souza e Prof. Henry Daniel Ruiz Alba, pela orientação científica e pelo aprendizado.

A Ana Paula Gomes, Aroldo Brandão e José Queiroz, pela ajuda constante e pela inspiração como profissionais e pessoas. Ao senhor Aragão, pela disponibilidade e apoio.

Por fim, agradeço às agências de fomento CAPES, CNPq e FAPESB pelo suporte financeiro e institucional que possibilitou a realização desta pesquisa.

BIOGRAFIA

JOYANNE MIRELE DE SOUSA FERREIRA, filha de Marli de Sousa Ferreira e José Ferreira de Sousa Neto, nasceu em Pombal, no estado da Paraíba, no dia 17 de abril de 1989.

Em maio de 2008, ingressou no curso de Zootecnia, no Centro de Ciências Agrárias da Universidade Federal da Paraíba, concluindo em dezembro de 2012.

Em fevereiro de 2013, ingressou no Programa de Pós-Graduação em Zootecnia, em nível de Mestrado, área de concentração Sistemas Agrossilvipastoril, na Universidade Federal de Campina Grande, concluindo em fevereiro de 2015.

Em abril de 2022, iniciou estudos no Programa de Pós-Graduação em Zootecnia, em nível de Doutorado, área de concentração Produção de Ruminantes, na Universidade Estadual do Sudoeste da Bahia, realizando estudos na área de Nutrição e Alimentação Animal.

Sumário

RESUMO GERAL	12
GENERAL ABSTRACT	13
1. CAPÍTULO I.....	14
Referencial Teórico	14
1.1. Introdução	14
1.2. Metabolismo do amido em pequenos ruminantes: da ingestão ao aproveitamento energético	16
1.3. Metabolismo e aplicação dos lipídios protegidos em pequenos ruminantes.....	21
1.4. Interação entre amido e lipídios protegidos em dietas para pequenos ruminantes	23
1.5. Efeito do amido e da gordura protegida sobre características de carcaça e qualidade da carne de pequenos ruminantes	25
REFERÊNCIAS	28
2. OBJETIVOS	35
3. CAPÍTULO II.....	36
ABSTRACT	37
3.1. Introduction.....	38
3.2. Materials and Methods.....	40
3.3. Results	46
3.4. Discussion	55
3.5. Conclusions and perspectives.....	59
References	60
4. CAPÍTULO III	66
Abstract	68
4.1. Introduction	69
4.2. Materials and Methods	70
4.3. Results	75
4.4. Discussion	81
4.5. Conclusions.....	85
References	86
5. CONCLUSÃO GERAL	92

LISTA DE TABELAS

I – REFERENCIAL TEÓRICO

Tabela 1. Principais estudos avaliando o efeito de níveis de amido sobre parâmetros nutricionais e produtivos em pequenos ruminantes	20
Tabela 2. Síntese de estudos experimentais sobre a suplementação com sais de cálcio de ácidos graxos e fontes lipídicas em pequenos ruminantes	22
Tabela 3. Interações Amido + SCAGP	24
Tabela 4. Síntese Integrativa	25
Tabela 5. Principais resultados dos estudos sobre amido e gordura protegida em ruminantes	27

II - Dietary starch level determines the efficacy of calcium salts of palm fatty acids on nitrogen use efficiency and performance in feedlot lambs.

Table 1. Ingredients and chemical composition of experimental diets containing low or high starch (S) levels and with or without calcium salts of palm fatty acids (CSPFA; g/kg).	41
Table 2. Nutrient intake and apparent digestibility coefficients in feedlot lambs fed diets containing low (220 g/kg) or high (420 g/kg) starch levels with (30g/kg DM) or without (0g/kg DM) calcium salts of palm fatty acids (CSPFA).	46
Table 3. Nitrogen balance, urinary purine derivatives, and microbial nitrogen synthesis in feedlot lambs fed diets containing low (220 g/kg) or high (420 g/kg) starch levels with (30g/kg DM) or without (0g/kg DM) calcium salts of palm fatty acids (CSPFA)	48
Table 4. Ingestive behavior patterns, feeding and ruminating efficiencies, and chewing activity of feedlot lambs fed diets containing low (220 g/kg) or high (420 g/kg) starch levels with (30g/kg DM) or without (0g/kg DM) calcium salts of palm fatty acids.....	51
Table 5. Serum metabolite profile, liver function enzymes, and lipid indicators of feedlot lambs fed diets containing low (220 g/kg) or high (420 g/kg) starch levels with (30g/kg DM) or without (0g/kg DM) calcium salts of palm fatty acids (CSPFA).	52
Table 6. Growth performance and feed efficiency of feedlot lambs fed diets containing low (220 g/kg) or high (420 g/kg) starch levels with (30g/kg DM) or without (0g/kg DM) calcium salts of palm fatty acids (CSPFA).	54

III - Effects of Dietary Starch Level and Calcium Salts of Palm Fatty Acids on Carcass Traits and Meat Quality of Lambs.

Table 1. Proportion of ingredients and chemical composition of experimental diets.....	71
Table 2. Slaughter and carcass traits of lambs fed diets with starch and calcium salts of palm fatty acids.	76
Table 3. Half carcass composition of feedlot lambs fed starch and calcium salts of palm fatty acids.	76

Table 4. Carcass fat depots and meat quality traits of feedlot lambs fed starch and calcium salts of palm fatty acids.....	77
Table 5. Fatty acid profile of the Longissimus dorsi muscle of lambs fed diets with different starch levels and calcium salts of palm fatty acids.....	79
Table 6. Interaction effects of dietary starch level and calcium salts of palm fatty acids on individual fatty acids of the lamb Longissimus dorsi muscle.	80
Table 7. Lipid profile and health indices of lamb Longissimus dorsi muscle as affected by dietary starch and calcium salts of palm fatty acids.....	81
Table 8. Desaturase activity indices in the Longissimus dorsi muscle of lambs fed diets with different starch levels and calcium salts of palm fatty acids.....	81

LISTA DE FIGURAS

II - Dietary starch level determines the efficacy of calcium salts of palm fatty acids on nitrogen use efficiency and performance in feedlot lambs.

- Figure 1. Interaction effects between dietary starch level (Low: 220 g/kg; High: 420 g/kg DM) and calcium salts of palm fatty acids (CSPFA; 0 or 30 g/kg DM) on (A) Ether extract intake, (B) Starch intake, and (C) Starch digestibility in feedlot lambs. Bars represent least square means with values displayed on top; error bars indicate the standard error of the mean (SEM). Dotted lines indicate specific pairwise contrasts with their respective P-values. Note: Starch digestibility is expressed in g/kg.....47
- Figure 2. Interaction effects between dietary starch level (Low: 220 g/kg; High: 420 g/kg DM) and calcium salts of palm fatty acids (CSPFA; 0 or 30 g/kg DM) on nitrogen metabolism parameters: (A) Nitrogen retained, (B) Uric acid excretion, (C) Total purine derivatives, and (D) Microbial nitrogen synthesis in feedlot lambs. Bars represent least square means with numerical values; error bars indicate the standard error of the mean (SEM). Dotted lines indicate pairwise P-values for specific contrasts.49
- Figure 3. Interaction effects between dietary starch level (Low: 220 g/kg; High: 420 g/kg DM) and calcium salts of palm fatty acids (CSPFA; 0 or 30 g/kg DM) on serum metabolites: (A) Creatinine and (B) Urea concentrations in feedlot lambs. Bars represent least square means with numerical values; error bars indicate the standard error of the mean (SEM). Dotted lines indicate pairwise P-values for specific contrasts.....53
- Figure 4. Interaction effects between dietary starch level (Low: 220 g/kg; High: 420 g/kg DM) and calcium salts of palm fatty acids (CSPFA; 0 or 30 g/kg DM) on growth performance: (A) Final body weight, (B) Average daily gain (ADG), and (C) Feeding efficiency in feedlot lambs. Bars represent least square means with numerical values; error bars indicate the standard error of the mean.....54

III - Effects of Dietary Starch Level and Calcium Salts of Palm Fatty Acids on Carcass Traits and Meat Quality of Lambs.

- Figure 1. Interactive Effects of Dietary Starch and Calcium Salts of Palm Fatty Acids on 24-h Carcass Temperature (A) and Polyunsaturated Fatty Acids in Muscle (B) of Lambs.78

RESUMO GERAL

Este estudo avaliou os efeitos interativos da concentração de amido dietético (220 ou 420 g/kg de MS) e da suplementação com sais de cálcio de ácidos graxos de palma (CSPFA; 0 ou 30 g/kg de MS) sobre o metabolismo de nutrientes, o desempenho produtivo e as características de carcaça de cordeiros confinados. Trinta e dois cordeiros Dorper × Santa Inês foram distribuídos em delineamento inteiramente casualizado, em arranjo fatorial 2 × 2, por 44 dias. Foi observada interação significativa entre o teor de amido e a suplementação com CSPFA para o metabolismo do nitrogênio (N) e o desempenho, indicando que os efeitos do CSPFA dependem do suprimento energético da dieta. Em dietas com alto teor de amido, a suplementação com CSPFA reduziu a concentração de ureia sérica em 43%, aumentou o fluxo de N microbiano em 29% e elevou a retenção de N em 22%. Essas respostas metabólicas foram vinculadas a incrementos de 35% no ganho médio diário e de 22% na eficiência alimentar. Em contraste, em dietas com baixo teor de amido, a suplementação com CSPFA não promoveu melhorias no desempenho, possivelmente em função da limitação no suprimento de energia fermentescível para a síntese de proteína microbiana. Em relação às características de carcaça, o rendimento não foi influenciado pelos tratamentos (média de 49,4%). No entanto, a suplementação com CSPFA aumentou a deposição de gordura, duplicando a espessura de gordura subcutânea (3,8 vs. 1,9 mm) e elevando a massa de gordura perirrenal. Observou-se ainda interação para o resfriamento da carcaça, na qual dietas com alto teor de amido reduziram a temperatura após 24 horas apenas nos cordeiros não suplementados com CSPFA. Embora a suplementação com CSPFA tenha melhorado o acabamento e o isolamento térmico da carcaça, também promoveu alterações no perfil de ácidos graxos do músculo, caracterizadas pelo aumento de C16:0 e dos ácidos graxos saturados totais, concomitantemente à redução dos ácidos graxos poli-insaturados. Em síntese, o CSPFA derivado do óleo de palma melhora a eficiência do uso do nitrogênio e o desempenho produtivo sob dietas com alto teor de amido e aumenta o acabamento de gordura da carcaça independentemente do nível de amido.

Palavras-chave: amido dietético; carcaça de cordeiros; eficiência do nitrogênio; gordura protegida; proteína microbiana.

GENERAL ABSTRACT

This study evaluated the interactive effects of dietary starch concentration (220 or 420 g/kg DM) and supplementation with calcium salts of palm fatty acids (CSPFA; 0 or 30 g/kg DM) on nutrient metabolism, productive performance, and carcass characteristics of feedlot lambs. Thirty-two Dorper $\times$ Santa Inês lambs were randomly assigned to a fully randomized design in a 2×2 factorial arrangement for 44 days. A significant interaction between dietary starch level and CSPFA supplementation was observed for nitrogen (N) metabolism and performance, indicating that the metabolic responses to CSPFA depend on dietary energy supply. In high-starch diets, CSPFA supplementation reduced serum urea concentration by 43%, increased microbial N flow by 29%, and improved N retention by 22%. These metabolic responses were associated with increases of 35% in average daily gain and 22% in feed efficiency. In contrast, under low-starch conditions, CSPFA supplementation did not enhance performance, likely due to limitations in fermentable energy availability for microbial protein synthesis. Regarding carcass traits, dressing percentage was not affected by the treatments (mean = 49.4%). However, CSPFA supplementation increased fat deposition, doubling subcutaneous fat thickness (3.8 vs. 1.9 mm) and increasing perirenal fat mass. An interaction was also detected for carcass chilling, whereby high-starch diets reduced 24-h carcass temperature only in lambs that did not receive CSPFA. Although CSPFA supplementation improved carcass finish and thermal insulation, it also altered the muscle fatty acid profile by increasing C16:0 and total saturated fatty acids while reducing polyunsaturated fatty acids. In conclusion, CSPFA derived from palm oil improves nitrogen use efficiency and growth performance under high-starch diets and enhances carcass fatness regardless of starch level; however, it may negatively affect the nutritional quality of lamb meat by modifying its fatty acid composition.

Keywords: carcass; dietary starch; lamb; microbial protein; nitrogen use efficiency; protected fat;

1. CAPÍTULO I

Referencial Teórico

1.1. Introdução

A intensificação dos sistemas de produção de ovinos tem imposto desafios crescentes relacionados à eficiência de utilização dos nutrientes, ao desempenho produtivo e à sustentabilidade dos sistemas, especialmente em regimes de confinamento. Nesse contexto, a formulação de dietas com elevada densidade energética assume papel central, uma vez que o suprimento adequado de energia é determinante para sustentar altas taxas de crescimento, eficiência alimentar e respostas zootécnicas consistentes. A eficiência nutricional, portanto, emerge como um eixo estratégico na ovinocultura intensiva moderna, exigindo arranjos dietéticos que maximizem o aproveitamento dos nutrientes sem comprometer a estabilidade metabólica e ruminal.

Entre os nutrientes dietéticos, a energia fermentescível destaca-se como o principal fator regulador da atividade microbiana no rúmen e, consequentemente, do desempenho animal. Em sistemas intensivos, o amido constitui a principal fonte de energia dietética, sendo amplamente utilizado para atender à elevada demanda energética associada ao rápido crescimento e à intensificação da produção (Ma *et al.*, 2022). No entanto, a elevada taxa fermentativa desses carboidratos impõe um desafio metabólico relevante: a redução do pH ruminal, que pode comprometer a estabilidade do ecossistema microbiano. Esse ambiente desfavorável promove uma assincronia entre a liberação de amônia oriunda da degradação ruminal da proteína e a disponibilidade de ATP para os microrganismos, reduzindo a eficiência da síntese de proteína microbiana no rúmen e refletindo negativamente sobre o consumo, a digestibilidade dos nutrientes e o desempenho animal (Seo *et al.*, 2010; Chen *et al.*, 2022; de Oliveira *et al.*, 2025).

Além de seus efeitos sobre a fermentação ruminal e a eficiência microbiana, dietas ricas em amido influenciam diretamente o metabolismo lipídico no rúmen. A instabilidade do ambiente ruminal altera as vias de biohidrogenação, modificando o fluxo de isômeros de ácido linoleico conjugado (CLA) e de ácidos graxos poli-insaturados (AGPI) para o duodeno, o que pode repercutir sobre o particionamento energético, a deposição de gordura nos tecidos e, consequentemente, sobre o perfil lipídico e a qualidade da carne (Toral *et al.*, 2018; Van Le *et al.*, 2019). Assim, embora o amido seja essencial para elevar a

densidade energética da dieta, sua utilização em níveis elevados demanda estratégias complementares que atenuem seus efeitos adversos sobre a estabilidade ruminal.

Nesse cenário, a suplementação com sais de cálcio de ácidos graxos (SCAG) surge como uma alternativa tecnológica para aumentar o aporte energético da dieta sem intensificar o desafio fermentativo imposto pelo amido. Em condições adequadas, os SCAG contornam a biohidrogenação ruminal, permitindo o fornecimento de ácidos graxos de cadeia longa diretamente ao intestino delgado, contribuindo para o suprimento energético, o particionamento de nutrientes e a deposição lipídica nos tecidos corporais (Lashkari *et al.*, 2020; Wang *et al.*, 2020). Todavia, a eficácia dessa estratégia é estritamente dependente da homeostase ruminal. Sob condições de pH ácido ($\text{pH} < 5,8$), frequentemente associadas a dietas com elevado teor de amido, ocorre a dissociação precoce dos sais de cálcio, com liberação de ácidos graxos livres capazes de exercer toxicidade microbiana, comprometer a digestibilidade dos nutrientes e reduzir a previsibilidade das respostas produtivas (Frank *et al.*, 2022; Shpirer *et al.*, 2024).

Além do aporte energético, a utilização de SCAG pode influenciar diretamente o particionamento de energia e a composição dos tecidos. Nesse contexto, a fonte lipídica empregada na produção dos SCAG torna-se relevante, uma vez que sais derivados de óleo de palma, caracterizados por maior proporção de ácidos graxos saturados, especialmente o ácido palmítico (C16:0), podem afetar de forma distinta a deposição de gordura, o perfil de ácidos graxos da carne e as características de carcaça, quando comparados a sais oriundos de fontes mais insaturadas. Dessa forma, a interação entre o nível de amido da dieta e a suplementação com SCAG, particularmente aqueles derivados de óleo de palma, assume importância não apenas para o desempenho produtivo, mas também para a qualidade do produto final.

Nesse arranjo nutricional, o nitrogênio assume papel integrador. Estimativas indicam que entre 60% e 80% do nitrogênio ingerido por ruminantes é excretado, resultando em perdas econômicas e impactos ambientais relevantes, como emissões de amônia e lixiviação de nitratos (Hutchings *et al.*, 2020; Plomaritou *et al.*, 2025). Considerando que a síntese de proteína microbiana no rúmen responde por aproximadamente 50% a 80% da proteína metabolizável absorvida pelo animal (Lima *et al.*, 2023; de Oliveira *et al.*, 2025), a eficiência de utilização do nitrogênio passa a ser diretamente dependente da sincronia entre a oferta de energia fermentescível, principalmente na forma de amido, e a estabilidade ruminal necessária para a ação efetiva dos SCAG. Assim, ajustes no nível de amido e na suplementação lipídica têm potencial

para modular simultaneamente a fermentação ruminal, o aproveitamento energético e a eficiência de utilização do nitrogênio (Rodrigues *et al.*, 2026; Scoresby *et al.*, 2025).

Apesar da relevância prática dessa interação nutricional, a literatura científica disponível permanece fragmentada, abordando predominantemente os efeitos isolados do nível de amido ou da suplementação com gordura protegida sobre variáveis específicas, como fermentação ruminal, consumo, desempenho animal ou qualidade dos produtos de origem animal (Costa *et al.*, 2017; Orzuna-Orzuna *et al.*, 2023). Persiste, portanto, uma lacuna relevante quanto à compreensão integrada dos efeitos interativos entre o nível de amido da dieta e a suplementação com SCAG, especialmente no que se refere às respostas conjuntas de consumo, digestibilidade, desempenho produtivo, eficiência de utilização do nitrogênio, características de carcaça e qualidade da carne.

Dessa forma, o presente referencial teórico tem como objetivo integrar e sistematizar os conhecimentos atuais sobre (1.2) o metabolismo do amido em pequenos ruminantes, (1.3) o metabolismo e a aplicação dos lipídios protegidos, (1.4) as interações entre amido e sais de cálcio de ácidos graxos em dietas para pequenos ruminantes, e (1.5) o efeito do amido e da gordura protegida sobre características de carcaça e qualidade da carne de pequenos ruminantes. Essa abordagem fornece a base conceitual necessária para a compreensão dos mecanismos nutricionais e metabólicos avaliados nos capítulos subsequentes desta tese, os quais investigam, respectivamente, os efeitos dessa interação sobre o consumo, a digestibilidade, o desempenho e a eficiência de utilização do nitrogênio em cordeiros confinados, bem como sobre as características de carcaça e a qualidade da carne, com destaque para a utilização de SCAG derivados de óleo de palma.

1.2. Metabolismo do amido em pequenos ruminantes: da ingestão ao aproveitamento energético

Os carboidratos representam a fração quantitativamente predominante da matriz vegetal e constituem a principal fonte de energia para ruminantes. Sob a ótica nutricional, distingue-se entre os carboidratos estruturais, componentes da parede celular vegetal, como celulose e hemicelulose, e os carboidratos não fibrosos (CNF), que compreendem as reservas energéticas intracelulares e as frações solúveis, incluindo a pectina (Ponnampalam *et al.*, 2024). Entre os CNF, o amido destaca-se pela elevada fermentabilidade ruminal e pelo expressivo potencial glicogênico pós-absortivo, desempenhando papel central no suporte ao desempenho produtivo em sistemas intensivos. Entretanto, o aumento da densidade energética da dieta por meio do amido impõe limites fisiológicos claros, uma

vez que seus efeitos positivos dependem da manutenção da estabilidade do ambiente ruminal.

Quimicamente, o amido é constituído por α -glucanos organizados em duas frações estruturais distintas: amilose, de cadeia linear, e amilopectina, de cadeia altamente ramificada. Entre os cereais utilizados na alimentação de pequenos ruminantes, o milho (*Zea mays L.*) consolidou-se como a principal fonte de amido em dietas para ovinos (Valadares Filho *et al.*, 2006). Todavia, o valor nutricional do amido não é determinado apenas pelo seu teor na matéria seca, mas sobretudo pela sua taxa e extensão de degradação ruminal. Embora milho e sorgo apresentem teores semelhantes de amido, próximos a 72% da matéria seca, o sorgo apresenta menor digestibilidade e menor velocidade de degradação, resultando em menor aproveitamento energético (Gao *et al.*, 2022). Em contrapartida, fontes como trigo e cevada exibem taxas de degradação ruminal mais elevadas, variando entre 75% e 90%, o que promove maior disponibilidade imediata de substrato fermentescível (Ma *et al.*, 2022; Shipandeni *et al.*, 2023).

A intensificação da fermentação ruminal associada ao aumento da disponibilidade de amido reflete-se em respostas produtivas e metabólicas que variam conforme o nível de inclusão e a fonte amilácea. Resultados sintetizados na Tabela 1 demonstram que dietas contendo amido em concentrações variando aproximadamente de 27,1% a 43,6% da matéria seca estão associadas ao aumento da fermentação ruminal e a melhorias no ganho médio diário e na conversão alimentar (Guo *et al.*, 2021; Monjezi *et al.*, 2022). Entretanto, à medida que o teor de amido se aproxima dos valores mais elevados desse intervalo, observam-se efeitos adversos sobre o consumo e, principalmente, sobre a digestibilidade da fração fibrosa. Em ovinos e caprinos alimentados com dietas contendo 42% de amido, Rodrigues *et al.* (2026) relataram redução significativa da digestibilidade da fibra em detergente neutro corrigida para cinzas e proteína (FDNcp), de 0,74 para 0,61, concomitantemente à redução do pH ruminal, evidenciando o impacto da intensificação fermentativa sobre o aproveitamento da fibra.

No ambiente ruminal, a degradação do amido é conduzida predominantemente por bactérias amilolíticas (Zhang *et al.*, 2019), resultando na produção de ácidos graxos de cadeia curta (AGCC), principalmente acetato, propionato e butirato, que constituem a principal fonte de energia metabolizável para o animal. O perfil de AGCC produzido varia de acordo com a fonte amilácea e exerce influência direta sobre o metabolismo energético. Dietas compostas por grãos de rápida degradação, como trigo e cevada, tendem a gerar proporções aproximadas de 55:35:10 (acetato:propionato:butirato), elevando a produção

de propionato, principal precursor da gliconeogênese hepática, enquanto dietas baseadas em milho moído grosso produzem perfil mais acetogênico, próximo de 65:25:10 (Ma *et al.*, 2022; Zhang *et al.*, 2022). Esse desvio do perfil fermentativo contribui para explicar os resultados observados por Monjezi *et al.* (2022), nos quais variações no teor de amido entre 27,1% e 43,6% da matéria seca não alteraram o consumo de matéria seca, mas promoveram melhora no ganho médio diário e na conversão alimentar (Tabela 1).

Além da produção de AGCC, a fermentação do amido fornece ATP para a microbiota ruminal, estimulando a síntese de proteína microbiana (Toral *et al.*, 2018). Contudo, esse benefício energético está diretamente associado ao risco de ocorrência da acidose ruminal subaguda (SARA), condição caracterizada pela redução prolongada do pH ruminal para valores inferiores a 5,8–5,6 (Kleen *et al.*, 2003; Abdela, 2016). Assim, dietas com alta proporção de amidos rapidamente degradáveis podem elevar a produção total de ácidos graxos voláteis (AGVs) e reduzir o pH ruminal para valores críticos, o que pode levar ao acúmulo transitório de ácido lático em situações de desequilíbrio fermentativo, como relatado em sistemas com elevada inclusão de trigo (Abdela, 2016; Guo *et al.*, 2021; Shipandeni *et al.*, 2023). No entanto, em condições de confinamento, o principal desafio metabólico é o acúmulo de AGVs e a manutenção do pH acima de 5,8 para preservar a microbiota fibrolítica.

Clinicamente, a SARA manifesta-se por redução do consumo voluntário, diarreia, laminite e maior incidência de abscessos hepáticos (Kleen *et al.*, 2003; Frank *et al.*, 2022). Esses efeitos ajudam a interpretar os resultados contrastantes observados nos estudos compilados na Tabela 1, nos quais dietas com maior teor de amido aumentam o ganho médio diário e o consumo de matéria seca (Guo *et al.*, 2021), mas podem comprometer a eficiência alimentar quando a digestibilidade da fibra é severamente reduzida, como observado em dietas contendo 42% de amido (Rodrigues *et al.*, 2026). Embora ocorram adaptações fisiológicas e microbianas, como aumento da área das papilas ruminais e maior participação de bactérias amilolíticas do gênero *Prevotella*, tais mecanismos não conferem proteção completa contra a SARA quando o manejo dietético é inadequado (Farenzena *et al.*, 2017; Guo *et al.*, 2021).

Paralelamente, a fração do amido que escapa da degradação ruminal, denominada amido de escape, é digerida no intestino delgado, fornecendo glicose de absorção direta (Urrutia *et al.*, 2020). Evidências experimentais indicam que a modulação da fonte amilácea pode influenciar esse equilíbrio, como demonstrado por Zhang *et al.* (2022), que observaram melhora da fermentação ruminal com a substituição de 33% do amido de milho

por amido de cevada, sem alteração no consumo de matéria seca (Tabela 1). Isso ocorre, devido à melhora na fermentação ruminal observada com a substituição parcial do amido de milho por amido de cevada, que pode ser atribuída à maior taxa de degradação ruminal da cevada, que proporciona uma melhor sincronia entre a disponibilidade de energia fermentescível e o nitrogênio degradável no rúmen (Huntington, 1997; Offner; Bach; Sauvant, 2003). Essa sincronia otimiza a síntese de proteína microbiana e a produção de ácidos graxos voláteis, especialmente o propionato, aumentando o aporte energético para o animal sem a necessidade de elevar o consumo de matéria seca.

Dessa forma, o aproveitamento eficiente do amido em pequenos ruminantes depende da adoção de estratégias integradas de nutrição de precisão, que envolve a escolha da fonte amilácea, o nível de inclusão e o processamento físico dos grãos. O milho, por apresentar taxa de degradação moderada, ou a combinação de fontes com diferentes velocidades fermentativas constitui estratégia fundamental para mitigar riscos metabólicos (Pittaluga *et al.*, 2022). O processamento físico deve ser ajustado criteriosamente, sendo essencial para elevar a digestibilidade do sorgo, mas potencialmente deletério quando acelera excessivamente a fermentação de grãos como trigo e cevada (Gao *et al.*, 2022; Shipandeni *et al.*, 2023). O uso de aditivos moduladores em conjunto com práticas adequadas de manejo alimentar, como adaptação gradual às dietas e fracionamento das refeições, complementa esse conjunto de estratégias (Zhang *et al.*, 2024).

Em síntese, o metabolismo do amido em pequenos ruminantes evidencia que respostas produtivas positivas ocorrem em uma faixa relativamente ampla de inclusão, entre 27,1% e 43,6% da matéria seca, porém com custos metabólicos crescentes à medida que o teor de amido se aproxima de níveis mais elevados.

Tabela 1. Principais estudos avaliando o efeito de níveis de amido sobre parâmetros nutricionais e produtivos em pequenos ruminantes

Referência	Espécie / Raça	Tratamentos/ Dietas	Efeito/ Conclusão
(Rodrigues <i>et al.</i> , 2026)	Ovino Dorper / Caprino Boer	22% amido; 42% amido; 22% amido + 3% SCAGP; 42% amido + 3% SCAGP	O nível de amido na dieta influenciou a ingestão de fibra em detergente neutro. Animais alimentados com dietas contendo 42% de amido passaram mais tempo se alimentando e ruminando do que com dietas contendo 22% de amido. O aumento da concentração de amido na dieta intensifica a fermentação ruminal e reduz a digestibilidade da fibra.
(Guo <i>et al.</i> , 2021)	Ovino Suffolk	Fontes de Fibra: Polpa de beterraba (BP) vs. Casca de soja (SH). • Níveis de Amido: Baixo (22%), Médio (25%) e Alto (27%). • Arranjo: Fatorial 2x3 (6 tratamentos no total).	Para a dieta alto amido, o consumo de matéria seca e o ganho médio diário aumentaram. A concentração de $\text{NH}_3 \cdot \text{N}$ no fluido ruminal foi maior em ovelhas alimentadas com a dieta de silagem de alfafa do que com a dieta de feno de alfafa, e maior na dieta de alto amido em relação à dieta de baixo amido.
(Zhang <i>et al.</i> , 2022)	Ovino Hu	100% amido de milho; 67% AM + 33% AC; 33% AM + 67% AC; 100% amido de cevada	A substituição de 33% do amido de milho por amido de cevada melhorou a fermentação ruminal. Não houve diferença no consumo de matéria seca entre os tratamentos.
(Monjezi <i>et al.</i> , 2022)	Ovino Árabe	Alto amido (43,6% MS); médio amido (35,4% MS); baixo amido (27,1% MS)	Variações no teor de amido (27,1% a 43,6% MS) não influenciaram o consumo de matéria seca. Houve melhora no ganho total, ganho médio diário e conversão alimentar.
(Lunesu <i>et al.</i> , 2021)	Ovino Sarda / Caprino Saanen	Dieta com alto teor de amido (20% MS) vs. dieta com baixo teor de amido (7,8% MS)	Em cabras, a dieta com 20% de amido favoreceu a produção de leite; em ovelhas, favoreceu o acúmulo de reservas corporais. A substituição parcial do amido por fibra de alta digestibilidade aumentou a partição de energia para produção de leite nas ovelhas.

¹Sais de cálcio de ácidos graxos da palma, ²Amido degradável no rúmen, AM: amido de milho; AC: amido de cevada; AA: alto amido; MA: médio amido; BA: baixo amido; CMS: consumo de matéria seca; MS: matéria seca.

1.3. Metabolismo e aplicação dos lipídios protegidos em pequenos ruminantes

A suplementação lipídica em dietas para ovinos visa, primordialmente, elevar a densidade energética da ração. No entanto, essa estratégia encontra uma barreira fisiológica determinante: o fornecimento de lipídios convencionais em níveis superiores a 5% da matéria seca (MS) exerce efeito tóxico sobre a microbiota ruminal, inibindo predominantemente as bactérias celulolíticas e comprometendo o consumo de MS (Gümüş *et al.*, 2022). Diante desse impasse, as tecnologias de proteção ruminal, como os Sais de Cálcio de Ácidos Graxos (SCAG), consolidaram-se como a alternativa mais difundida para permitir o *bypass* da biohidrogenação (Bulcão *et al.*, 2021).

A relevância funcional desta tecnologia reside na sua proteção seletiva dependente de pH: os complexos permanecem inertes no ambiente ruminal, dissociando-se apenas no meio ácido do abomaso para posterior absorção intestinal (Lashkari *et al.*, 2020). A relevância funcional da gordura protegida reside na sua proteção seletiva dependente de pH: os complexos permanecem estáveis no ambiente ruminal ($\text{pH} > 6,0$), mas iniciam sua dissociação à medida que o pH declina para valores inferiores a 5,5. A liberação completa dos ácidos graxos ocorre no ambiente altamente ácido do abomaso ($\text{pH} 2,0$ a $3,0$), onde a alta concentração de íons de hidrogênio promove a quebra das ligações entre o cálcio e os grupos carboxila dos ácidos graxos, permitindo sua posterior absorção no intestino delgado (Jenkins, 1993).

Todavia, a eficiência desse processo depende da estabilidade do ambiente fermentativo. Em dietas com alta densidade energética, a interação entre carboidratos e lipídios torna-se complexa; Rodrigues *et al.* (2026) demonstraram que caprinos e ovinos apresentam ajustes distintos no metabolismo pós-ruminal ao integrarem a energia derivada do amido (22% vs. 42%) com o suprimento de 3% de SCAG, evidenciando respostas espécie-específicas na cinética digestiva (Tabela 2).

O mecanismo de ação dos SCAG é, primariamente, indireto e substitutivo. Ao permitirem a redução da carga de carboidratos de rápida fermentação, contribuem para a estabilização do pH (Shpirer *et al.*, 2024). Evidências experimentais compiladas na Tabela 2 corroboram que, em níveis adequados, os SCAG mantêm a homeostase ruminal. Simionatto *et al.* (2024), ao testarem a inclusão de gordura protegida (GP) em níveis de 2%, 4% e 6% da MS, não observaram alterações na ingestão de nutrientes ou no pH ruminal, embora tenham relatado aumento na concentração de nitrogênio amoniacal. De forma análoga, Behan *et al.* (2019) demonstraram que a inclusão de aproximadamente 5% de gordura granulada, ou GP, não afetou o consumo e a digestibilidade de MS, MO e

frações fibrosas (FDN e FDA), reforçando a característica inerte desses compostos frente à microbiota fibrolítica.

A resposta produtiva à suplementação é expressiva em sistemas intensivos. A inclusão de 3,5% de SCAG na dieta de cordeiros confinados resultou em aumento no peso de abate e rendimento de carcaça, além de melhoria no acabamento (Alba *et al.*, 2021). No metabolismo sistêmico, a GP (3% da MS) tendeu a elevar o ganho médio diário (GMD) e incrementou as concentrações sanguíneas de glicose e colesterol (Kandi *et al.*, 2020). Em fêmeas lactantes, os benefícios estendem-se à persistência e qualidade do produto: a inclusão de 2% de GP promoveu melhora na condição corporal, enquanto o nível de 6% elevou a produção e o teor de gordura do leite em ovelhas Lacaune (Bianchi *et al.*, 2018; Tabela 2).

Conforme evidenciado pelos estudos sumarizados na Tabela 2, a utilização de gordura protegida via sais de cálcio contorna as limitações biológicas da suplementação lipídica convencional. A manutenção da estabilidade ruminal, aliada ao aumento do fluxo de energia pós-ruminal, consolida essa tecnologia como uma ferramenta eficaz para a maximização do desempenho em sistemas intensivos de produção de pequenos ruminantes.

Tabela 1. Síntese de estudos experimentais sobre a suplementação com sais de cálcio de ácidos graxos e fontes lipídicas em pequenos ruminantes.

Referência	Espécie/ Raça	Tratamentos/ Dietas	Efeito/ Conclusão
(Rodrigues <i>et al.</i> , 2026)	Ovino Dorper / Caprino Boer	22% amido; 42% amido; 22% amido + 3% SCAGP; 42% amido + 3% SCAGP	A suplementação com SCAGP e a espécie revelaram ajustes distintos na fermentação ruminal e no metabolismo pós-ruminal, indicando que caprinos e ovinos diferem não apenas na cinética digestiva, mas também na forma como integram a energia derivada do amido com o suprimento de lipídios.
(Simionatto <i>et al.</i> , 2024)	SRD	Dieta controle sem GP; inclusão de GP a 2%, 4% e 6% da MS	A adição de SCAGP não afetou a ingestão de nutrientes, digestibilidade, balanço de nitrogênio ou pH ruminal, mas aumentou a concentração de nitrogênio amoniacal. Recomenda-se a inclusão de até 6% de GP na MS.
(Alba <i>et al.</i> , 2021)	Ovino Dorper x Santa Inês	Sem adição de gordura; soja integral; SCAG (3,5% da MS); óleo de soja; gérmen de milho	A inclusão de 3,5% de SCAG na dieta total de cordeiros confinados aumentou o peso de abate, o peso e o rendimento da carcaça, além de melhorar a conformação e o acabamento.
(Kandi <i>et al.</i> , 2020)	Ovino Farahan i	Dietas com diferentes níveis de PB (18% e 21%), com ou sem GP (3% da MS)	A suplementação com GP tendeu a aumentar o GMD e elevou as concentrações sanguíneas de glicose e colesterol. Não houve influência sobre o CMS.

(Behan <i>et al.</i> , 2019)	Ovino Dorper	Dieta basal sem GP (controle); gordura granulada (~5% da MS); gordura + lecitina; GP	Os consumos e digestibilidades de MS, MO, PB, FDN e FDA não foram afetados. CMS e relação ganho/ração também não apresentaram diferenças entre os tratamentos.
(Bianchi <i>et al.</i> , 2018)	Ovino Lacaune	Inclusão de 2%, 4% e 6% de GP na MS	A adição de 2% de GP promoveu ganho de peso e melhoria da condição corporal. A inclusão de 6% aumentou a produção e o teor de gordura do leite.

SRD: sem raça definida; GP: gordura protegida; SCAGP: sais de cálcio de ácidos graxos da palma; GMD: ganho médio diário; CMS: consumo de matéria seca; IFDN: ingestão de fibra em detergente neutro; MS: matéria seca; MO: matéria orgânica; PB: proteína bruta; FDN: fibra em detergente neutro; FDA: fibra em detergente ácido, GP: gordura protegida.

1.4. Interação entre amido e lipídios protegidos em dietas para pequenos ruminantes

A interação entre carboidratos não fibrosos (CNF), como o amido, e lipídios protegidos na forma de sais de cálcio de ácidos graxos (SCAG) constitui um eixo central na modulação do metabolismo ruminal e na eficiência energética de dietas para ovinos de alto desempenho (Ferraretto *et al.*, 2013). Mais do que uma simples soma nutricional, essa relação pode gerar efeitos sinérgicos ou antagônicos, dependendo do equilíbrio dietético e da resposta do ecossistema ruminal.

Sob a perspectiva bioquímica, parte do NADH gerado na fermentação do amido pode ser desviada da rota típica de biohidrogenação pela presença de SCAG, favorecendo maior fluxo de ácidos graxos poli-insaturados (AGPI) para o trato pós-ruminal (Lashkari *et al.*, 2020; Toral *et al.*, 2018). Conforme evidenciado na Tabela 3, essa associação promoveu incremento de até 15% na passagem de AGPI para o intestino. Além disso, respostas metabólicas variam entre espécies: caprinos apresentaram maior turnover lipídico e níveis plasmáticos de triglicerídeos superiores aos dos ovinos (Rodrigues *et al.*, 2026), conforme apresentado na Tabela 3.

No nível fermentativo, os SCAG atuam como moduladores da fermentação amilolítica através de mecanismos de substituição e diluição. Por serem fontes densas e não fermentescíveis, reduzem a carga fermentativa e a taxa de acumulação de ácidos orgânicos. Níveis de amido considerados moderados (aprox. 25% da MS) favorecem a produção de propionato sem comprometer severamente o pH ruminal, o que, em sinergia com os SCAG (~30 g/kg MS), resultou em redução de até 12% na emissão de metano entérico. Em condições de equilíbrio, essa sinergia potencializou o desempenho: o uso de concentrado associado a SCAG elevou o ganho médio diário de 200 g/dia para 230 g/dia

(Nobre *et al.*, 2016), conforme mostrado na Tabela 3, enquanto o amido isolado aumentou em até 50 g/dia, embora com maior risco de acidose (Tabela 4).

Entretanto, a eficácia dos SCAG depende da homeostase ruminal. Em ambientes com $\text{pH} < 5,8$, sua estabilidade é comprometida, reduzindo a eficácia em cerca de 20% devido à dissociação precoce, como evidenciado na Tabela 3 (Lashkari *et al.*, 2020). Uma vez dissociados, os ácidos graxos livres podem recobrir fisicamente partículas e exercer toxicidade sobre a microbiota fibrolítica. Além disso, níveis elevados de suplementação podem dificultar a fermentação do amido ao limitar o acesso enzimático ao substrato.

Diferentemente dos óleos vegetais livres, que podem recobrir fisicamente as partículas de fibra e exercer toxicidade direta sobre a microbiota fibrolítica devido ao seu alto teor de ácidos graxos poli-insaturados, a utilização de sais de cálcio de ácidos graxos de palma (SCAGP) minimiza esses efeitos. Por serem predominantemente saturados e apresentarem-se na forma de sabões insolúveis no pH ruminal, os SCAGP permanecem inertes, evitando a inibição da digestão da fibra e permitindo o aumento da densidade energética da dieta sem prejuízos fermentativos (Santos *et al.*, 2020)

Portanto, a otimização metabólica requer o balanceamento dos níveis de amido e SCAG em associação à fração fibrosa da dieta. Conforme sintetizado na Tabela 4, essa interação promove ganhos produtivos e enriquecimento lipídico, como o aumento de 18% no CLA, garantindo que os pilares energéticos atuem de forma convergente e não em desbalanço metabólico (Huntington, 1997; Owens *et al.*, 1998).

Tabela 2. Interações Amido + SCAGP

Referência	Variável	Resultados principais
(Rodrigues <i>et al.</i> , 2026)	Parâmetros sanguíneos	Caprinos apresentam maior turnover lipídico e níveis plasmáticos de triglicerídeos do que ovinos.
(Lashkari <i>et al.</i> , 2020)	Estabilidade dos SCAG	Comprometida em $\text{pH} < 5,8$; eficácia reduzida em 20%.
(Toral <i>et al.</i> , 2018)	Passagem de PUFA	↑ em 15% para intestino com SCAGP + amido
(NOBRE <i>et al.</i> , 2016)	Ganho de peso	↑ de 200 g/dia para 230 g/dia com concentrado + SCAGP

Tabela 3. Síntese Integrativa

Nutriente	Benefícios	Riscos/limitações	Estratégia prática
Amido	↑ Ganho de peso em até 50 g/dia; propionato ↑ 7%; pH pode cair para 5,7.	Acidose, redução da diversidade microbiana.	Uso moderado + correção da fibra
SCAGP	↑ Ganho de peso em 30 g/dia; CLA ↑ 18%; metano ↓ 12%.	Estabilidade comprometida em pH baixo; custo.	Inclusão controlada (~30 g/kg MS)
Amido + SCAGP	↑ PUFA em 15%; ↑ ganho de peso em 30 g/dia.	Acidose pode reduzir a eficácia dos SCAGP.	Balancear níveis de amido e SCAGP + fibra

1.5. Efeito do amido e da gordura protegida sobre características de carcaça e qualidade da carne de pequenos ruminantes

A manipulação da dieta de ruminantes em terminação é uma estratégia central para melhorar tanto o desempenho produtivo quanto o valor nutricional da carne. Entre os recursos mais estudados estão o uso de gordura protegida no rúmen (sabão de cálcio de ácidos graxos), a substituição parcial de amido por fontes alternativas de energia e a inclusão de aditivos funcionais como probióticos ou colina protegida. Os estudos recentes analisados nesta revisão permitem distinguir claramente os efeitos sobre carcaça e sobre qualidade da carne, conforme sintetizado na Tabela 5.

O encapsulamento de cevada e farelo de soja com sabão de cálcio de óleo de girassol (Karimi *et al.*, 2022) demonstrou reduzir a degradabilidade ruminal e aumentar a digestibilidade pós-ruminal, sem alterar desempenho ou características de carcaça. Esse resultado confirma que tecnologias de proteção lipídica podem ser aplicadas a carboidratos e proteínas sem comprometer parâmetros produtivos.

De forma semelhante, a suplementação materna com sais de cálcio de óleo de palma (Brochini *et al.*, 2022) não prejudicou o desempenho ou a carcaça da progênie, mas promoveu alterações metabólicas de longo prazo, resultando em carne com perfil lipídico otimizado, caracterizado pela redução proporcional de ácidos graxos saturados e pelo enriquecimento em CLA. Esse achado reforça que a dieta materna influencia a composição da carne dos descendentes.

Já dietas ricas em forragem e óleo, com baixo amido (Jerónimo *et al.*, 2026), mantiveram o ganho de peso e características de carcaça, mas foram menos eficientes em conversão alimentar e aumentaram a gordura perirrenal. Apesar do custo alimentar mais elevado, o perfil lipídico da carne foi superior, com maiores níveis de CLA e ômega-3.

A suplementação conjunta de gordura protegida e colina protegida (Liu *et al.*, 2025) mostrou que a colina atua como modulador metabólico, revertendo efeitos adversos da gordura isolada (como o aumento de ácidos graxos *trans* e redução do consumo). O resultado foi uma carne com perfil lipídico mais saudável ($\uparrow$ C16:1, $\downarrow$ C18:2n-6t) e sem prejuízo de desempenho ou carcaça.

A associação de *Clostridium butyricum* com gordura protegida (Zang *et al.*, 2024) evidenciou um efeito sinérgico: o probiótico reduziu a oxidação lipídica e aumentou a maciez, neutralizando o efeito pró-oxidante da gordura protegida. A carne resultante apresentou maior marmoreio, mais CLA e AGMI, além de maior vida de prateleira.

Os estudos analisados demonstram que a gordura protegida é eficaz para enriquecer a carne com ácidos graxos insaturados e CLA, mas pode resultar em efeitos adversos (oxidação, redução do consumo). Esses efeitos podem ser mitigados por aditivos funcionais (colina, probióticos), que modulam o metabolismo hepático ou aumentam a capacidade antioxidante do músculo.

Em relação às características de carcaça, as tecnologias de encapsulamento e a substituição parcial do amido demonstraram-se seguras, preservando o rendimento e a conformação dos cordeiros. O nível de amido dietético atua como o principal indutor da deposição de tecidos, uma vez que o maior fluxo de propionato ruminal estimula a secreção de insulina, criando um ambiente anabólico que favorece tanto a hipertrofia muscular quanto a deposição de gordura cavitária e subcutânea (Lima *et al.*, 2002). A relevância da interação entre o amido e a gordura protegida reside na sinergia metabólica, onde o amido provê o suporte energético necessário para a deposição dos ácidos graxos provenientes da suplementação lipídica. Complementarmente, do ponto de vista da qualidade da carne, a associação de gordura protegida com antioxidantes dietéticos permite a modulação do perfil lipídico para um produto mais saudável e funcional, sem comprometer a estabilidade oxidativa e a vida de prateleira (Jerónimo *et al.*, 2010).

Tabela 4. Principais resultados dos estudos sobre amido e gordura protegida em ruminantes.

Referências	Espécies	Tratamento	Principais Resultados
Karimi <i>et al.</i> , 2022	Cabritos Mahabadi	Encapsulação de cevada e soja com SCAG	↓ degradabilidade ruminal; ↑ digestibilidade pós-ruminal; desempenho e carcaça inalterados; ↑ proteína plasmática
Brochini <i>et al.</i> , 2022	Ovelhas (progênie)	Suplementação materna com Ca-PO	↑ CLA e AG insaturados na carne da progênie; ↓ AG saturados; carne mais funcional sem prejuízo sensorial.
Liu <i>et al.</i> , 2025	Cordeiros	Gordura protegida isolada vs. + colina protegida	Colina ↑ consumo; ↓ ácidos graxos <i>trans</i> ; ↑ C16:1; perfil lipídico mais saudável; carcaça inalterada.
Zang <i>et al.</i> , 2024	Cabras	Gordura protegida ± <i>Clostridium butyricum</i>	GPR ↑ marmoreio e CLA mas ↑ oxidação; CB ↑ maciez; carne mais macia, mais CLA, maior vida útil.
Jerónimo <i>et al.</i> , 2026	Cordeiros Merino Branco	Dieta alta forragem, baixo amido, + óleo de soja.	Desempenho mantido; ↑ gordura perirrenal; carne com perfil lipídico superior (↑ CLA, ↑ ômega-3, ↑ ácido vacênico, ↓ risco de <i>t10-shift</i>); qualidade sensorial preservada; custo alimentar ↑ 13%.

REFERÊNCIAS

ABDELA, Nejash. Sub-acute Ruminal Acidosis (SARA) and its Consequence in Dairy Cattle: A Review of Past and Recent Research at Global Prospective. **Achievements in the Life Sciences**, [N.p.], vol. 10, no. 2, pp. 187–196, Oct. 2016. Available at: <https://doi.org/10.1016/j.als.2016.11.006>.

ALBA, Henry D R; DE FREITAS JÚNIOR, José E; LEITE, Laudi C; AZEVÊDO, José A G; SANTOS, Stefanie A; PINA, Douglas S; CIRNE, Luís G A; RODRIGUES, Carlindo S; SILVA, Willian P; LIMA, Victor G O; TOSTO, Manuela S L; DE CARVALHO, Gleidson G P. Protected or Unprotected Fat Addition for Feedlot Lambs: Feeding Behavior, Carcass Traits, and Meat Quality. **Animals**, [N.p.], vol. 11, no. 2, p. 328, Oct. 2021. Available at: <https://doi.org/10.3390/ani11020328>.

BEHAN, Atique A; LOH, Teck Chwen; FAKURAZI, Sharida; KAKA, Ubedullah; KAKA, Asmatullah; SAMSUDIN, Anjas Asmara. Effects of Supplementation of Rumen Protected Fats on Rumen Ecology and Digestibility of Nutrients in Sheep. **Animals**, [N.p.], vol. 9, no. 7, p. 400, Oct. 2019. Available at: <https://doi.org/10.3390/ani9070400>.

BIANCHI, Anderson Elias; DE PAULO MACEDO, Vicente; SILVA, Aleksandro Schafer Da; DA SILVEIRA, André Luís Finkler; HILL, João Ari Gualberto; ZORTÉA, Talyta; ROSSI, Robson Marcelo; BATISTA, Rafael. Effect of the addition of protected fat from palm oil to the diet of dairy sheep. **Revista Brasileira de Zootecnia**, [N.p.], vol. 47, no. 0, Oct. 2018. Available at: <https://doi.org/10.1590/rbz4720160137>.

BROCHINE, Luciano; DOS SANTOS, Fernanda Ferreira; MOREIRA, Flávia Mallaco; DO VALLE DE ZOPPA, André Luis; LEME, Paulo Roberto; TEDESCHI, Luis Orlindo; GALLO, Sarita Bonagurio. The Impact of Fetal Programming in Ewe Nutrition with Chromium Propionate or Calcium Salts of Palm Oil on the Meat Quality and Bone of the Progeny. **Biological Trace Element Research**, [N.p.], vol. 201, no. 5, pp. 2331–2340, 27 May 2023. Available at: <https://doi.org/10.1007/s12011-022-03344-x>.

CHEN, Panliang; LI, Yan; SHEN, Yizhao; CAO, Yufeng; LI, Qiufeng; WANG, Meimei; LIU, Mingchao; WANG, Zhiyuan; HUO, Zihan; REN, Shuai; GAO, Yanxia; LI, Jianguo. Effect of Dietary Rumen-Degradable Starch to Rumen-Degradable Protein Ratio on In Vitro Rumen Fermentation Characteristics and Microbial Protein Synthesis. **Animals**, [N.p.], vol. 12, no. 19, p. 2633, 30 Sep. 2022. Available at: <https://doi.org/10.3390/ani12192633>.

COSTA, M.; ALVES, S. P.; FRANCISCO, A.; ALMEIDA, J.; ALFAIA, C. M.; MARTINS, S. V.; PRATES, J. A. M.; SANTOS-SILVA, J.; DORAN, O.; BESSA, R. J. B. The reduction of starch in finishing diets supplemented with oil does not prevent the accumulation of trans-10 18:1 in lamb meat1. **Journal of Animal Science**, [N.p.], vol. 95, no. 8, pp. 3745–3761, 1 Aug. 2017. Available at: <https://doi.org/10.2527/jas.2017.1578>.

DE ARAGÃO BULÇÃO, Lucas Fialho; ALBA, Henry Daniel Ruiz; DE CARVALHO, Gleidson Giordano Pinto; DE ARAÚJO, Maria Leonor Garcia Melo Lopes; GANDRA, Jefferson Rodrigues; RIBEIRO, Cláudio Vaz Di Mambro; DE FREITAS JÚNIOR, José Esler. Digestion, ruminal metabolism, and feeding behavior of buffaloes fed diets supplemented with soybean oil, whole and raw soybean, and calcium salts of fatty acids. **Tropical Animal Health and Production**, [N.p.], vol. 53, no. 2, p. 216, 20 May 2021. Available at: <https://doi.org/10.1007/s11250-021-02654-x>.

DE C VALADARES FILHO, S. **Tabelas brasileiras de composição de alimentos para bovinos**. [N.p.]: UFV, 2006.

DE OLIVEIRA, M.; COSTA, C.; FERNANDES, T. Graduate Student Literature Review: Concepts and challenges of amino acid supply and nitrogen metabolism in dairy cattle. **Journal of Dairy Science**, [N.p.], vol. 108, no. 7, pp. 6906–6916, Jul. 2025. Available at: <https://doi.org/10.3168/jds.2024-26136>.

DE SOUSA NOBRE, Ismael; DE SOUZA, Bonifácio Benício; DE ASSIS MARQUES, Bennio Alexandre; DE AZEVEDO, Aderbal Marcos; DE PÁDUA ARAÚJO, Rafael; DA SILVA GOMES, Thiago Lima; BATISTA, Luanna Figueiredo; DE ASSIS SILVA, Gustavo. Avaliação dos níveis de concentrado e gordura protegida sobre o desempenho produtivo e termorregulação de ovinos. **Revista Brasileira de Saúde e Produção Animal**, [N.p.], vol. 17, no. 1, pp. 116–126, Oct. 2016. Available at: <https://doi.org/10.1590/S1519-99402016000100011>.

FARENZENA, R.; KOZLOSKI, G. V.; GINDRI, M.; STEFANELLO, S. Minimum length of the adaptation and collection period in digestibility trials with sheep fed *ad libitum* only forage or forage plus concentrate. **Journal of Animal Physiology and Animal Nutrition**, [N.p.], vol. 101, no. 5, pp. 1057–1066, 24 Oct. 2017. Available at: <https://doi.org/10.1111/jpn.12550>.

FERRARETTO, L F; CRUMP, P M; SHAVER, R D. Effect of cereal grain type and corn grain harvesting and processing methods on intake, digestion, and milk production by dairy cows through a meta-analysis. **Journal of Dairy Science**, [N.p.], vol. 96, no. 1, pp. 533–550, Oct. 2013. Available at: <https://doi.org/10.3168/jds.2012-5932>.

LIMA, Láiße Garcia de; NUSSIO, Luiz Gustavo; GONÇALVES, José Renato Silva; SIMAS, José Manuel Correia de; PIRES, Alexandre Vaz; SANTOS, Flávio Augusto Portela. Fontes de amido e proteína para vacas leiteiras em dietas à base de capim-elefante. *Scientia Agricola*, v. 59, n. 1, p. 19-27, jan./mar. 2002.

FRANK, Eyal; LIVSHITZ, Lilya; PORTNICK, Yuri; KAMER, Hadar; ALON, Tamir; MOALLEM, Uzi. The Effects of High-Fat Diets from Calcium Salts of Palm Oil on Milk Yields, Rumen Environment, and Digestibility of High-Yielding Dairy Cows Fed Low-Forage Diet. **Animals**, [N.p.], vol. 12, no. 16, p. 2081, 15 Aug. 2022. Available at: <https://doi.org/10.3390/ani12162081>.

GAO, Chang; GAO, Dong; ZHANG, Qiuxu; WANG, Yuan; GAO, Aiqin. Performance, meat quality, intramuscular fatty acid profile, rumen characteristics and serum parameters of lambs fed microencapsulated or conventional linseed oil. **Czech Journal of Animal Science**, [N.p.], vol. 67, no. 9, pp. 365–373, 30 Sep. 2022. Available at: <https://doi.org/10.17221/77/2022-CJAS>.

GÜMÜŞ, Hıdır; KARAKAS OGUZ, Fatma; OĞUZ, Mustafa Numan; BUĞDAYCI, Kadir Emre; DAĞLI, Hüseyin. Effects of replacing grain feed with rumen-protected fat on feedlot performance, ruminal parameters and blood metabolites in growing Merino lambs' diets during the hot season. **Ankara Üniversitesi Veteriner Fakültesi Dergisi**, [N.p.], vol. 69, no. 2, pp. 131–138, 25 Mar. 2022. Available at: <https://doi.org/10.33988/auvfd.856477>.

GUO, Tongqing; WANG, Zhi Lan; GUO, Long; LI, Fadi; LI, Fei. Effects of supplementation of nonforage fiber source in diets with different starch levels on growth performance, rumen fermentation, nutrient digestion, and microbial flora of Hu lambs. **Translational Animal Science**, [N.p.], vol. 5, no. 2, Oct. 2021. Available at: <https://doi.org/10.1093/tas/txab065>.

HUNTINGTON, G B. Starch utilization by ruminants: from basics to the bunk. **Journal of Animal Science**, [N.p.], vol. 75, no. 3, p. 852, 1997. Available at: <https://doi.org/10.2527/1997.753852x>.

HUTCHINGS, Nicholas J.; SØRENSEN, Peter; CORDOVIL, Cláudia M.d.S.; LEIP, Adrian; AMON, Barbara. Measures to increase the nitrogen use efficiency of European agricultural production. **Global Food Security**, [N.p.], vol. 26, p. 100381, Sep. 2020. Available at: <https://doi.org/10.1016/j.gfs.2020.100381>.

JENKINS, T.C. Lipid Metabolism in the Rumen. **Journal of Dairy Science**, [N.p.], vol. 76, no. 12, pp. 3851–3863, Dec. 1993. Available at: [https://doi.org/10.3168/jds.S0022-0302\(93\)77727-9](https://doi.org/10.3168/jds.S0022-0302(93)77727-9).

JERÓNIMO, Eliana; GUERREIRO, Olinda; SILVA, Andreia; LAGE, Patrícia; ALVES, Hélder; ALMEIDA, João M.; ALVES, Susana P.; BESSA, Rui J. B.; SANTOS-SILVA, José. Growth Performance, Carcass Characteristics, and Meat Quality of Lambs Fed a High-Forage, Low-Starch, High-Oil Diet. **Foods**, [N.p.], vol. 15, no. 2, p. 193, 6 Jan. 2026. Available at: <https://doi.org/10.3390/foods15020193>.

KANDI, M.; KAZEMI-BONCHENARI, M.; HOSSEINYAZDI, M.; MIRZAEI, M. Effects of Ca-salt of linseed oil supplementation and protein content in diet on performance, ruminal fermentation, microbial protein yield, and blood metabolites in young lambs. **Small Ruminant Research**, [N.p.], vol. 193, p. 106257, Dec. 2020. Available at: <https://doi.org/10.1016/j.smallrumres.2020.106257>.

KARIMI, M.; GANJKHANLOU, M.; ZALI, A.; PARNIAN-KHAJEHDIZAJ, F.; KARIMI-AZANDARIYANI, S.; FATEHI, F.; TOWHIDI, A.; ZAKARIAPOUR, H.; KHANAKI, H. Encapsulation of soybean meal and barley grain with calcium soap of sunflower fatty acids: Effects on growth performance and carcass characteristics in

Mahabadi kids. **Small Ruminant Research**, [N.p.], vol. 215, p. 106764, Oct. 2022. Available at: <https://doi.org/10.1016/j.smallrumres.2022.106764>.

KLEEN, J. L.; HOOIJER, G. A.; REHAGE, J.; NOORDHUIZEN, J. P. T. M. Subacute Ruminant Acidosis (SARA): a Review. **Journal of Veterinary Medicine Series A**, [N.p.], vol. 50, no. 8, pp. 406–414, 20 Oct. 2003. Available at: <https://doi.org/10.1046/j.1439-0442.2003.00569.x>.

LASHKARI, Saman; BONEFELD PETERSEN, Majbritt; KROGH JENSEN, Søren. Rumen biohydrogenation of linoleic and linolenic acids is reduced when esterified to phospholipids or steroids. **Food Science & Nutrition**, [N.p.], vol. 8, no. 1, pp. 79–87, 6 Jan. 2020. Available at: <https://doi.org/10.1002/fsn3.1252>.

LIMA, Joana; INGABIRE, Winfred; ROEHE, Rainer; DEWHURST, Richard James. Estimating Microbial Protein Synthesis in the Rumen—Can ‘Omics’ Methods Provide New Insights into a Long-Standing Question? **Veterinary Sciences**, [N.p.], vol. 10, no. 12, p. 679, 27 Nov. 2023. Available at: <https://doi.org/10.3390/vetsci10120679>.

LIU, Haitao; LI, Fadi; LI, Fei; MA, Zhiyuan; WANG, Tao; LI, Qinwu; WANG, Xinji; LI, Kaidong. Rumen-Protected Fat and Rumen-Protected Choline Co-Supplementation: Impacts on Performance and Meat Quality of Growing Lambs. **Veterinary Sciences**, [N.p.], vol. 12, no. 6, p. 525, 28 May 2025. Available at: <https://doi.org/10.3390/vetsci12060525>.

LUNESU, Mondina Francesca; DECANDIA, Mauro; MOLLE, Giovanni; ATZORI, Alberto Stanislao; BOMBOI, Giovanni Cristoforo; CANNAS, Antonello. Dietary Starch Concentration Affects Dairy Sheep and Goat Performances Differently during Mid-Lactation. **Animals**, [N.p.], vol. 11, no. 5, p. 1222, Oct. 2021. Available at: <https://doi.org/10.3390/ani11051222>.

MA, Xiaowen; ZHOU, Wenjing; GUO, Tongqing; LI, Fei; LI, Fadi; RAN, Tao; ZHANG, Zhian; GUO, Long. Effects of Dietary Barley Starch Contents on the Performance, Nutrient Digestion, Rumen Fermentation, and Bacterial Community of Fattening Hu Sheep. **Frontiers in Nutrition**, [N.p.], vol. 8, 27 Jan. 2022. Available at: <https://doi.org/10.3389/fnut.2021.797801>.

MONJEZI, Yaser; SARI, Mohsen; CHAJI, Morteza; FERRET, Alfred. Effects of concentrate starch level and free-choice provision of straw on performance, feeding behaviour and feed sorting of fattening lambs. **Applied Animal Behaviour Science**, [N.p.], vol. 256, p. 105773, Nov. 2022. Available at: <https://doi.org/10.1016/j.applanim.2022.105773>.

OFFNER, Anne; BACH, Alex; SAUVANT, Daniel. Quantitative review of in situ starch degradation in the rumen. **Animal Feed Science and Technology**, [N.p.], vol. 106, nos. 1–4, pp. 81–93, Apr. 2003. Available at: [https://doi.org/10.1016/S0377-8401\(03\)00038-5](https://doi.org/10.1016/S0377-8401(03)00038-5).

ORZUNA-ORZUNA, José Felipe; HERNÁNDEZ-GARCÍA, Pedro Abel; CHAY-CANUL, Alfonso Juventino; DÍAZ GALVÁN, Cesar; RAZO ORTÍZ, Pablo Benjamín. Microalgae as a dietary additive for lambs: A meta-analysis on growth performance, meat quality, and meat fatty acid profile. **Small Ruminant Research**, [N.p.], vol. 227, p. 107072, Oct. 2023. Available at: <https://doi.org/10.1016/j.smallrumres.2023.107072>.

OWENS, F N; SECRIST, D S; HILL, W J; GILL, D R. Acidosis in cattle: a review. **Journal of Animal Science**, [N.p.], vol. 76, no. 1, p. 275, 1998. Available at: <https://doi.org/10.2527/1998.761275x>.

PLOMARITOU, Antigoni; HANLON, Mikenzie; KANTAS, Dimitrios; GEORGAKOUDIS, Konstantinos; DOVOLOU, Eleni; FOSKOLOS, Andreas. A Review of Nitrogen Use Efficiency of Dairy Replacement Heifers: Improving Management Practices and Minimizing Nitrogen Losses. **Animals**, [N.p.], vol. 15, no. 7, p. 1031, 3 Apr. 2025. Available at: <https://doi.org/10.3390/ani15071031>.

PONNAMPALAM, Eric; PRIYASHANTHA, Hasitha; VIDANARACHCHI, Janak; KIANI, Ali; HOLMAN, Benjamin. Effects of Nutritional Factors on Fat Content, Fatty Acid Composition, and Sensorial Properties of Meat and Milk from Domesticated Ruminants: An Overview. **Animals**, [N.p.], vol. 14, no. 6, p. 840, 8 Mar. 2024. Available at: <https://doi.org/10.3390/ani14060840>.

RODRIGUES, Hérick Pachêco; FERREIRA, Joyanne Mirelle de Sousa; PEREIRA, Maria Izabel Batista; TRAJANO, Lais Santos; SOUZA, Ligia Lins; DOS SANTOS PINA, Douglas; DE FREITAS JUNIOR, José Esler; DE CARVALHO, Gleidson Giordano Pinto; SANTOS, Stefanie Alvarenga; AZEVÊDO, José Augusto Gomes. Levels of starch and supplementation with calcium salts of palm oil fatty acids in the diets of goats and sheep. **Small Ruminant Research**, [N.p.], vol. 254, p. 107661, Jan. 2026. Available at: <https://doi.org/10.1016/j.smallrumres.2025.107661>.

SANTOS, F. A. P.; CARVALHO, G. G. P.; AZEVÊDO, J. A. G.; ALBA, H. D. R.; SOUZA, L. L.; FERREIRA, J. M. S.; RODRIGUES, H. P.; PEREIRA, M. I. B.; TRAJANO, L. S.; FREITAS JUNIOR, J. E.; PINA, D. S.; SANTOS, S. A. Effects of starch sources and lipid supplementation on performance and carcass traits of feedlot lambs. *Animal Feed Science and Technology*, v. 246, p. 1-12, 2019. DOI: 10.1016/j.anifeedsci.2018.10.015.

SCORESBY, Daniel; TEIXEIRA, Izabelle A. M. A.; CHAHINE, Mireille. Effects of Corn Silage and Alfalfa Hay on Production and Nitrogen Excretion in Lactating Dairy Cows. **Nitrogen**, [N.p.], vol. 6, no. 2, p. 43, 10 Jun. 2025. Available at: <https://doi.org/10.3390/nitrogen6020043>.

SEO, Ja Kyeom; YANG, Jiyoung; KIM, Hyun J.; UPADHAYA, Santi Devi; CHO, W. M.; HA, Jong K. Effects of Synchronization of Carbohydrate and Protein Supply on Ruminal Fermentation, Nitrogen Metabolism and Microbial Protein Synthesis in Holstein Steers. **Asian-Australasian Journal of Animal Sciences**, [N.p.], vol. 23, no. 11, pp. 1455–1461, 25 Oct. 2010. Available at: <https://doi.org/10.5713/ajas.2010.10247>.

SHIPANDENI, Maria N T; PAULA, Eduardo M; ESPOSITO, Giulia; FACIOLA, Antonio P; RAFFRENATO, Emiliano. Effects of starch sources varying in particle sizes on ruminal fermentation, nutrient flow, starch digestibility, and lactation performance of dairy cows. **Journal of Animal Science**, [N.p.], vol. 101, 3 Jan. 2023. Available at: <https://doi.org/10.1093/jas/skad147>.

SHPIRER, Jen; LIVSHITS, Lilya; KAMER, Hadar; ALON, Tamir; PORTNIK, Yuri; MOALLEM, Uzi. Effects of the palmitic-to-oleic ratio in the form of calcium salts of fatty acids on the production and digestibility in high-yielding dairy cows. **Journal of Dairy Science**, [N.p.], vol. 107, no. 9, pp. 6785–6796, Sep. 2024. Available at: <https://doi.org/10.3168/jds.2023-24382>.

SIMIONATTO, Marcio; MAEDA, Emilyn M; DA SILVEIRA, Magali F; DE P. MACEDO, Vicente; DE PAULA, Fabiana L M; HILL, João A G. Effect of adding different levels of palm oil-protected fat in the diet of lambs concerning rumen parameters. **Animal Feed Science and Technology**, [N.p.], vol. 310, p. 115929, Oct. 2024. Available at: <https://doi.org/10.1016/j.anifeedsci.2024.115929>.

TORAL, P.G.; MONAHAN, F.J.; HERVÁS, G.; FRUTOS, P.; MOLONEY, A.P. Review: Modulating ruminal lipid metabolism to improve the fatty acid composition of meat and milk. Challenges and opportunities. **Animal**, [N.p.], vol. 12, pp. s272–s281, 2018. Available at: <https://doi.org/10.1017/S1751731118001994>.

URRUTIA, Olaia; MENDIZABAL, José Antonio; ALFONSO, Leopoldo; SORET, Beatriz; INSAUSTI, Kizkitza; ARANA, Ana. Adipose Tissue Modification through Feeding Strategies and Their Implication on Adipogenesis and Adipose Tissue Metabolism in Ruminants. **International Journal of Molecular Sciences**, [N.p.], vol. 21, no. 9, p. 3183, 30 Apr. 2020. Available at: <https://doi.org/10.3390/ijms21093183>.

VAN LE, Hung; NGUYEN, Don Viet; VU NGUYEN, Quang; MALAU-ADULI, Bunmi Sherifat; NICHOLS, Peter David; MALAU-ADULI, Aduli Enoch Othniel. Fatty acid profiles of muscle, liver, heart and kidney of Australian prime lambs fed different polyunsaturated fatty acids enriched pellets in a feedlot system. **Scientific Reports**, [N.p.], vol. 9, no. 1, p. 1238, 4 Feb. 2019. Available at: <https://doi.org/10.1038/s41598-018-37956-y>.

WANG, Xue; MARTIN, Graeme B.; WEN, Qi; LIU, Shulin; LI, Yinhao; SHI, Binlin; GUO, Xiaoyu; ZHAO, Yanli; GUO, Yangdong; YAN, Sumei. Palm oil protects α -linolenic acid from rumen biohydrogenation and muscle oxidation in cashmere goat kids. **Journal of Animal Science and Biotechnology**, [N.p.], vol. 11, no. 1, p. 100, 5 Dec. 2020. Available at: <https://doi.org/10.1186/s40104-020-00502-w>.

ZHANG, Meimei; ZHANG, Zhiyue; ZHANG, Xinlong; LU, Changming; YANG, Wenzhu; XIE, Xiaolai; XIN, Hangshu; LU, Xiaotan; NI, Mingbo; YANG, Xinyue; LV, Xiaoyang; JIAO, Peixin. Effects of dietary *Clostridium butyricum* and rumen protected fat on meat quality, oxidative stability, and chemical composition of finishing goats. **Journal**

of Animal Science and Biotechnology, [*N.p.*], vol. 15, no. 1, p. 3, 15 Jan. 2024. Available at: <https://doi.org/10.1186/s40104-023-00972-8>.

ZHANG, Xiu Min; MEDRANO, Rodolfo F; WANG, Min; BEAUCHEMIN, Karen A; MA, Zhi Yuan; WANG, Rong; WEN, Jiang Nan; LUKUYU, Bernard A; TAN, Zhi Liang; HE, Jian Hua. Corn oil supplementation enhances hydrogen use for biohydrogenation, inhibits methanogenesis, and alters fermentation pathways and the microbial community in the rumen of goats. **Journal of Animal Science**, [*N.p.*], vol. 97, no. 12, pp. 4999–5008, 17 Dec. 2019. Available at: <https://doi.org/10.1093/jas/skz352>.

ZHANG, Zhian; LI, Fei; MA, Xiaowen; LI, Fadi; WANG, Zongli. Effects of Barley Starch Level in Diet on Fermentation and Microflora in Rumen of Hu Sheep. **Animals**, [*N.p.*], vol. 12, no. 15, p. 1941, 30 Jul. 2022. Available at: <https://doi.org/10.3390/ani12151941>.

2. OBJETIVOS

2.1. Objetivo geral:

Avaliar os efeitos interativos entre concentrações de amido dietético (220 vs. 420 g/kg de MS) e a suplementação com sais de cálcio de ácidos graxos de palma (SCAGP; 0 vs. 30 g/kg de MS) sobre a eficiência metabólica, o desempenho produtivo, o perfil de ácidos graxos do músculo *Longissimus dorsi* e a excreção de nitrogênio em cordeiros terminados em confinamento.

2.2. Objetivos específicos:

1. Investigar o impacto da interação amido $\times$ SCAGP sobre o consumo e a digestibilidade aparente dos nutrientes.
2. Analisar o metabolismo de nitrogênio (N), incluindo a síntese de proteína microbial, a eficiência de retenção de nitrogênio e a excreção de N urinário.
3. Avaliar as alterações no comportamento ingestivo (alimentação, ruminação e ócio) e na eficiência de mastigação causadas pelos níveis de amido e pela gordura protegida.
4. Monitorar os perfis de metabólitos séricos e de enzimas hepáticas para verificar a saúde metabólica e a função hepática sob a influência dessas dietas.
5. Determinar o desempenho de crescimento (ganho médio diário e eficiência alimentar) em função da disponibilidade de substrato fermentável e da suplementação lipídica.
6. Avaliar as características quantitativas da carcaça, como rendimentos, composição dos cortes comerciais e os padrões de deposição de gordura (perirrenal, renal e subcutânea).
7. Examinar os parâmetros físico-químicos *post-mortem* da carcaça, incluindo a dinâmica de resfriamento (temperatura), as perdas por frio e a evolução do pH.
8. Determinar o perfil de ácidos graxos do músculo *Longissimus dorsi*, com ênfase na proporção de ácidos graxos saturados (SFA), monoinsaturados (MUFA), poli-insaturados (PUFA) e isômeros de ácido linoleico conjugado (CLA).

3. CAPÍTULO II

Artigo submetido na revista Animal Science

Dietary starch level determines the efficacy of calcium salts of palm fatty acids on nitrogen use efficiency and performance in feedlot lambs

J. M. De S. Ferreira^a, H. P. Rodrigues^b, H. D. R. Alba^c, A. P. G. Da Silva^a, A. B. De Oliveira^a, R. R. Silva^a, L. L. Souza^d, G. G. P. De Carvalho^e, D. Dos S. Pina^e, S. A. Santos^e and J. A. G. Azevêdo^{f*}

^a *Postgraduate Program in Animal Science, Universidade Estadual do Sudoeste da Bahia, Itapetinga, 45700000, Brazil.*

^b *Postgraduate Program in Animal Science, Universidade Estadual de Santa Cruz, Ilhéus, Bahia, 45662900, Brazil.*

^c *Universidade Federal do Oeste do Pará, Santarém, Pará.*

^d *Department of Exact and Technological Sciences, Universidade Estadual do Sudoeste da Bahia, Vitória da Conquista, Bahia, 45083900, Brazil.*

^e *Department of Animal Science, Universidade Federal da Bahia, Salvador, Bahia, 40170110, Brazil.*

^f *Department of Agricultural and Environmental Sciences, Universidade Estadual de Santa Cruz, Ilhéus, Bahia, 45662900, Brazil *Corresponding author. E-mail address: augustog@uesc.br (José Augusto Gomes Azevêdo).*

ABSTRACT

This study evaluated the interaction between dietary starch concentration and calcium salts of palm fatty acids (CSPFA) on nutrient intake, digestibility, nitrogen (N) metabolism, ingestive behavior, serum and liver metabolite profiles, and growth performance in feedlot lambs. Thirty-two Dorper $\times$ Santa Inês lambs (25.0 ± 2.85 kg) were distributed in a completely randomized 2×2 factorial design. The treatments consisted of two starch levels (low: 220 g/kg DM or high: 420 g/kg DM) and two lipid supplementations (0 or 30 g/kg DM of CSPFA). A starch $\times$ CSPFA interaction was detected for intake, N metabolism ($P < 0.05$), and performance ($P < 0.05$). In low-starch diets, CSPFA reduced starch intake ($P < 0.001$) due to dietary dilution rather than physiological satiety, as the total dry matter intake remained unaffected. Consequently, CSPFA failed to improve N retained or growth in an energy-limited environment. Conversely, in high-starch diets, CSPFA maintained fiber digestibility, reduced serum urea by 43% ($P = 0.005$), increased microbial N flow by 29% ($P = 0.007$), and improved N retained by 22% ($P = 0.021$). The systemic markers (AST, ALT, and lipid profiles) were unchanged. As a result, CSPFA increased average daily gain (+35%, $P < 0.015$) and feed efficiency (+22%, $P < 0.018$) exclusively in lambs fed the high-starch diet. The metabolic efficacy of CSPFA depends on the substrate. Supplementing high-starch diets with CSPFA enhanced nitrogen retention, average daily gain, and feed efficiency in feedlot lambs. Conversely, no performance benefits were noted with CSPFA supplementation in low-starch diets, highlighting a significant interaction between CSPFA and dietary starch level.

Keywords: Microbial protein; Nitrogen retention efficiency; Protected fat; Rumen synchrony

Implications

These findings demonstrate a clear interaction of starch $\times$ CSPFA in feedlot lambs. CSPFA increased nitrogen retention, microbial N supply, and growth only under high-starch conditions, acting as a context-dependent modulator of ruminal fermentation rather than a general energy source. Strategically applying CSPFA to high-starch finishing diets can improve nitrogen-use efficiency and reduce urinary nitrogen losses—key contributors to ammonia emissions—without impairing hepatic or renal function. In contrast, CSPFA offers minimal metabolic or performance benefits in low-starch diets. Overall, the results provide a mechanistic basis for more targeted use of protected fats to align fermentable energy, microbial growth, and nitrogen efficiency in small ruminants.

3.1. Introduction

Improving nitrogen-use efficiency (NUE) in ruminant production systems has become a vital priority, as recent assessments have shown that ruminants lose 60–80% of the nitrogen they consume through urine and feces. This loss significantly contributes to ammonia emissions, nitrate leaching, and the buildup of reactive nitrogen within the nitrogen cascade (Hutchings et al., 2020; Plomaritou et al 2025). Modern analyses have demonstrated that relatively small shifts in diet formulation lead to proportional changes in nitrogen intake, utilization efficiency, and excretion (Rodrigues et al., 2026; Scoresby et al., 2025). Since microbial protein synthesis (MPS) provides 50–80% of the metabolizable protein available to ruminants (Lima et al., 2023; de Oliveira et al., 2025), optimizing rumen fermentation is essential for reducing nitrogen losses and enhancing overall animal efficiency.

High-starch diets are commonly used in feedlot lambs to provide rapidly fermentable energy (Ma et al., 2022; Zebeli et al., 2012). The increased fermentability of these diets reduces fiber digestion, destabilizes rumen pH, and disrupts the synchronization between ammonia release and microbial ATP availability (Ma et al., 2022). Asynchrony between nitrogen and carbohydrate release can reduce microbial protein synthesis by 11–

20% and increase nitrogen loss (Chen et al., 2022; Ren et al., 2022). When ammonia accumulates beyond the microbial assimilation capacity, postprandial peaks elevate hepatic ureagenesis and urinary N excretion (de Oliveira et al., 2025; Seo et al., 2010), contributing to low NUE and increasing the environmental footprint of feedlot systems.

Lipid supplementation has been proposed as a strategy to modulate rumen fermentation, partially suppress protozoal populations, and enhance the microbial amino acid supply, particularly when rumen-protected or unsaturated lipids are used (Dewanckele et al., 2020; Sears et al., 2024). In high-starch diets, sunflower oil increased microbial N flow by approximately 34% and enhanced urea-N recycling while reducing urinary N output (Doranalli and Mutsvangwa, 2011). However, these benefits are highly dependent on diet; in low-starch diets, limited fermentable energy restricts microbial ATP production, and lipids, especially unsaturated forms, can depress fiber digestion and microbial efficiency (Palmquist and Jenkins, 1980; WACHIRA et al., 2000).

Despite extensive research on lipid–rumen interactions, it remains unknown whether the metabolic efficacy of rumen-protected fats, specifically calcium salts of palm fatty acids (CSPFA), directly depends on dietary starch concentration. This uncertainty persists because mechanistic models predict that improvements in MPS and NUE occur only when fermentable starch supplies sufficient energy to support enhanced urea assimilation (Jenkins, 1993; Zhang et al., 2024). Consequently, it remains unclear whether CSPFA functions primarily as an energy-dense nutrient or as a conditional modulator of rumen fermentation that requires a high-starch context.

We hypothesized that CSPFA would enhance microbial nitrogen capture, urea-N recycling, and nitrogen retained only when fermentable energy availability is increased, thereby increasing metabolizable amino acid flow and improving growth performance. Therefore, this study aimed to evaluate the effects of dietary starch concentration and

CSPFA supplementation on nutrient intake, digestibility, nitrogen balance, and growth performance in feedlot lambs. A primary objective was to determine whether the efficacy of CSPFA is dependent on the dietary concentration of fermentable starch.

3.2. Materials and Methods

3.2.1. Ethical approval

All procedures involving animals complied with Brazilian legislation on the ethical use of animals in research (Law No. 11.794, October 8, 2008). The experimental protocol was reviewed and approved by the Ethics Committee on Animal Use of the State University of Santa Cruz (UESC), Ilhéus, Bahia, Brazil (protocol number 008/2025).

3.2.2. Animals, housing, and management

The experiment was conducted at the Laboratory for Research in Nutrition and Feeding of Ruminants at UESC, Ilhéus, Bahia, Brazil. The experimental facility comprised a covered barn (ceiling height 3.5 m) equipped with lateral curtains, natural and artificial lighting, and 40 individual pens (1.20 × 0.80 m) each fitted with suspended wooden slatted floors, individual feed bunks, and 5-L water buckets. During the experimental period, the ambient temperature ranged from 23 to 29 °C and the relative humidity from 70% to 86%, which is characteristic of a humid tropical climate (Köppen Af classification).

Thirty-two castrated Dorper × Santa Inês male lambs (approximately 120 ± 5 days old; 25.0 ± 2.85 kg initial body weight) were used in this study. Upon arrival, animals were identified, dewormed using Albendathor Oral 10% (albendazole 10%; Tortuga Companhia Zootécnica Agrária, Brazil) at 0.75–1.0 mL per 20 kg BW administered orally with a drenching gun, and vaccinated against clostridial diseases using Sintoxan Polivalente T (Merial Saúde Animal Ltda., Paulínia, Brazil), following the manufacturer's recommendations.

The animals were then subjected to a 15-day adaptation period. During this period and throughout the 44-day feeding trial, diets were offered twice a day (07:30 and 14:00 h) to allow for ad libitum intake. The initial amount of feed offered was calculated to be 2.5% of the lambs' body weight. Thereafter, the daily feed offered was adjusted for each lamb to ensure that refusals were approximately 10% of the as-fed amount, guaranteeing unrestricted access to feed. At the end of the adaptation phase, the lambs underwent a 12-

h solid-feed withdrawal and were weighed to determine the initial body weight for the experimental period. After weighing, the lambs were randomly assigned to treatments using a completely randomized design.

3.2.3. Dietary Treatments and Feeding Management

The experiment followed a completely randomized design in a 2×2 factorial arrangement, testing two starch levels (220 or 420 g/kg DM) and the inclusion (30 g/kg DM) or absence of calcium salts of palm fatty acids (CSPFA; Nutri Gordura Lac, Nutricorp Inc., Araras, SP, Brazil). Diets were formulated according to the recommendations of BR-Caprinus and Ovinos (Pereira et al., 2024), using a target average daily gain of 200 g/animal/day as a reference for nutrient adequacy.

Corn silage served as the roughage source, and the concentrate was composed of ground corn, soybean meal, soybean hulls, urea, limestone, and mineral salt (Table 1).

Table 1. Ingredients and chemical composition of experimental diets containing low or high starch (S) levels and with or without calcium salts of palm fatty acids (CSPFA; g/kg).

Item	Low-S + CSPFA	Low-S	High-S + CSPFA	High-S
Ingredients g/kg				
Corn silage	400	400	400	400
Ground corn	190.4	187.3	485	483.2
Soy hull	322.6	314.1	0	12.7
Soybean meal	32.1	74.6	60.2	74.6
Urea	9.9	3	10	7
Limestone	6.5	12	9.7	14
Mineral mixture ¹	8.5	9	5.1	8.5
CSPFA ²	30	0	30	0
Chemical composition g/kg				
Dry matter (as-fed basis)	663.3	662.2	664.8	653.8
Organic matter	961.1	955.5	961.9	953.5
Ether extract	54.45	22.23	60.4	36.5
Crude protein	135	135	135	136.1
Neutral detergent fiber	466	474	323.4	330.5
Starch	220	220	420	420
Residual organic matter	85.7	104.3	23.2	30.4
Metabolizable energy (MJ/kg DM) ³	11.9	11.3	12.4	11.8
Fatty acid profile, mg/100g DM				
Caprylic (C8:0)	0.03	0.03	0.03	0.03

Capric (C10:0)	0.15	0.15	0.15	0.15
Lauric (C12:0)	0.30	0.31	0.30	0.31
Myristic (C14:0)	1.99	1.81	1.78	1.62
Palmitic (C16:0)	15.08	14.50	15.34	15.14
Stearic (C18:0)	4.39	4.71	4.38	4.69
Palmitoleic (C16:1)	0.38	0.36	0.38	0.36
Oleic (C18:1 n-9)	28.10	28.09	28.09	27.94
Linoleic (C18:2 n-6)	46.61	47.13	46.58	46.87
α -linolenic (C18:3 n-3)	2.96	2.90	2.96	2.88

1 Mineral premix supplied (per kg product): Ca 110–13 g; P 87 g; S 18 g; Na 147 g; Co 15 mg; Cu 590 mg; Cl 20 mg; I 50 mg; Mn 2,000 mg; Mo 300 mg; Se 20 mg; Zn 3,800 mg; Fl 870 mg. 2 Calcium salts of palm fatty acids (Nutri Gordura Lac, Nutricorp Inc., Araras, São Paulo, Brazil): 84.9% total fatty acids (48.6% C16:0, 4.40% C18:0, 34.3% C18:1 cis-9, 5.5% C18:2 cis-9 cis-12, and others <2% each). 3 Estimated according to Cruz et al. (2021).

The four dietary treatments were as follows: low starch + CSPFA: 220 g/kg DM starch + 30 g/kg DM CSPFA; Low starch – CSPFA: 220 g/kg DM starch, without CSPFA; High starch + CSPFA: 420 g/kg DM starch + 30 g/kg DM CSPFA; High starch – CSPFA: 420 g/kg DM starch, without CSPFA. Diets were offered twice daily (07:30 and 14:00 h), with daily adjustments to allow for 10–20% feed refusal. Fresh water was provided ad libitum and was replenished daily.

3.2.4. Chemical Analyses

Representative samples of the diets and ingredients were collected, dried at 60 °C for 72 h, and ground using a 1 mm sieve. All laboratory analyses were performed in duplicate. Dry Matter (DM), Crude Protein (CP), Ether Extract (EE), and ash contents were determined according to the Association of Official Analytical Chemists (AOAC, 2016) procedures. The concentrations of Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were analyzed according to the method described by Van Soest et al. (1991), with heat-stable alpha-amylase and omitting sodium sulfite.

Starch quantification was performed colorimetrically at the 3rlab - Agricultural Analysis Laboratory (Lavras, MG, Brazil) according to the method described by Dische (1962). The Residual Organic Matter was calculated following the proposal by Tebbe et al. (2017). The metabolizable energy (ME) values were estimated based on the ingredient composition and nutrient content, according to da Cruz et al. (2021). The fatty acid profiles of the ingredients and diets were determined as described in Section 2.5. The composition of the CSPFA (Nutri Gordura Lac) supplement was verified by laboratory analysis to confirm compliance with the manufacturer's specifications prior to the feeding trial.

3.2.5. *Intake and Apparent Digestibility*

Feed offered, and refusals were recorded daily for each animal in the study. Representative samples (500 g) of feed ingredients, diets, and refusals were collected daily, stored at -20°C , and later pre-dried in a forced-air oven (60°C for 72 h). Dried samples were ground through a 1-mm screen and composited for a period of 28 days based on the dry matter.

Fecal samples were collected directly from the rectal ampulla over four consecutive days (days 24–27 of each period), identified, frozen at -20°C , and processed for chemical analysis. Apparent nutrient digestibility was estimated using indigestible neutral detergent fiber (iNDF) as an internal digestibility marker. Diet, refusals, and fecal samples were incubated in situ for 288 h, following the methodology described by Reis et al. (2017) and dos Santos et al. (2025). The digestibility coefficient (DC) of each nutrient was calculated using the following formula: $\text{DC} = (\text{nutrient consumed} - \text{nutrient excreted}) / \text{nutrient consumed}$.

3.2.6. *Nitrogen partitioning, urinary purine derivatives, and microbial nitrogen synthesis*

Urine sampling was performed on days 18–22 and 40–44 during the experimental period. Spot urine samples were collected once daily and stored at -20°C according to the methodology of Rennó et al. (2008). Daily urinary volume (L/d) was estimated using the ratio between daily creatinine excretion (mg/kg BW) and creatinine concentration (mg/dL) in urine samples, following the procedures recommended by Santos et. al., (2018).

The urinary concentrations of urea, creatinine, and uric acid were determined using Bioclin commercial kits. Urea nitrogen (N-urea) was calculated by multiplying the urea values by 0.4667. Total urinary nitrogen (N) was determined according to the Kjeldahl method (AOAC, 2016).

Allantoin concentration was quantified using a colorimetric method, and xanthine and hypoxanthine levels were measured using an enzymatic assay (Chen and Gomes, 1992). The total purine derivatives were calculated as the sum of allantoin, uric acid, hypoxanthine, and xanthine.

Absorbed microbial purines (mmol/day) were estimated from total purine derivatives using the equations of Chen and Gomes (1992) for sheep as follows: Microbial nitrogen synthesis (g/day) was calculated assuming an N content of 70 mg/mmol of the absorbed purines.

3.2.7. *Blood Sampling and Biochemical Analyses*

Blood sampling was performed to assess the metabolic profile, nitrogen metabolism markers, and liver function in response to the dietary treatments. Samples were collected on day 21 of each experimental period by jugular venipuncture using Vacutainer tubes without an anticoagulant, four hours after the morning feeding.

Approximately 3 mL of blood was collected from each lamb. Samples were allowed to clot at room temperature and subsequently centrifuged at $3500 \times g$ (Refrigerated Centrifuge Excelsa 4, Mod. 280 R, Fanem, São Paulo, SP, Brazil). The resulting serum was aliquoted into Eppendorf tubes and stored at -20°C until further analyses.

Biochemical determinations included: nitrogen metabolism indicators: urea, creatinine; liver function enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT); energy–lipid profile: glucose, triglycerides, cholesterol, very low-density lipoprotein (VLDL). All serum metabolites were analyzed using Bioclin commercial kits (Quibasa-Bioclin, Belo Horizonte, Brazil). VLDL concentration was estimated according to Friedewald et al. (1972), and globulin concentration as total protein–albumin.

3.2.8. *Feeding Behavior*

Ingestive behavior was evaluated to characterize the effects of starch level and CSPFA inclusion on feeding, rumination, and idling patterns. Observations were conducted over a continuous 24-hour period on day 20 of each experimental phase. Behavioral monitoring followed the methodology described by Carvalho et al. (2004).

Animals were visually observed every 10 min, and the predominant activity in each interval (feeding, ruminating, or idling) was recorded using an ethogram developed specifically for the experiment, following the methodology described by Carvalho et al. (2004). During nighttime observations, the barn was maintained under artificial lighting to which the animals had been previously habituated to avoid interference with their natural behavior.

Rumination dynamics were assessed by quantifying the characteristics of the ruminated bolus. The number of chews per bolus and the time spent ruminating each bolus (s/bolus) were recorded using a digital stopwatch. These measurements were obtained by collecting three ruminated boluses at three distinct periods of the day (10:00–12:00, 14:00–16:00, and 18:00–20:00), in accordance with Bürger et al. (2000) and Carvalho et al. (2007, 2004).

Chewing and ingestion efficiencies were calculated based on dry matter (DM) and NDFap intakes per bolus and per unit of feeding or rumination time, as recommended by Carvalho et al. (2007, 2004). The number of feeding, rumination, and idling periods was determined by counting each uninterrupted sequence of each activity within a 24-hour dataset.

3.2.9. Performance Evaluation

Initial body weight (IBW) was recorded at the beginning of the trial after a 16 h fast (only solids). The final body weight (FBW) was recorded after 44 days. Average daily gain (ADG) was calculated as (FBW – IBW) divided by the number of feeding days. The feed conversion ratio (FCR) and feed efficiency (FE) were computed as $FCR = DMI / ADG$ and $FE = ADG / DMI$, respectively.

3.2.10. Statistical Analyses

The experiment followed a completely randomized 2×2 factorial arrangement with two starch levels (220 and 420 g/kg DM) and two CSPFA levels (0 and 30 g/kg DM), with eight replicates per treatment.

Data were checked for normality and homogeneity using the UNIVARIATE procedure in SAS 9.4 (SAS - Statistical Analysis System, version 9.4, SAS Institute Inc., Cary, NC, 2013). The MIXED procedure was used for analysis of variance (ANOVA) according to the following model:

$$Y_{ijk} = \mu + S_i + C_j + (S \times C)_{ij} + \epsilon_{ijk}$$

where Y_{ijk} = observed value; μ = overall mean; S_i = starch level; C_j = CSPFA inclusion; $(S \times C)_{ij}$ = interaction; and ϵ_{ijk} = residual error.

Performance variables were analyzed using initial body weight as a covariate:

$$Y_{ijk} = \mu + \beta(X_{ij} - \bar{X}) + S_i + C_j + (S \times C)_{ij} + \epsilon_{ijk}$$

where β is the regression coefficient for the covariate, X_{ij} is the observed covariate value, and $\bar{X}$ is the mean covariate value.

Significant effects were declared at $P \leq 0.05$, and interactions were further explored using the SLICE option of SAS (MIXED procedure) software. Data are presented as least square means with standard error of the mean (SEM).

3.3. Results

3.3.1 Nutrient intake and apparent digestibility

A starch $\times$ CSPFA interaction was detected for ether extract (EE) intake ($P=0.045$), starch intake ($P=0.050$), and starch digestibility ($P=0.025$) (Table 2 and Fig 1). Under the low-starch diet (220 g/kg DM), CSPFA increased EE intake ($P<0.001$) but reduced starch intake ($P<0.001$) while maintaining a slightly lower starch digestibility than the unsupplemented diet ($P=0.003$). In contrast, under the high-starch diet (420 g/kg DM), CSPFA increased EE intake ($P<0.001$), increased starch intake ($P=0.010$), and improved starch digestibility ($P=0.015$) (Fig 1).

Table 2. Nutrient intake and apparent digestibility coefficients in feedlot lambs fed diets containing low (220 g/kg) or high (420 g/kg) starch levels with (30g/kg DM) or without (0g/kg DM) calcium salts of palm fatty acids (CSPFA).

Item	Starch (S)		CSPFA (FA)		SEM	<i>P</i> -value		
	Low-S	High-S	Without	With		S	FA	S×FA
Nutrient intake, g/day								
Dry matter	1237.9	1170.4	1190.7	1217.6	23.49	0.152	0.564	0.183
Ether extract	48.1	58.0	34.8	71.2	2.56	<0.001	<0.001	0.045
NDF	568.9	364.8	466.8	466.9	16.28	<0.001	0.998	0.305
Starch	291.6	504.7	399.3	397.0	15.46	<0.001	0.882	0.050
Nutrient digestibility, g/kg								
Dry matter	770.3	712.8	732.4	750.8	8.60	<0.001	0.249	0.745
Organic matter	791.3	733.0	751.5	772.8	8.00	<0.001	0.138	0.511
Ether extract	852.3	850.7	799.1	903.9	14.24	0.946	<0.001	0.587
NDF ¹	596.3	490.4	555.62	531.04	16.41	<0.001	0.162	0.717
Starch	986.2	969.0	975.8	979.4	19.30	<0.001	0.104	0.025

¹ NDF: Neutral detergent fiber. SEM: Standard error of the mean. S: Starch effect; FA: Fatty acid (CSPFA) effect; S $\times$ FA: Interaction between starch and CSPFA. Significance was declared at $P \leq 0.05$.

When analyzing the effect of CSPFA across starch levels, diets without lipid supplementation showed higher EE ($P<0.001$) and starch ($P<0.001$) intakes when combined with high starch levels, but starch digestibility was reduced ($P<0.001$).

Conversely, in diets with CSPFA, low-starch diets promoted a lower ($P < 0.001$) starch intake but a greater ($P < 0.001$) starch digestibility than high-starch diets (Fig 1).

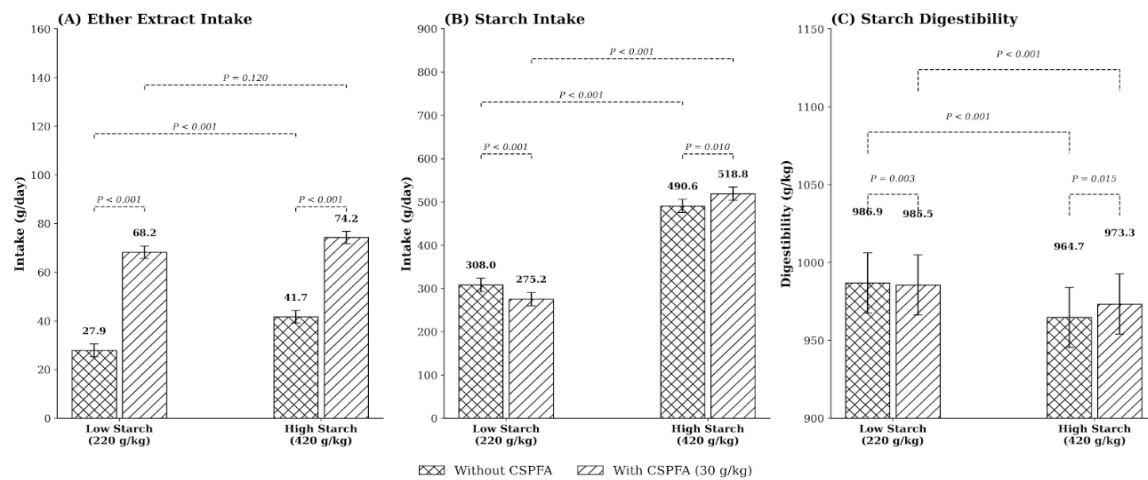


Figure 1. Interaction effects between dietary starch level (Low: 220 g/kg; High: 420 g/kg DM) and calcium salts of palm fatty acids (CSPFA; 0 or 30 g/kg DM) on (A) Ether extract intake, (B) Starch intake, and (C) Starch digestibility in feedlot lambs. Bars represent least square means with values displayed on top; error bars indicate the standard error of the mean (SEM). Dotted lines indicate specific pairwise contrasts with their respective P-values. Note: Starch digestibility is expressed in g/kg.

These reciprocal effects highlight substrate-dependent adjustments. In the absence of CSPFA, high-starch diets increased starch flow but reduced its utilization efficiency, whereas CSPFA promoted a compensatory effect, lowering starch intake at low starch levels and enhancing starch capture under high-starch conditions. This indicates that lipid supplementation modulates carbohydrate utilization in response to fermentative load, supporting the synchrony between energy and nitrogen availability in the rumen.

For the main effects, CSPFA did not affect the intake or digestibility of DM, OM, or NDF ($P > 0.05$) but increased EE digestibility (799 vs. 904 g/kg; $P < 0.001$) (Table 2). High-starch diet lowered NDF intake (569 vs. 365 g/d; $P < 0.001$) and reduced the digestibility of DM (770 vs. 713 g/kg; $P < 0.001$), OM (791 vs. 733 g/kg; $P < 0.001$), and NDF (596 vs. 490 g/kg; $P < 0.001$) (Table 2). The depression in cell wall digestion with high starch is expected; lipid protection selectively improved fat use and, at high starch levels, supported starch capture.

3.3.2. Nitrogen metabolism and microbial nitrogen synthesis

A significant starch $\times$ CSPFA interaction was observed for nitrogen retained ($P = 0.021$), uric acid (0.034), total purine derivatives ($P = 0.008$), and microbial N synthesis ($P = 0.005$) (Table 3 and Fig 2).

Table 3. Nitrogen balance, urinary purine derivatives, and microbial nitrogen synthesis in feedlot lambs fed diets containing low (220 g/kg) or high (420 g/kg) starch levels with (30g/kg DM) or without (0g/kg DM) calcium salts of palm fatty acids (CSPFA).

Item	Starch (S)		CSPFA (FA)		SEM	<i>P</i> -value		
	Withou					S×F		
	Low-S	High-S	t	With		S	FA	A
N intake, g/d	25.79	26.00	25.76	26.04	0.09	0.230	0.124	0.862
N fecal, g/d	5.65	5.87	5.85	5.67	0.14	0.434	0.535	0.118
N urinary, g/d	6.77	7.65	7.91	6.52	0.39	0.206	0.056	0.111
N retained, g/d	13.36	12.48	12.00	13.84	0.42	0.159	0.009	0.021
N absorbed/N intake, g/kg	781.02	774.20	773.09	782.13	5.16	0.503	0.378	0.106
Hypoxanthine+xanthine, mmol/d	2.68	2.87	2.62	2.92	0.12	0.434	0.220	0.152
Uric acid, mmol/d	1.70	1.75	1.56	1.89	0.07	0.652	0.010	0.034
Allantoin, mmol/d	5.23	5.23	4.80	5.66	0.24	0.996	0.058	0.056
Total purines, mmol/d	9.61	9.85	8.98	10.47	0.34	0.618	0.008	0.008
Microbial N, g/d	10.65	11.12	10.35	11.41	0.32	0.320	0.036	0.005

SEM: Standard error of the mean. S: Starch effect; FA: Fatty acid (CSPFA) effect; S×FA: Interaction between starch and CSPFA. Significance was declared at $P \leq 0.05$.

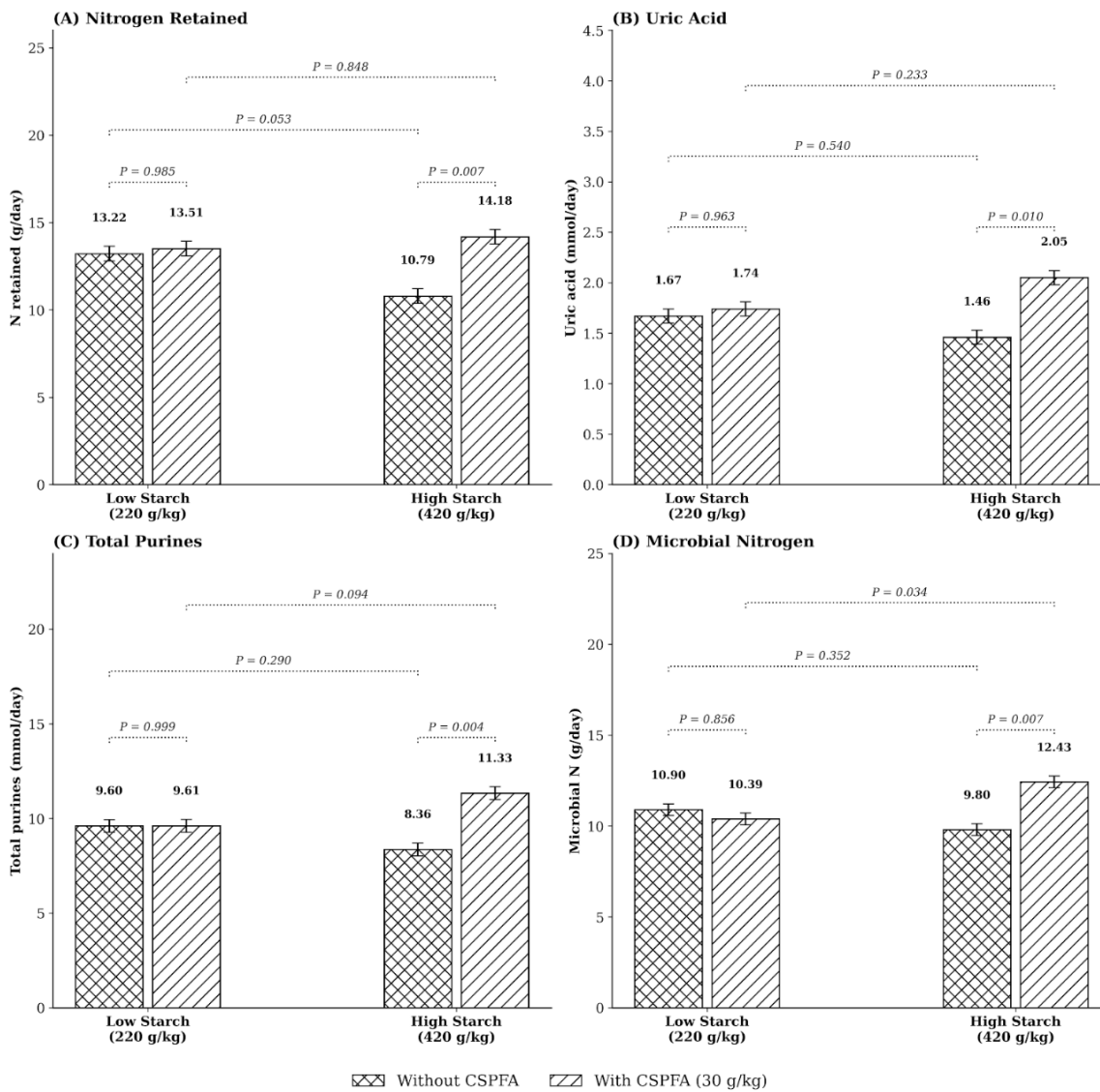


Figure 2. Interaction effects between dietary starch level (Low: 220 g/kg; High: 420 g/kg DM) and calcium salts of palm fatty acids (CSPFA; 0 or 30 g/kg DM) on nitrogen metabolism parameters: (A) Nitrogen retained, (B) Uric acid excretion, (C) Total purine derivatives, and (D) Microbial nitrogen synthesis in feedlot lambs. Bars represent least square means with numerical values; error bars indicate the standard error of the mean (SEM). Dotted lines indicate pairwise P-values for specific contrasts.

Under a low-starch diet (220 g/kg DM), CSPFA supplementation did not affect nitrogen retained, uric acid, total purines, or microbial N flow ($P > 0.05$). However, under a high-starch diet (420 g/kg DM), the inclusion of CSPFA markedly increased N retained (14.18 vs. 10.79 g/d; $P = 0.007$), uric acid (2.05 vs. 1.46 mmol/d; $P = 0.010$), total purines (11.33 vs. 8.36 mmol/d; $P = 0.004$), and microbial N synthesis (12.43 vs. 9.80 g/d; $P = 0.007$).

The main effects showed that CSPFA inclusion tended to decrease urinary N excretion (7.91 vs. 6.52 g/d; $P = 0.056$) and allantoin (10.47 vs. 8.98 mmol/d; $P = 0.58$).

3.3.3. Feeding behavior

Feeding behavior was significantly affected by the main effects of dietary starch level and calcium salts of palm fatty acids (CSPFA) inclusion, with no interaction detected between factors ($P > 0.05$; Table 4). Lambs fed the low-starch diet spent most time of the day feeding (18.77% vs. 15.16%; $P = 0.004$) and less time idling (50.32% vs. 56.27%; $P = 0.004$) than those receiving the high-starch diet. Likewise, CSPFA supplementation increased feeding activity (18.29% vs. 15.64%; $P = 0.029$) and reduced idling time (51.06% vs. 55.53%; $P = 0.026$), while rumination activity remained unaffected ($P > 0.05$).

High-starch diets enhanced feeding efficiency for both dry matter (DM) (446.8 vs. 289.2 g/h; $P < 0.001$) and neutral detergent fiber (NDF) (177.4 vs. 115.8 g/h; $P < 0.001$), and increased ruminating efficiency for DM (220.4 vs. 171.5 g/h; $P < 0.001$) and NDF (87.9 vs. 71.3 g/h; $P = 0.048$). The inclusion of CSPFA did not affect DM feeding efficiency ($P = 0.576$) but markedly improved NDF feeding efficiency (176.7 vs. 116.5 g/h; $P < 0.001$). Similarly, CSPFA increased ruminating efficiency for DM (218.1 vs. 173.7 g/h; $P = 0.010$).

Dietary starch level also affected chewing parameters. High-starch diets increased chewing time per bolus (40.66 vs. 37.51 s/bolus; $P = 0.026$) but reduced the total chewing time (10.49 vs. 11.92 h/d; $P = 0.004$). Conversely, CSPFA supplementation prolonged total chewing time (11.74 vs. 10.67 h/d; $P = 0.026$).

Table 4. Ingestive behavior patterns, feeding and ruminating efficiencies, and chewing activity of feedlot lambs fed diets containing low (220 g/kg) or high (420 g/kg) starch levels with (30g/kg DM) or without (0g/kg DM) calcium salts of palm fatty acids.

Item	Starch (S)		CSPFA (FA)		SEM	<i>P</i> -value		
	Low-S	High-S	Without	With		S	FA	S×FA
Activity, %/day								
Feeding	18.77	15.16	15.64	18.29	0.64	0.004	0.029	0.827
Rumination	30.9	28.55	28.81	30.64	0.74	0.112	0.215	0.406
Idling	50.32	56.27	55.53	51.06	1.07	0.004	0.026	0.628
Feeding efficiency, g/h								
Dry matter	289.2	446.8	357	379.1	21.84	<0.001	0.576	0.28
NDF ¹	115.8	177.4	116.5	176.7	9.49	<0.001	<0.001	0.184
Ruminating efficiency, g/h								
Dry matter	171.5	220.4	173.7	218.1	9.17	<0.001	0.01	0.487
NDF ¹	71.29	87.94	67.5	63.12	1.09	0.048	0.387	0.635
Chewing activity								
Chewing per bolus, n	37.51	40.66	39.14	39.03	0.7	0.026	0.934	0.836
Chewing, h/d	11.92	10.49	10.67	11.74	0.26	0.004	0.026	0.628

¹ NDF: Neutral detergent fiber. SEM: Standard error of the mean. S: Starch effect; FA: Fatty acid (CSPFA) effect; S×FA: Interaction between starch and CSPFA. Significance was declared at $P \leq 0.05$.

3.3.4. Serum metabolite and liver function

A starch × CSPFA interaction was observed for serum urea ($P=0.002$) and creatinine ($P=0.032$) concentrations (Table 5). The breakdown of this interaction (Fig 3) showed that CSPFA inclusion did not affect urea levels in lambs fed the low-starch diet ($P=0.696$), whereas in the high-starch diet, CSPFA reduced serum urea by 43.2% (35.97 vs. 20.44 mg/dL; $P=0.005$). Additionally, lambs fed high-starch diets without CSPFA supplementation had higher serum urea levels than those on the low-starch diet (35.97 vs. 20.51 mg/dL; $P=0.05$).

Table 5. Serum metabolite profile, liver function enzymes, and lipid indicators of feedlot lambs fed diets containing low (220 g/kg) or high (420 g/kg) starch levels with (30g/kg DM) or without (0g/kg DM) calcium salts of palm fatty acids (CSPFA).

Item	Starch (S)		CSPFA (FA)		SEM	<i>P</i> -value		
	Low-S	High-S	Without	With		S	FA	S×FA
Nitrogen metabolism								
Urea, mg/dL	22.93	28.20	28.24	22.89	1.76	0.098	0.093	0.002
Creatinine, mg/dL	1.59	1.65	1.66	1.58	0.02	0.189	0.085	0.032
Liver function								
AST ¹ , IU/L	38.51	37.01	40.42	35.1	2.45	0.766	0.293	0.991
ALT ² , IU/L	26.25	25.41	24.96	26.7	0.73	0.577	0.247	0.958
Energy–lipid profile (safety endpoints)								
Glucose, mg/dL	67.29	66.66	70.2	63.75	2.23	0.889	0.153	0.286
Triglycerides, mg/dL	22.34	27.25	24.01	25.58	5.35	0.657	0.887	0.633
Cholesterol, mg/dL	63.3	62.22	61.65	63.87	1.2	0.647	0.352	0.063
VLDL, mg/dL	4.47	5.70	5.05	5.12	1.09	0.584	0.978	0.561

¹ AST: Aspartate aminotransferase. ² ALT: Alanine aminotransferase. VLDL: Very low-density lipoprotein. SEM: Standard error of the mean. S: Starch effect; FA: Fatty acid (CSPFA) effect; S×FA: Interaction between starch and CSPFA. Significance was declared at $P \leq 0.05$.

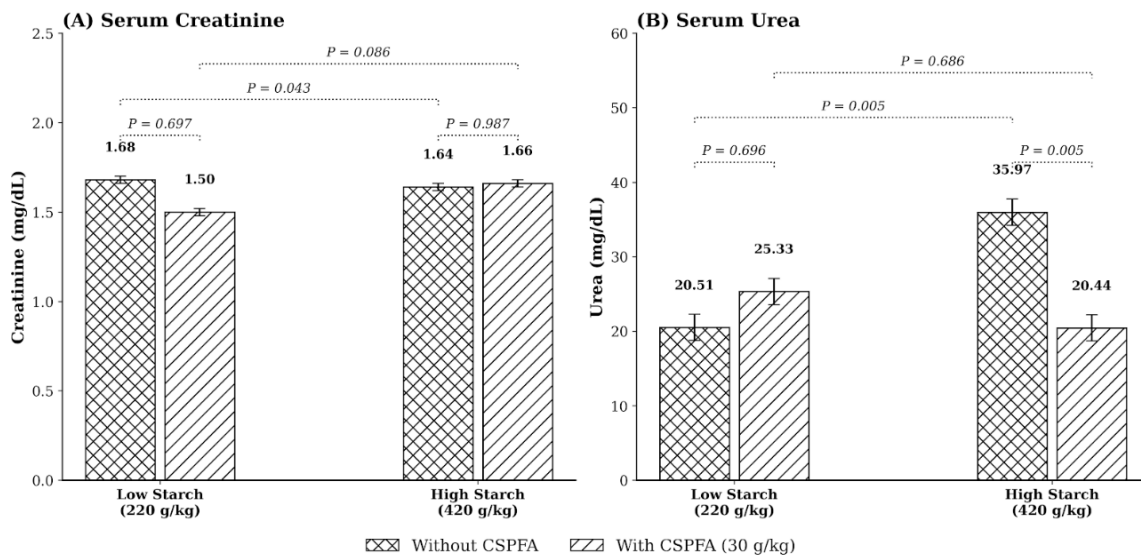


Figure 3. Interaction effects between dietary starch level (Low: 220 g/kg; High: 420 g/kg DM) and calcium salts of palm fatty acids (CSPFA; 0 or 30 g/kg DM) on serum metabolites: (A) Creatinine and (B) Urea concentrations in feedlot lambs. Bars represent least square means with numerical values; error bars indicate the standard error of the mean (SEM). Dotted lines indicate pairwise P-values for specific contrasts.

For creatinine, lambs fed low-starch diets without CSPFA exhibited higher concentrations than those fed high-starch diets (1.68 vs. 1.64 mg/dL; $P=0.043$). No effects of starch level, CSPFA inclusion, or their interaction were observed for liver enzymes (AST and ALT) or for energy–lipid profile markers (glucose, triglycerides, cholesterol, and very low density lipoprotein (VLDL); $P>0.05$; Table 5).

3.3.5. Growth performance

A significant starch $\times$ CSPFA interaction was observed for final body weight (FBW), average daily gain (ADG), and feeding efficiency (FE) ($P < 0.05$; Table 6). The breakdown of this interaction (Fig. 4) revealed that lambs fed the high-starch diet (420 g/kg DM), with CSPFA supplementation, significantly improved all performance metrics. Lambs receiving CSPFA showed higher FBW (+9.1%; 37.45 vs. 34.32 kg; $P = 0.002$), ADG (+34.9%; 274.84 vs. 203.71 g/day; $P = 0.0024$), and FE (+22.5%; 222.82 vs. 181.93 g/kg DMI; $P = 0.012$) compared to those without supplementation.

Table 6. Growth performance and feed efficiency of feedlot lambs fed diets containing low (220 g/kg) or high (420 g/kg) starch levels with (30g/kg DM) or without (0g/kg DM) calcium salts of palm fatty acids (CSPFA).

Item	Starch (S)		CSPFA (FA)		SEM	P-value		
	Low-S	High-S	Without	With		S	FA	S×FA
Initial body weight, kg	25.44	25.28	25.56	25.15	0.49	-	-	-
Final body weight, kg	36.67	35.88	35.8	36.75	0.54	0.161	0.092	0.001
ADG ¹ (g/d)	257.11	239.27	237.34	259.05	7.72	0.801	0.394	0.015
Feeding efficiency, g/kg DMI ²	214.49	202.37	205.35	211.52	5.74	0.164	0.74	0.018

¹ADG: Average daily gain. ²DMI: Dry matter intake. SEM: Standard error of the mean. S: Starch effect; FA: Fatty acid (CSPFA) effect; S×FA: Interaction between starch and CSPFA.

In diets without CSPFA, lambs fed the low-starch diet outperformed those fed the high-starch diet, showing greater FBW (37.28 vs. 34.32 kg; $P = 0.003$), ADG (270.98 vs. 203.71 g/d; $P = 0.0038$), and FE (228.76 vs. 181.93 g/kg DMI; $P = 0.003$; Fig 4), highlighting that excess starch alone impaired performance in the absence of supplemental fat.

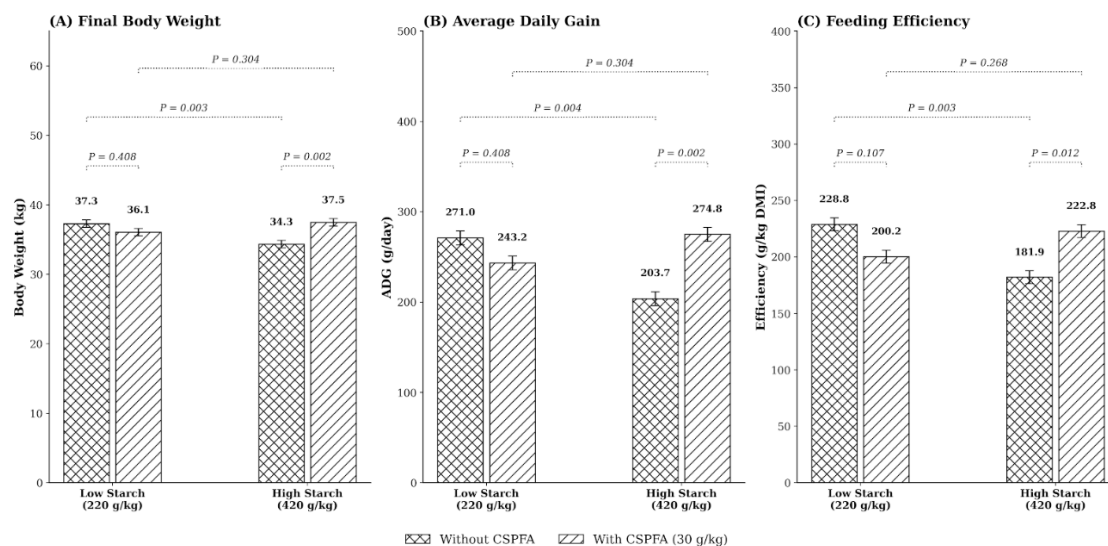


Figure 4. Interaction effects between dietary starch level (Low: 220 g/kg; High: 420 g/kg DM) and calcium salts of palm fatty acids (CSPFA; 0 or 30 g/kg DM) on growth performance: (A) Final body weight, (B) Average daily gain (ADG), and (C) Feeding efficiency in feedlot lambs. Bars represent least square means with numerical values; error bars indicate the standard error of the mean.

3.4. Discussion

The present study demonstrated that dietary starch concentration and calcium salts of palm fatty acids (CSPFA) interact to modify digestion, nitrogen partitioning, and the performance of feedlot lambs. The consistent pattern across response variables indicates that CSPFA does not act as a simple additional energy source; its advantages only become apparent when the fermentable energy supply is sufficiently high to promote intensive microbial growth (Yang and Beauchemin, 2006; Beauchemin, 2018).

3.4.1. Intake and digestibility: shifts in ruminal energetics

Increasing dietary starch from 220 to 420 g/kg DM reduced NDF digestibility (from 658 to 490 g/kg) and total chewing activity (from 11.92 to 10.49 h/d). These responses are compatible with the well-documented inhibitory effect of rapidly fermentable starch on fibrolytic bacteria and chewing behaviors. High-starch diets accelerate acid production, lower mean ruminal pH, and prolong the duration below pH 5.8, a range that selectively inhibits cellulolytic taxa such as *Ruminococcus albus* and *Fibrobacter succinogenes* (Ma et al., 2022; Zhang et al., 2024). The observed reduction in feeding time (from 18.77% to 15.16% of the day) is consistent with lower physically effective NDF (peNDF) and decreased salivary buffering.

Despite these challenges, CSPFA inclusion (30 g/kg DM) did not further depress NDF digestibility, which remained within a narrow range (570–578 g/kg) across all treatments. This outcome is unlikely to result from the complete ruminal inertness of palmitic acid alone because the supplement also contains a substantial proportion of oleic acid (34% C18:1). Under lower pH, typical of high-starch diets, the calcium salts of long-chain fatty acids, particularly Ca-oleate, can partially dissociate, releasing free unsaturated fatty acids into rumen fluid (Jenkins, 1993; Palmquist and Jenkins, 1980). Free oleic acid exerts selective antiprotozoal effects, reducing protozoal biomass and thereby decreasing bacterial turnover through predation (Doranalli and Mutsvangwa, 2011; Machmüller et al., 1998). Such partial defaunation can increase the net survival of fibrolytic and amylolytic bacteria, helping to maintain fiber digestion even when peNDF is reduced. This interpretation aligns with in vitro evidence that palmitic acid (C16:0) minimally inhibits cellulolytic taxa, whereas oleic acid (C18:1) can influence protozoal activity and reshape microbial interactions (Sears et al., 2024).

CSPFA also increased ether extract digestibility (from 799 to 904 g/kg) and improved starch digestibility within the high-starch diet (from 806 to 840 g/kg DM). These improvements align with a scenario where limited Ca-soap dissociation releases sufficient

oleic acid to suppress protozoa and reduce bacterial recycling, allowing a larger fraction of the amylolytic biomass to reach the lower gut (Doranalli and Mutsvangwa, 2011; Jenkins, 1993). Although rumen pH and protozoal counts were not measured, the combination of preserved NDF digestibility and higher starch digestibility suggests that CSPFA shifted microbial competition in favor of bacteria that convert fermentable starch into microbial biomass rather than simply altering total intake.

In low-starch diets, CSPFA reduced starch intake (from 308 to 275 g/d) without changing dry matter intake. Thus, the lower starch intake reflects the dilution of dietary starch density by the lipid supplement, not a physiological depression of appetite. Because the fermentable carbohydrate supply was already limited in the low-starch diet, this dilution further constrained microbial ATP availability and prevented improvements in microbial protein synthesis or in retained nitrogen. This agrees with synchrony theory, which predicts that when fermentable energy is limited, microbial growth is ATP-constrained, regardless of the additional lipid supply (Firkins, 1996; Nocek and Russell, 1988).

3.4.2. *Nitrogen metabolism and microbial protein synthesis*

The significant interaction between starch and CSPFA in nitrogen metabolism indicates that nitrogen utilization efficiency is highly dependent on the dietary energy source. In high-starch diets, CSPFA supplementation optimized nitrogen partitioning by shifting nitrogen excretion from urine to microbial protein synthesis, as evidenced by increased microbial nitrogen flow and higher nitrogen retention. Since nitrogen intake remained constant across treatments, these findings suggest that CSPFA acts as a metabolic modulator, reorganizing nitrogen flows and enhancing the incorporation of ammonia into microbial biomass when fermentable energy is abundant.

These responses are consistent with the concept of energy–nitrogen synchrony. Microbial protein synthesis requires a simultaneous supply of rumen-degradable nitrogen and fermentable carbohydrates to generate ATP for ammonia assimilation. When this temporal alignment is poor, ruminal $\text{NH}_3\text{-N}$ can rise above the 3–7 mM range considered optimal for microbial capture, increasing ammonia absorption, ureagenesis, and urinary N loss, thereby depressing microbial protein yield and overall NUE (Chen et al., 2022; Ren et al., 2022). Recent studies using rumen simulation systems have confirmed that higher synchrony indices between energy and nitrogen release enhance microbial protein synthesis and improve fermentation profiles (Chen et al., 2022; Ren et al., 2022). In our study, the high-starch diet without CSPFA exhibited the classical inefficiency signature, with higher serum urea, greater urinary N excretion, and lower microbial N flow.

CSPFA supplementation altered this pattern only when the starch supply was high. In the high-starch + CSPFA treatment, the combination of improved starch digestibility and partial defaunation likely increased ATP availability for bacteria and reduced protozoal predation, enhancing the capture of ammonia into microbial protein. Under these conditions, microbial growth is limited primarily by nitrogen availability rather than ATP (Firkins, 1996; Silva et al., 2014); therefore, improved synchrony can markedly increase microbial N flow. In contrast, in low-starch diets, microbial growth is ATP-limited; therefore, CSPFA cannot enhance microbial N capture, even if some protozoal suppression occurs. This explains the absence of CSPFA effects on nitrogen balance under low-starch supply conditions.

The concurrent reductions in serum urea and urinary nitrogen excretion, together with higher microbial N flow, are consistent with the increased efficiency of ruminal urea recycling (Lapierre and Lobley, 2001). Although urea kinetics were not directly measured, the nitrogen balance data indicated that a larger fraction of circulating urea was redirected for microbial assimilation rather than lost in urine, reinforcing the conclusion that CSPFA improved ammonia utilization rather than overall nitrogen intake.

3.4.3. *Serum metabolites and liver function*

Serum metabolites provide systemic support for nitrogen-use responses. The marked reduction in serum urea with high-starch + CSPFA reflects lower ammonia overflow and reduced hepatic ureagenesis, both of which are energetically costly (Firkins, 1996). Importantly, this decline occurred without any indication of hepatic or renal impairment in the patient. The activities of AST and ALT remained within physiological ranges and were unaffected by starch level, CSPFA inclusion, or their interaction, indicating preserved hepatocellular integrity (Dewanckele et al., 2020; Tedeschi et al., 2021).

Serum creatinine was slightly higher in lambs fed with the low-starch diet without CSPFA than in those fed with the high-starch diet, but CSPFA itself had no effect. As creatinine concentration reflects muscle mass when renal function is normal (Valkeners et al., 2006), this pattern is compatible with the greater nitrogen retained and lean tissue accretion observed in the high-starch + CSPFA group.

Finally, serum glucose, triglycerides, cholesterol, and VLDL levels remained stable and within normal physiological limits across all treatments. The maintenance of glycemic and lipid homeostasis indicates that including 30 g/kg CSPFA did not cause lipotoxicity, excessive fat mobilization, or dyslipidemia, which are adverse effects sometimes linked to

unprotected high-fat diets (Caixeta and Omontese, 2021; Heeren and Scheja 2021). Overall, the serum profile showed that CSPFA improved nitrogen efficiency under high-starch feeding without imposing additional metabolic load on the liver or kidney.

3.4.4. *Behavioural responses as integrators of digestion kinetics*

Ingestive behavior responses reflected shifts in dietary starch and peNDF. Lambs fed the high-starch diet spent less time feeding and more time idling than those on the low-starch diet, which is consistent with faster particle breakdown, reduced rumen mat formation, and lower chewing requirements in high-concentrate systems (Monjezi et al., 2022). Such reductions in chewing are relevant because they decrease salivary buffer secretion and, thereby, the rumen's capacity to neutralize acids produced during rapid starch fermentation (Allen, 1997; Owens et al., 1998).

CSPFA inclusion did not exacerbate starch-induced behavioral changes. Instead, it slightly increased feeding time and reduced idleness while maintaining the overall rumination time. More importantly, CSPFA increased the amount of dry matter processed per hour of rumination (173.7 to 218.1 g/h) and nearly doubled the NDF processed per hour (56.4 to 102.9 g/h). These improvements suggest a more efficient use of rumination time and align with the observed maintenance of NDF digestibility in CSPFA-supplemented diets. The lack of any reduction in NDF processing supports the idea that, at the inclusion level tested, partial Ca-soap dissociation and the presence of free fatty acids did not impair fibrolytic function (Tedeschi et al., 2021).

Chewing time per bolus was longer in lambs fed high-starch diets, which was associated with a smaller particle size and less cohesive rumen mat (Zhang et al., 2023). Nevertheless, the total daily chewing time remained within physiological ranges (≈ 11 – 12 hours per day), indicating that CSPFA did not cause severe behavioral disruption. Overall, the behavioral data aligned with the digestion and nitrogen results, supporting that CSPFA maintained fiber processing while improving the utilization of high-starch diets.

3.4.5. *Growth performance: nitrogen retained as the driver of productivity*

Growth performance was integrated with the cumulative effects of starch concentration and CSPFA. In high-starch diets, CSPFA increased final body weight (34.32 to 37.45 kg), average daily gain (203.71 to 274.84 g/d), and feed efficiency (181.93 to 222.82 g/kg DMI). These results are similar to those reported for finishing lambs supplemented with lipids under energy-dense conditions (Mierlita et al., 2010). In contrast, CSPFA did not improve performance on low-starch diets, indicating that its effectiveness depends on the fermentative energy context.

The performance responses aligned with the improvements observed in nitrogen metabolism. Microbial amino acids are the primary source of metabolizable protein in ruminants, typically providing 50–80% of the amino acids absorbed in the small intestine (Lima et al., 2023; de Oliveira et al., 2025). In sheep, microbial protein synthesis commonly reaches 40–45 g/d, with efficiencies of approximately 150–200 g microbial protein per kg of ruminally digested organic matter, values that have recently been reaffirmed in controlled feeding studies (Lima et al., 2023; de Oliveira et al., 2025). Contemporary microbiome analyses have further shown that increases in the abundance and activity of key bacterial taxa, particularly *Prevotella spp.*, which are major contributors to amino acid biosynthesis, are strongly associated with higher microbial protein flow and improved nitrogen-use efficiency (Xue et al., 2020).

Therefore, even modest enhancements in microbial protein synthesis, such as those observed in the high-starch + CSPFA treatment, can substantially increase the supply of metabolizable amino acids and support greater tissue accretion and growth. In the high-starch + CSPFA treatment, higher microbial N flow, greater purine derivative excretion, and higher nitrogen retained indicated that more dietary nitrogen was converted into tissue protein rather than being lost as urea. These patterns resemble classical reports in which partial defaunation, including that induced by dietary lipids, increased the microbial N supply by approximately 30–35% and improved nitrogen retention (Doranalli and Mutsvangwa, 2011; Eugène et al., 2004; Newbold et al., 2015).

Therefore, the lack of performance response to low-starch diets is not a failure of the lipid source per se but rather a consequence of ATP limitation and reduced protozoal abundance under high-fiber conditions (Jouany, 1996; Russell and Wallace, 1997). Under these circumstances, lipid supplementation cannot meaningfully increase microbial protein synthesis, and no gain in growth performance is expected.

3.5. Conclusions and perspectives

This study demonstrated that the metabolic fate of nitrogen in feedlot lambs fed calcium salts of palm fatty acids (CSPFA) is determined by the availability of fermentable carbohydrates. CSPFA supplementation enhances nitrogen retention, microbial protein synthesis, and growth performance only when dietary starch levels are high. This substrate dependency indicates that CSPFA functions not only as an energy source but also as a modulator of ruminal synchrony, likely through partial defaunation and improved ammonia

capture. The lack of response to low-starch diets confirms that lipid supplementation cannot overcome the limitations of the microbial ATP supply. Furthermore, CSPFA supports high-performance finishing without compromising hepatic or renal function, validating its use as a strategic tool for maximizing nitrogen use efficiency in intensive feeding systems.

References

Allen MS. Relationship Between Fermentation Acid Production in the Rumen and the Requirement for Physically Effective Fiber. *J Dairy Sci* 1997;80:1447–62. [https://doi.org/10.3168/jds.S0022-0302\(97\)76074-0](https://doi.org/10.3168/jds.S0022-0302(97)76074-0).

AOAC. Association of official analytical chemists. Official Methods of Analysis, 20th Ed Arlington, VA: Journal - Association of Official Analytical Chemists 2016.

Bürger PJ, Pereira JC, Queiroz AC de, Coelho da Silva JF, Valadares Filho S de C, Cecon PR, et al. Comportamento ingestivo em bezerros holandeses alimentados com dietas contendo diferentes níveis de concentrado. *Revista Brasileira de Zootecnia* 2000;29:236–42. <https://doi.org/10.1590/S1516-35982000000100031>.

Caixeta LS, Omontese BO. Monitoring and Improving the Metabolic Health of Dairy Cows during the Transition Period. *Animals* 2021;11:352. <https://doi.org/10.3390/ani11020352>.

Carvalho GGP de, Pires AJV, Silva FF da, Veloso CM, Silva RR, Silva HG de O, et al. Comportamento ingestivo de cabras leiteiras alimentadas com farelo de cacau ou torta de dendê. *Pesqui Agropecu Bras* 2004;39:919–25. <https://doi.org/10.1590/S0100-204X2004000900012>.

Carvalho GGP de, Pires AJV, Silva RR, Carvalho BMA de, Silva HG de O, Carvalho LM de. Aspectos metodológicos do comportamento ingestivo de ovinos alimentados com capim-elefante amonizado e subprodutos agroindustriais. *Revista Brasileira de Zootecnia*

2007;36:1105–12. <https://doi.org/10.1590/S1516-35982007000500017>.

Chen P, Li Y, Shen Y, Cao Y, Li Q, Wang M, et al. Effect of Dietary Rumen-Degradable Starch to Rumen-Degradable Protein Ratio on In Vitro Rumen Fermentation Characteristics and Microbial Protein Synthesis. *Animals* 2022;12:2633. <https://doi.org/10.3390/ani12192633>.

Chen XB, Gomes M. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives: an overview of the technical details 1992.

da Cruz CH, Santos SA, de Carvalho GGP, Azevedo JAG, Detmann E, Valadares Filho S de C, et al. Estimating digestible nutrients in diets for small ruminants fed with tropical forages. *Livest Sci* 2021;249:104532. <https://doi.org/10.1016/j.livsci.2021.104532>.

Dewanckele L, Toral PG, Vlaeminck B, Fievez V. Invited review: Role of rumen biohydrogenation intermediates and rumen microbes in diet-induced milk fat depression: An update. *J Dairy Sci* 2020;103:7655–81. <https://doi.org/10.3168/jds.2019-17662>.

Dische Z. General color reactions. *Methods in Carbohydrate Chemistry* 1962;1:478–512.

Doranalli K, Mutsvangwa T. Feeding sunflower oil to partially defaunate the rumen increases nitrogen retention, urea-nitrogen recycling to the gastrointestinal tract and the anabolic use of recycled urea-nitrogen in growing lambs. *British Journal of Nutrition* 2011;105:1453–64. <https://doi.org/10.1017/S0007114510005155>.

Eugène M, Archimède H, Sauvant D. Quantitative meta-analysis on the effects of defaunation of the rumen on growth, intake and digestion in ruminants. *Livest Prod Sci* 2004;85:81–97. [https://doi.org/10.1016/S0301-6226\(03\)00117-9](https://doi.org/10.1016/S0301-6226(03)00117-9).

Firkins JL. Maximizing Microbial Protein Synthesis in the Rumen. *J Nutr* 1996;126:1347S-1354S. https://doi.org/10.1093/jn/126.suppl_4.1347S.

- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. *Clin Chem* 1972;18:499–502. <https://doi.org/10.1093/clinchem/18.6.499>.
- Heeren J, Scheja L. Metabolic-associated fatty liver disease and lipoprotein metabolism. *Mol Metab* 2021;50:101238. <https://doi.org/10.1016/j.molmet.2021.101238>.
- Hutchings NJ, Sørensen P, Cordovil CM d. S, Leip A, Amon B. Measures to increase the nitrogen use efficiency of European agricultural production. *Glob Food Sec* 2020;26:100381. <https://doi.org/10.1016/j.gfs.2020.100381>.
- Jenkins TC. Lipid Metabolism in the Rumen. *J Dairy Sci* 1993;76:3851–63. [https://doi.org/10.3168/jds.S0022-0302\(93\)77727-9](https://doi.org/10.3168/jds.S0022-0302(93)77727-9).
- Jouany J-P. Effect of Rumen Protozoa on Nitrogen Utilization by Ruminants. *J Nutr* 1996;126:1335S-1346S. https://doi.org/10.1093/jn/126.suppl_4.1335S.
- Lapierre H, Lobley GE. Nitrogen Recycling in the Ruminant: A Review. *J Dairy Sci* 2001;84:E223–36. [https://doi.org/10.3168/jds.S0022-0302\(01\)70222-6](https://doi.org/10.3168/jds.S0022-0302(01)70222-6).
- Lima J, Ingabire W, Roehe R, Dewhurst RJ. Estimating Microbial Protein Synthesis in the Rumen—Can ‘Omics’ Methods Provide New Insights into a Long-Standing Question? *Vet Sci* 2023;10:679. <https://doi.org/10.3390/vetsci10120679>.
- Ma X, Zhou W, Guo T, Li Fei, Li Fadi, Ran T, et al. Effects of Dietary Barley Starch Contents on the Performance, Nutrient Digestion, Rumen Fermentation, and Bacterial Community of Fattening Hu Sheep. *Front Nutr* 2022;8. <https://doi.org/10.3389/fnut.2021.797801>.
- Machmüller A, Ossowski DA, Wanner M, Kreuzer M. Potential of various fatty feeds to reduce methane release from rumen fermentation in vitro (Rusitec). *Anim Feed Sci Technol* 1998;71:117–30. [https://doi.org/10.1016/S0377-8401\(97\)00126-0](https://doi.org/10.1016/S0377-8401(97)00126-0).

Mierlita D, Padeanu I, Daraban S, Lup F, Maerescu C, Chereji I. Effect of dietary supplementation with different types of protected fat on bioproductive performances and quality of carcass in sheeps. 2010.

Monjezi Y, Sari M, Chaji M, Ferret A. Effects of concentrate starch level and free-choice provision of straw on performance, feeding behaviour and feed sorting of fattening lambs. *Appl Anim Behav Sci* 2022;256:105773. <https://doi.org/10.1016/j.applanim.2022.105773>.

Newbold CJ, de la Fuente G, Belanche A, Ramos-Morales E, McEwan NR. The Role of Ciliate Protozoa in the Rumen. *Front Microbiol* 2015;6. <https://doi.org/10.3389/fmicb.2015.01313>.

Nocek JE, Russell JB. Protein and Energy as an Integrated System. Relationship of Ruminant Protein and Carbohydrate Availability to Microbial Synthesis and Milk Production. *J Dairy Sci* 1988;71:2070–107. [https://doi.org/10.3168/jds.S0022-0302\(88\)79782-9](https://doi.org/10.3168/jds.S0022-0302(88)79782-9).

de Oliveira M, Costa C, Fernandes T. Graduate Student Literature Review: Concepts and challenges of amino acid supply and nitrogen metabolism in dairy cattle. *J Dairy Sci* 2025;108:6906–16. <https://doi.org/10.3168/jds.2024-26136>.

Owens FN, Secrist DS, Hill WJ, Gill DR. Acidosis in cattle: a review. *J Anim Sci* 1998;76:275. <https://doi.org/10.2527/1998.761275x>.

Palmquist DL, Jenkins TC. Fat in Lactation Rations : Review. *J Dairy Sci* 1980;63:1–14. [https://doi.org/10.3168/jds.S0022-0302\(80\)82881-5](https://doi.org/10.3168/jds.S0022-0302(80)82881-5).

Pereira ES, Teixeira IAMA, Azevêdo JAG, Santos SA. Exigências Nutricionais de Caprinos e Ovinos – BR-Caprinos & Ovinos. São Carlos: Editora Scienza; 2024.

Plomaritou A, Hanlon M, Kantas D, Georgakoudis K, Dovolou E, Foskolos A. A Review of Nitrogen Use Efficiency of Dairy Replacement Heifers: Improving Management

Practices and Minimizing Nitrogen Losses. *Animals* 2025;15:1031.
<https://doi.org/10.3390/ani15071031>.

Reis MJ, Santos SA, Prates LL, Detmann E, Carvalho GGP, Santos ACS, et al. Comparing sheep and cattle to quantify internal markers in tropical feeds using in situ ruminal incubation. *Anim Feed Sci Technol* 2017;232:139–47.
<https://doi.org/10.1016/j.anifeedsci.2017.08.013>.

Ren G, Hao X, Zhang X, Liu S, Zhang J. Effects of guanidinoacetic acid and betaine on growth performance, energy and nitrogen metabolism, and rumen microbial protein synthesis in lambs. *Anim Feed Sci Technol* 2022;292:115402.
<https://doi.org/10.1016/j.anifeedsci.2022.115402>.

Rodrigues HP, Ferreira JM de S, Pereira MIB, Trajano LS, Souza LL, dos Santos Pina D, et al. Levels of starch and supplementation with calcium salts of palm oil fatty acids in the diets of goats and sheep. *Small Ruminant Research* 2026;254:107661.
<https://doi.org/10.1016/j.smallrumres.2025.107661>.

Russell JB, Wallace RJ. Energy-yielding and energy-consuming reactions. The rumen microbial ecosystem, Springer; 1997, p. 246–82.

dos Santos ACS, dos Santos GR, da Silva MP, Mariz LDS, Alba HDR, Tosto MSL, et al. Estimation of indigestible NDF by in situ procedure in sheep: number of bags and fabric type. *Frontiers in Animal Science* 2025;6. <https://doi.org/10.3389/fanim.2025.1547776>.

SAS - Statistical Analysis System, version 9.4, SAS Institute Inc., Cary, NC. 2013.

Scoresby D, Teixeira IAMA, Chahine M. Effects of Corn Silage and Alfalfa Hay on Production and Nitrogen Excretion in Lactating Dairy Cows. *Nitrogen* 2025;6:43.
<https://doi.org/10.3390/nitrogen6020043>.

Sears A, Hentz F, de Souza J, Wenner B, Ward RE, Batistel F. Supply of palmitic, stearic,

and oleic acid changes rumen fiber digestibility and microbial composition. *J Dairy Sci* 2024;107:902–16. <https://doi.org/10.3168/jds.2023-23568>.

Seo JK, Yang J, Kim HJ, Upadhaya SD, Cho WM, Ha JK. Effects of Synchronization of Carbohydrate and Protein Supply on Ruminal Fermentation, Nitrogen Metabolism and Microbial Protein Synthesis in Holstein Steers. *Asian-Australas J Anim Sci* 2010;23:1455–61. <https://doi.org/10.5713/ajas.2010.10247>.

Silva AC da, Figueiredo MP de, Bonomo P, Pereira MLA, Luz YDS, Santos EDJ. Microbial protein synthesis and nitrogen metabolism in cows bred on tropical pasture and fed on cassava root and corn. *Acta Sci* 2014;36:185. <https://doi.org/10.4025/actascianimsci.v36i2.22161>.

Van Soest PJ, Robertson JB, Lewis BA. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *J Dairy Sci* 1991;74:3583–97. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2).

Tebbe AW, Faulkner MJ, Weiss WP. Effect of partitioning the nonfiber carbohydrate fraction and neutral detergent fiber method on digestibility of carbohydrates by dairy cows. *J Dairy Sci* 2017;100:6218–28. <https://doi.org/10.3168/jds.2017-12719>.

Tedeschi LO, Muir JP, Naumann HD, Norris AB, Ramírez-Restrepo CA, Mertens-Talcott SU. Nutritional Aspects of Ecologically Relevant Phytochemicals in Ruminant Production. *Front Vet Sci* 2021;8. <https://doi.org/10.3389/fvets.2021.628445>.

Valkeners D, Théwis A, Amant S, Beckers Y. Effect of various levels of imbalance between energy and nitrogen release in the rumen on microbial protein synthesis and nitrogen metabolism in growing double-muscled Belgian Blue bulls fed a corn silage-based diet1. *J Anim Sci* 2006;84:877–85. <https://doi.org/10.2527/2006.844877x>.

WACHIRA AM, SINCLAIR LA, WILKINSON RG, HALLETT K, ENSER M, WOOD

JD. Rumen biohydrogenation of $n-3$ polyunsaturated fatty acids and their effects on microbial efficiency and nutrient digestibility in sheep. *J Agric Sci* 2000;135:419–28. <https://doi.org/10.1017/S0021859699008370>.

Xue M-Y, Sun H-Z, Wu X-H, Liu J-X, Guan LL. Multi-omics reveals that the rumen microbiome and its metabolome together with the host metabolome contribute to individualized dairy cow performance. *Microbiome* 2020;8:64. <https://doi.org/10.1186/s40168-020-00819-8>.

Zebeli Q, Aschenbach JR, Tafaj M, Boguhn J, Ametaj BN, Drochner W. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. *J Dairy Sci* 2012;95:1041–56. <https://doi.org/10.3168/jds.2011-4421>.

Zhang Z, Wang L, Li Q, Li Fei, Ma Z, Li Fadi, et al. Effects of dietary forage neutral detergent fiber and rumen degradable starch ratios on chewing activity, ruminal fermentation, ruminal microbes and nutrient digestibility of *Hu* sheep fed a pelleted total mixed ration. *J Anim Sci* 2024;102. <https://doi.org/10.1093/jas/skae100>.

Zhang ZA, Li F, Ma ZY, Li FD, Wang ZL, Li SR, et al. Variability in chewing, ruminal fermentation, digestibility and bacterial communities between subacute ruminal acidosis-susceptible and acidosis-tolerant sheep. *Animal* 2023;17:100902. <https://doi.org/10.1016/j.animal.2023.100902>.

4. CAPÍTULO III

Article published in the journal Agriculture (2-18)

Doi:10.3390/agriculture16010098

Effects of Dietary Starch Level and Calcium Salts of Palm Fatty Acids on Carcass Traits and Meat Quality of Lambs

Joyanne Mirelle Sousa Ferreira¹, Herick Pachêco Rodrigues², Maria Izabel Batista Pereira², Lais Santos Trajano³, Ligia Lins Souza⁴, Henry Daniel Ruiz Alba⁵, Jose Esler de Freitas Junior⁶, Gleidson Giordano Pinto de Carvalho⁶, Douglas dos Santos Pina⁶, Stefanie Alvarenga Santos⁶ and Jose Augusto Gomes Azevêdo^{3,*}

1 Postgraduate Program in Animal Science, Universidade Estadual do Sudoeste da Bahia, Itapetinga 45700000, Bahia, Brazil; 2022m0099@uesb.edu.br

2 Postgraduate Program in Animal Science, Universidade Estadual de Santa Cruz, Ilheus 45662900, Bahia, Brazil; hprodrigues.ppgca@uesc.br (H.P.R.); mibpereira@uesc.br (M.I.B.P.)

3 Department of Agricultural and Environmental Sciences, Universidade Estadual de Santa Cruz, Ilheus 45662900, Bahia, Brazil; lstrajano.agr@uesc.br

4 Department of Exact and Technological Sciences, Universidade Estadual do Sudoeste da Bahia, Vitoria da Conquista 45083900, Bahia, Brazil; ligia.souza@uesb.edu.br

5 Department of Agronomy, Universidade Federal do Oeste do Para, Santarem 68040255, Para, Brazil; henry.alba@ufopa.edu.br

6 Department of Animal Science, Universidade Federal da Bahia, Salvador 40170110, Bahia, Brazil; jose.esler@ufba.br (J.E.d.F.J.); gleidsongiordano@ufba.br (G.G.P.d.C.); douglas.pina@ufba.br (D.d.S.P.);

stefanie.alvarenga@ufba.br (S.A.S.)

** Correspondence: augustog@uesc.br*

Abstract

This study aimed to investigate the interactive effects of dietary starch concentration (220 or 420 g/kg DM) and supplementation with calcium salts of palm fatty acids (CSPFAs; 0 or 30 g/kg DM) on carcass characteristics and meat quality in lambs. Thirty-two castrated Dorper $\times$ Santa Inês male lambs (initial body weight 25.0 $\pm$ 2.85 kg) were randomly assigned to four experimental diets in a 2 $\times$ 2 factorial arrangement for 44 days. Although carcass yield remained unaffected (average 49.4%; $p > 0.05$), CSPFA supplementation significantly increased fat deposition, including perirenal fat mass (590 vs. 400 g; $p = 0.005$), renal fat score (2.90 vs. 2.66; $p = 0.035$), and subcutaneous fat thickness (3.8 vs. 1.9 mm; $p = 0.017$). A starch $\times$ CSPFA interaction ($p = 0.014$) was observed for carcass cooling, where high-starch diets reduced the 24-hour temperature only in lambs not receiving CSPFA (7.45 vs. 8.48 °C; $p = 0.028$). CSPFA also altered the muscle fatty acid profile by increasing C16:0 and total saturated fatty acids (SFA) while reducing polyunsaturated fatty acids (PUFA). In conclusion, palm-oil-derived CSPFA enhances carcass fatness but compromises the nutritional value of lamb meat by promoting an unfavorable fatty acid profile.

Keywords: adipose tissue metabolism; nutritional quality; rumen-protected fat

4.1. Introduction

Intensive lamb production is expanding to meet market demands for a consistent supply and improved carcass yield. However, the lipid quality of lamb meat remains a significant concern due to its relatively high saturated fatty acid (SFA) content and low concentrations of long-chain polyunsaturated fatty acids (PUFAs), particularly n-3 PUFAs, which are associated with cardioprotective effects [1,2]. Consequently, enhancing the fatty acid profile of lamb meat without compromising growth performance is a key objective for innovation in ruminant nutrition [3,4].

Two major nutritional factors directly influence lipid metabolism in ruminants: dietary starch and rumen-protected fats. Starch serves as a key modulator of ruminal fermentation and biohydrogenation; elevated starch levels lower ruminal pH, shift microbial populations, and modify hydrogenation pathways that determine the formation of conjugated linoleic acid (CLA) and its intermediates [5,6]. In contrast, calcium salts of palm fatty acids (CSPFAs) enhance dietary energy density and escape ruminal biohydrogenation, delivering preformed long-chain fatty acids to the small intestine for post-absorptive metabolism [7–9]. However, CSPFAs derived from palm oil are particularly rich in palmitic acid (C16:0), raising concerns regarding increased SFA deposition and adverse impacts on health-related lipid indices [2,10].

Despite extensive research on these dietary components, the available evidence remains fragmented. Studies investigating CSPFA and other rumen-protected fats have reported alterations in tissue lipid composition and fat depot distribution, but effects on carcass yield have been inconsistent [10,11]. Similarly, dietary manipulation of starch has been shown to affect ruminal biohydrogenation, yet it has not consistently enhanced beneficial PUFA levels [12,13]. Notably, most studies have evaluated starch and CSPFA in isolation. To date, no research has examined the interactive effects of dietary starch $\times$ CSPFA on carcass traits, fat deposition patterns, post-mortem temperature dynamics, or intramuscular fatty acid composition. This lack of integrative analysis limits our understanding of whether high dietary starch facilitates or impairs the utilization of long-chain fatty acids supplied by CSPFA.

We hypothesized that dietary starch concentration and CSPFA supplementation interact to modulate energy partitioning and lipid metabolism, thereby influencing fat distribution, post-mortem carcass temperature, and the fatty acid profile of the Longissimus dorsi muscle. Accordingly, this study employed a 2×2 factorial design to evaluate the main and interactive effects of dietary starch level and CSPFA supplementation on lamb

carcass traits, physicochemical parameters, and intramuscular fatty acid composition in lambs.

4.2. Materials and Methods

4.2.1. *Animal Ethics*

All experimental procedures complied with Brazilian guidelines for the ethical use of animals in research (Law No. 11.794, 8 October 2008) [14] and were approved by the Ethics Committee on Animal Use (CEUA) of the Universidade Estadual de Santa Cruz (UESC), Ilheus, Bahia, Brazil (approval protocol number 008/2025, dated 6 May 2025).

4.2.2. *Animals, Experimental Design, and Diets*

The study was conducted at the Laboratório de Pesquisa em Nutrição e Alimentação de Ruminantes (LaPNAR/UESC, Ilheus, Bahia, Brazil). The experimental facility consisted of a covered, naturally ventilated barn (ceiling height: 3.5 m) equipped with 40 individual pens (1.20 × 0.80 m), each featuring slatted wooden flooring, individual feed bunks, and water buckets. During the experimental period, the average ambient temperature ranged from 23 to 29 °C, with relative humidity between 70% and 86%, which is typical of a humid tropical climate (Köppen classification: Af).

Thirty-two castrated male lambs (Dorper × Santa Inês), approximately 120 days old and with an initial average body weight of 25.0 ± 2.85 kg, were used. Animals were identified, dewormed, vaccinated against clostridial diseases, and acclimated to the experimental conditions for 15 days prior to the start of the feeding trial. The initial body weight reported corresponds to the beginning of the 44-day feeding period. Animals were assigned to treatments through stratified randomization based on initial body weight to maintain homogeneity across groups.

The experiment design was a completely randomized design with a 2×2 factorial arrangement, testing two starch levels (220 or 420 g/kg DM) and the inclusion (30g/kg DM) or absence of calcium salts of palm fatty acids (CSPFAs; Nutri Gordura LacR, Nutricorp Inc., Araras, SP, Brazil). Diets were formulated according to Exigências Nutricionais de Caprinos e Ovinos—BR-Caprinos & Ovinos [15] recommendations for finishing lambs, using a target average daily gain of 200 g/animal/day as a reference for nutrient adequacy. All diets were based on corn silage and a concentrate mixture comprising ground corn, soybean meal, soybean hulls, urea, limestone, and mineral salt (Table 1). Metabolizable energy (ME) values were estimated according to Cruz et al. [16],

based on the proportions of ingredients and the chemical composition of the experimental diets.

Tabela 1. Proportion of ingredients and chemical composition of experimental diets.

Treatments	Experimental diets			
Starch level, g/kg DM	220	220	420	420
CSPFA level, g/kg DM	30	0	30	0
Ingredients proportion, g/kg DM				
Corn silage	400.0	400.0	400.0	400.0
Ground corn	190.4	187.3	485.0	483.2
Soy hull	322.6	314.1	0.0	12.7
Soybean meal	32.1	74.6	60.2	74.6
Urea	9.9	3.0	10.0	7.0
Limestone	6.5	12.0	9.7	14.0
Mineral mixture ¹	8.5	9.0	5.1	8.5
CSPFA ²	30.0	0.0	30.0	0.0
Chemical composition, g/kg DM				
Dry matter, <i>as-fed</i> basis	663.3	662.2	664.8	653.8
Organic matter	961.1	955.5	961.9	953.5
Ether extract	54.45	22.23	60.4	36.5
Crude protein	135.0	135.0	135.0	136.1
Neutral detergent fiber	466.0	474.0	323.4	330.5
Starch	220.0	220.0	420.0	420.0
Residual organic matter	85.7	104.3	23.2	30.4
Metabolizable energy (Mcal/day) ³	2.85	2.69	2.96	2.81
Fatty acid profile, mg/100g DM				
Caprylic (C8:0)	0.03	0.03	0.03	0.03
Capric (C10:0)	0.15	0.15	0.15	0.15
Lauric (C12:0)	0.30	0.31	0.30	0.31
Myristic (C14:0)	1.99	1.81	1.78	1.62
Palmitic (C16:0)	15.08	14.50	15.34	15.14
Stearic (C18:0)	4.39	4.71	4.38	4.69
Palmitoleic (C16:1)	0.38	0.36	0.38	0.36
Oleic (C18:1 n-9)	28.10	28.09	28.09	27.94
Linoleic (C18:2 n-6)	46.61	47.13	46.58	46.87
α -linolenic (C18:3 n-3)	2.96	2.90	2.96	2.88

¹ Guaranteed levels (per kg of product): Calcium (min.) 110.0 g/kg; Calcium (max.) 135.0 g/kg; Phosphorus 87.0 g/kg; Sulfur 18.0 g/kg; Sodium 147.0 g/kg; Cobalt 15.0 mg/kg; Copper 590.0 mg/kg; Chromium 20.0 mg/kg; Iodine 50.0 mg/kg; Manganese 2,000.0 mg/kg; Molybdenum 300.0 mg/kg; Selenium 20.0 mg/kg; Zinc 3,800.0 mg/kg; Fluorine (max.) 870.0 mg/kg. ² Calcium salts of palm fatty acids (Nutri Gordura Lac, Nutricorp Inc., Araras, São Paulo, Brazil). Guaranteed composition: 84.9% total fatty acids (48.6% C16:0, 4.40% C18:0, 34.3% C18:1 cis-9, 5.5% C18:2 cis-9 cis-12, and others <2% each); minimum ether extract: 820 g/kg; minimum calcium: 67 g/kg; maximum moisture: 50 g/kg; maximum ash: 200 g/kg; maximum acid value: 10.0 mg NaOH/g; maximum peroxide value: 5.0 meq/kg. ³ estimated according to [15].

The composition of the CSPFA and the chemical composition supplement were provided by Nutri Gordura LacR (Nutricorp Inc., Araras, SP, Brazil) and verified through laboratory analysis before the feeding trial. The supplement contained 84.9% total fatty acids (48.6% C16:0, 34.3% C18:1 cis-9, 5.5% C18:2 cis-9,12, 4.4% C18:0, others <2%). Fatty acid concentrations were expressed as percentages of the total fatty acids in the supplement. Additionally, the proximate composition included a minimum ether extract of 820 g/kg, calcium content of 67 g/kg, maximum moisture of 50 g/kg, and maximum ash

content of 200 g/kg, with an acidity index of <10 mg NaOH/g and peroxide index of <5 meq/kg.

To determine the chemical composition of the experimental diets, feed samples were pre-dried at 60 °C for 72 hours and ground to pass through a 1 mm screen. Analyses for dry matter, crude protein, ether extract, and ash were performed in duplicate, following the established methodologies of the Association of Official Analytical Chemists (AOAC) [17]. Neutral and acid detergent fibers were determined according to Van Soest et al. [18], using heat-stable α -amylase and omitting sodium sulfite. Starch concentration was analyzed colorimetrically at the 3rLab Agricultural Analysis Laboratory (Lavras, MG, Brazil) following the method of Dische [19]. Residual organic matter was calculated as described by Tebbe et al. [20], and metabolizable energy was estimated according to Cruz et al. [16]. The fatty acid profiles of the feeds and diets were determined as described in Section 2.4.

Diets were offered twice a day (07:30 and 14:00 h), with daily adjustments to accommodate 10–20% refusals. Clean, fresh water was provided ad libitum in individual 5L buckets, which were cleaned and refilled daily.

4.2.3. *Slaughter and Carcass Evaluation*

At the end of the 44-day feeding period, lambs underwent a 16 h solid-feed withdrawal while retaining free access to water. Pre-slaughter body weight was recorded to determine slaughter live weight (SLW). Animals were humanely slaughtered via captive-bolt stunning followed by exsanguination, in accordance with Brazilian Regulation of Industrial and Sanitary Inspection of Products of Animal Origin (RIISPOA, 2017) [21].

Evisceration was performed, and the contents of the gastrointestinal tract, gallbladder, and urinary bladder were removed to determine empty body weight (EBW). Hot carcass weight (HCW) was recorded after removal of the head, feet, genital organs, and respiratory tract. Hot carcass yield ($\text{HCY} = \text{HCW}/\text{SLW} \times 100$) and true yield ($\text{TY} = \text{HCW}/\text{EBW} \times 100$) were calculated as described by Cezar and Sousa [22]. Carcasses were then chilled at -4 °C for 24 h in a chamber with an airflow of approximately 1.5 m/s and a minimum spacing of 15 cm between carcasses. Then, cold carcass weight (CCW) was measured, and cold carcass yield ($\text{CCY} = \text{CCW}/\text{SLW} \times 100$) and chilling loss ($\text{CL} = (\text{HCW} - \text{CCW})/\text{HCW} \times 100$) were calculated.

Carcass pH and internal temperature were measured using a digital thermometer with a penetration probe inserted 2.5 cm into the Longissimus dorsi at the 12th rib interface

approximately 30 min postmortem and after 24 h of chilling. A calibrated pH meter with a penetration electrode was used for the measurements.

Each carcass was split longitudinally and sectioned into six commercial cuts: shoulder, neck, rib, rib-flank, loin, and leg, according to the method described by Cezar and Sousa [22]. All cuts were weighed and stored at -20°C for subsequent analyses. Subcutaneous fat thickness (SFT) was measured using a digital caliper over the Longissimus dorsi at the 12th rib. Fat depth was measured 11 cm from the midline at the same anatomical location. The loin eye area was traced on transparent plastic film, digitized, and analyzed using AutoCADR software, version 2023 (Autodesk Inc., San Rafael, CA, USA).

4.2.4. *Lipid Extraction and Fatty Acid Profile*

Lipid composition was analyzed in the Longissimus dorsi, the reference muscle for intramuscular fat metabolism and meat quality studies in ruminants [23,24]. Samples were collected from the left half of each carcass, lyophilized, and finely ground prior to analysis. Total lipids were extracted from homogenized tissue using the chloroform–methanol method described by Bligh and Dyer [25], with minor modifications. Lipid content was determined gravimetrically following solvent evaporation in a rotary evaporator at $33\text{--}34^{\circ}\text{C}$. Extracts were stored in n-heptane at -20°C until further analysis.

Fatty acid methyl esters (FAME) were prepared by transesterifying approximately 150 mg of extracted lipids with 0.25 mol/L sodium methoxide in methanol–diethyl ether. FAME were then extracted with iso-octane and washed with saturated NaCl solution [26]. To validate the recovery of long-chain PUFAs, a subset of samples was also transesterified according to the method of O’Fallon et al. [27].

The fatty acid methyl esters were analyzed using both a Shimadzu GC-2010 Plus (Shimadzu Corp., Kyoto, Japan) and an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA), each equipped with a flame ionization detector (FID) and a high-polarity capillary column (Rt-2560, Restek Corp., Bellefonte, PA, USA, or CP-Sil 88, Agilent Technologies, Santa Clara, CA, USA; $100\text{ m} \times 0.25\text{ mm i.d.}$, $0.20\text{ }\mu\text{m}$ film thickness). The carrier gas was hydrogen (40 mL/min), with nitrogen as make-up gas (30 mL/min), and synthetic air (400 mL/min). The injector and detector temperatures were set to 225 and 260°C , respectively. The oven program initiated at 140°C (held for 5 min), ramped at 3°C/min to 245°C , and was held for 20 min, totaling a 60 min run time. Duplicate $1\text{ }\mu\text{L}$ injections were performed, and peak areas were integrated using GCSolutionR software, version 2.42 (Shimadzu Corp., Kyoto, Japan).

The fatty acid methyl esters peaks were identified by comparison with retention times of external standards (Sigma-Aldrich, Supelco 37 FAME Mix, CLA isomers, and trans-18:1 isomers; Sigma-Aldrich, St. Louis, MO, USA). Quantification was conducted using methyl tricosanoate (C23:0) as an internal standard [28]. Fatty acid concentrations were expressed as mg fatty acids/g total lipids. Analytical validation included determination of the detection limit (LOD = 0.001 g/100 g FA) and quantification limit (LOQ = 0.005 g/100 g FA), calibration coefficients ($R^2 > 0.995$), and analytical coefficients of variation ($CV < 5\%$).

Only major fatty acids and those of nutritional or technological importance are reported in the main results table. Quantification was based on authentic standards, including CLA isomers, EPA, and DHA. Fatty acid percentages were normalized to the sum of identified and quantified peaks. Trace fatty acids ($<0.05\%$ of total FA) and unresolved peaks near baseline noise were reported as not detected (ND), which may cause totals not to add up exactly to 100%. Following the identification of fatty acids, the $\Delta 9$ -desaturase indices were calculated according to the equations proposed by Bichi et al. [29] and Malau-Aduli et al. [30].

4.2.5. *Nutritional Quality Indices*

The nutritional quality of intramuscular fat was assessed using: atherogenicity Index (AI), thrombogenicity Index (TI) [31], hypocholesterolemic/hypercholesterolemic ratio (h: H) [29], and desirable fatty acids, expressed as the sum of C18:0 + MUFA + PUFA [32].

4.2.6. *Cholesterol Determination*

Cholesterol content was determined following the method of Saldanha et al. [33]. Briefly, 2 g of homogenized muscle were saponified with ethanolic KOH, and the unsaponifiable fraction was extracted with hexane. Quantification was performed using HPLC (Shimadzu; C18 column, 250×4.6 mm, $3.5 \mu\text{m}$). The mobile phase consisted of acetonitrile:isopropanol (85:15, v/v) at a flow rate of 2.0 mL/min. The injection volume was 20 μL , and detection was performed via UV absorbance at 202 nm. Cholesterol was identified based on retention time and quantified using external calibration curves.

4.2.7. Statistical Analysis

Data were analyzed using a 2×2 factorial arrangement in a completely randomized design to test the main effects of dietary starch level and CSPFA inclusion, as well as their interaction. The statistical model was:

$$Y_{ijk} = \mu + S_i + C_j + (S \times C)_{ij} + \epsilon_{ijk}$$

where μ = overall mean, S_i = fixed effect of starch level, C_j = fixed effect of CSPFA inclusion, $(S \times C)_{ij}$ = interaction effect, and ϵ_{ijk} = random error.

Analyses were conducted with the MIXED procedure in SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA) [34]. Fixed effects included starch level, CSPFA supplementation, and their interaction. The individual animal was treated as a random effect. The experimental unit for carcass traits and meat quality was the half carcass.

Model assumptions were assessed by residual diagnostics, including the Shapiro–Wilk test for normality and Levene’s test for homogeneity of variances. As each factor had only two levels, significant differences between means were assessed directly using the F-test, without the need for post hoc adjustments. Where significant interactions were observed ($p < 0.05$), simple effects were evaluated via pairwise contrasts between the two levels of each factor.

4.3. Results

4.3.1. Carcass Traits

Slaughter weight, empty body weight, hot and cold carcass weights, carcass yield (hot and cold), biological yield, and gastrointestinal tract components were not affected by the dietary starch level, CSPFA supplementation, or their interaction ($p > 0.05$; Table 2). These results indicate that neither a higher starch concentration nor the inclusion of CSPFA (30g/kg DM) altered nutrient utilization efficiency or overall tissue deposition during finishing.

Table 2. Slaughter and carcass traits of lambs fed diets with starch and calcium salts of palm fatty acids.

Item	Starch (S), g/kg		CSPFA (FA), g/kg		SEM	p Value		
	220	420	0	30		S	FA	SxFA
Weight, kg								
Slaughter weight	34.55	34.91	34.83	34.63	0.61	0.780	0.876	0.458
Empty body weight	31.85	32.16	32.04	31.97	0.58	0.807	0.959	0.403
Hot carcass weight	17.16	17.19	17.07	17.29	0.36	0.968	0.775	0.303
Cold carcass weight	16.47	16.37	16.47	16.37	0.35	0.894	0.891	0.353
Chilling loss, %	4.01	4.41	4.58	3.83	0.25	0.439	0.165	0.593
Carcass yield, kg/100 kg BW								
Hot carcass yield	49.63	49.20	49.01	49.82	0.46	0.645	0.395	0.339
Cold carcass yield	47.68	46.85	47.28	47.19	0.40	0.363	0.914	0.440
Biological yield	53.85	53.41	53.30	53.96	0.47	0.655	0.507	0.422
Non-carcass components, kg								
FGIT ¹	6.65	7.08	6.85	6.88	0.18	0.291	0.939	0.917
EGIT ²	2.66	2.72	2.76	2.62	0.12	0.826	0.600	0.783

¹Full gastrointestinal tract; ²Empty gastrointestinal tract.

4.3.2. Half Carcass Cuts

Diets containing 420g/kg starch increased flank rib yield compared to the 220g/kg starch diet ($p=0.048$). CSPFA supplementation increased the proportion of loin ($p=0.041$), whereas neck, shoulder, rib, and leg cuts were unaffected ($p>0.05$; Table 3). Higher starch concentration slightly favored muscle deposition in flank regions, whereas CSPFA promoted greater loin development without altering the overall carcass distribution.

Table 3. Half carcass composition of feedlot lambs fed starch and calcium salts of palm fatty acids.

Item	Starch (S), g/kg		CSPFA (FA), g/kg		SEM	p Value		
	220	420	0	30		S	FA	SxFA
Half carcass, kg								
Neck	0.33	0.31	0.31	0.33	0.01	0.121	0.237	0.512
Shoulder	1.45	1.38	1.36	1.46	0.04	0.477	0.314	0.995
Rib	1.56	1.41	1.41	1.56	0.06	0.259	0.277	0.559
Flank rib	1.62	1.77	1.70	1.69	0.06	0.267	0.943	0.205
Loin	0.63	0.59	0.67	0.54	0.03	0.659	0.111	0.408
Leg	2.67	2.67	2.64	2.69	0.06	0.964	0.714	0.863
Half carcass yield, %								
Neck	4.04	3.84	3.86	4.02	0.10	0.391	0.490	0.329
Shoulder	17.58	17.02	16.87	17.73	0.44	0.554	0.372	0.603
Rib	18.84	17.32	17.38	18.77	0.58	0.209	0.247	0.594
Flank rib	19.59	21.71	20.88	20.42	0.56	0.048	0.651	0.098
Loin	7.53	7.27	8.26	6.52	0.42	0.745	0.041	0.273
Leg	32.39	32.81	32.7	32.51	0.36	0.594	0.806	0.347

4.3.3. Internal Fat Depots and Fatness Indicators

Calcium salt of palm fatty acids supplementation significantly increased perirenal fat weight ($p=0.005$), renal fat score ($p=0.035$), and subcutaneous fat thickness ($p=0.017$) without affecting cavity fat, renal fat weight, or loin eye area ($p>0.05$; Table 4). This pattern indicates a preferential lipid deposition in perirenal and subcutaneous depots, reflecting Enhanced energy storage rather than muscle hypertrophy.

Table 4. Carcass fat depots and meat quality traits of feedlot lambs fed starch and calcium salts of palm fatty acids.

Item	Starch (S), g/kg		CSPFA (FA), g/kg		SEM	<i>p</i> Value		
	220	420	0	30		S	FA	SxFA
Internal fat depots								
Cavity fat, g	90	150	90	140	20	0.281	0.446	0.143
Perirenal fat, g	530	460	400	590	30	0.223	0.005	0.196
Renal fat, g	90	90	90	90	10	0.992	0.762	0.689
Fatness indicators								
RFS ¹ (1-3)	2.77	2.80	2.66	2.90	0.05	0.788	0.035	0.673
SFT ² , mm	2.87	2.78	1.86	3.80	0.40	0.899	0.017	0.988
Grade Rule means, mm	13.04	12.30	13.11	12.24	0.68	0.622	0.564	0.609
Loin eye area, cm ²	7.35	6.80	7.60	6.55	0.28	0.331	0.078	0.392
Physicochemical parameters								
pH at 30 min	6.54	6.56	6.56	6.54	0.05	0.854	0.889	0.587
pH at 24 h	5.62	5.67	5.64	5.65	0.05	0.592	0.972	0.426
Temp at 0 h, °C	32.08	31.83	32.21	31.70	0.23	0.584	0.266	0.072
Temp at 24 h, °C	8.26	7.87	7.96	8.17	0.13	0.118	0.406	0.014

¹Renal fat score, ²Subcutaneous fat thickness.

4.3.4. Physicochemical Parameters of Meat

Carcass pH at 30 min and 24h, as well as the initial carcass temperature, were not influenced by starch level, CSPFA inclusion, or their interaction ($p>0.05$). However, a significant starch×CSPFA interaction was detected for the carcass temperature at 24h ($p=0.014$; Table 4, Figure 1A). In unsupplemented diets, lambs fed 420g/kg starch showed a lower 24h carcass temperature than those fed 220g/kg (7.45 vs. 8.48 °C; $p=0.028$). No differences were observed between starch levels in the CSPFA-supplemented diets or between CSPFA levels within each starch level ($p>0.05$). The reduction in carcass temperature under high-starch, fat-free diets suggests faster post-mortem cooling, likely due to reduced subcutaneous insulation, whereas CSPFA supplementation mitigated this effect by increasing external fat deposition.

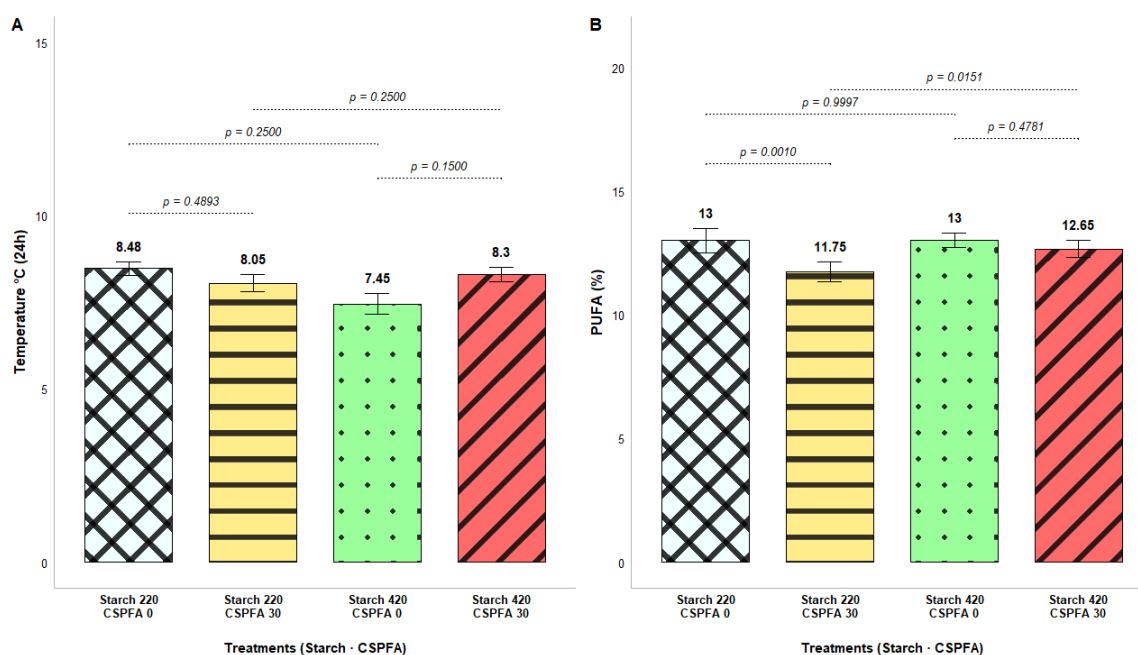


Figure 1. Interactive Effects of Dietary Starch and Calcium Salts of Palm Fatty Acids on 24-h Carcass Temperature (A) and Polyunsaturated Fatty Acids in Muscle (B) of Lambs.

4.3.5. Fatty Acid Profile of *Longissimus dorsi* and Desaturase Activity

A significant interaction between starch and CSPFA affected the composition of several major fatty acids ($p < 0.05$; Tables 5–7). In low-starch diets (220 g/kg DM), CSPFA supplementation reduced long-chain n-3 PUFA, particularly eicosatrienoic acid (C20:3n3) and eicosapentaenoic acid (EPA, C20:5n3). In contrast, under high-starch diets (420 g/kg DM), CSPFA increased EPA concentrations ($p < 0.05$), indicating that starch availability modulated post-ruminal absorption and tissue incorporation of PUFA (Figure 1-B).

Across the main effects, CSPFA supplementation increased palmitic (C16:0) and palmitoleic (C16:1) acids, and decreased stearic (C18:0), oleic (C18:1n9c), CLA isomers (c9t11 and t10c12), and docosahexaenoic acid (DHA, C22:6n3) (all $p < 0.05$). These shifts reflect the high palmitic acid content of palm oil and preferential incorporation of saturated fatty acids into intramuscular lipids. Conversely, high-starch diets increased the total PUFA and CLA concentrations while reducing C18:0 ($p < 0.05$), consistent with enhanced microbial desaturation and a shift in ruminal biohydrogenation toward more unsaturated intermediates.

Minor long-chain fatty acids (C20:0, C20:1, C22:1n9, and C24:1) represented less than 0.5% of the total intramuscular lipids and exhibited small, non-systematic variations across treatments ($p > 0.05$). Due to their minimal contribution to the overall lipid composition and negligible biological impact, they were grouped for concise reporting.

Table 5. Fatty acid profile of the Longissimus dorsi muscle of lambs fed diets with different starch levels and calcium salts of palm fatty acids.

Item	Starch (S), g/kg		CSPFA (FA), g/kg		SEM	<i>p</i> Value		
	220	420	0	30		S	FA	SxFA
Saturated fatty acids (SFA, %)								
Caproic (C6:0)	0.17	0.24	0.19	0.22	0.02	0.093	0.552	0.026
Capric (C10:0)	0.12	0.11	0.12	0.11	0.01	0.161	0.970	0.745
Lauric (C12:0)	0.25	0.23	0.23	0.26	0.01	0.288	0.221	0.630
Myristic (C14:0)	0.14	0.15	0.15	0.14	0.01	0.467	0.375	0.069
Pentadecanoic (C15:0)	0.27	0.31	0.28	0.30	0.01	0.203	0.581	0.748
Palmitic (C16:0)	22.95	23.66	21.01	25.61	0.82	0.441	<0.001	0.487
Heptadecanoic (C17:0)	1.17	1.20	1.52	0.84	0.09	0.467	<0.001	0.088
Stearic (C18:0)	23.48	19.93	20.78	21.62	0.11	0.003	0.496	0.153
Arachidic (C20:0)	0.18	0.19	0.19	0.18	0.01	<0.001	0.013	0.002
Monounsaturated fatty acids (MUFA, %)								
Palmitoleic (C16:1)	2.20	2.18	1.49	2.89	0.18	0.681	<0.001	0.050
Heptadecenoic (C17:1)	1.51	1.39	1.38	1.53	0.11	0.528	0.422	0.011
Elaidic (C18:1n9t)	3.27	3.14	3.16	3.25	0.06	0.362	0.498	0.440
Oleic (C18:1n9c)	31.72	32.94	34.79	29.88	1.16	0.463	0.010	0.152
Eicosenoic (C20:1)	0.34	0.46	0.33	0.47	0.02	<0.001	<0.001	0.015
Erucic (C22:1n9)	0.16	0.10	0.14	0.12	0.01	<0.001	<0.001	<0.001
Nervonic (C24:1)	0.12	0.17	0.14	0.15	0.01	<0.001	0.034	<0.001
Polyunsaturated fatty acids (PUFA, %)								
Linolelaidic (C18:2n6t)	9.00	8.77	8.81	8.96	0.07	0.110	0.298	0.390
α -Linolenic (C18:3n3)	1.44	1.45	1.53	1.36	0.05	0.929	0.203	0.482
Rumenic (C18:2c9t11)	0.90	1.11	1.32	0.70	0.08	0.004	<0.001	0.379
CLA (C18:2t10c12) ¹	0.20	0.25	0.23	0.21	0.01	<0.001	0.039	0.896
Eicosatrienoic (C20:3n3)	0.16	0.33	0.28	0.21	0.02	<0.001	<0.001	0.007
DHA (C22:6n3) ²	0.07	0.10	0.09	0.07	0.01	<0.001	<0.001	0.495
EPA (C20:5n3) ³	0.18	0.33	0.26	0.25	0.02	<0.001	0.023	<0.001

Table 6. Interaction effects of dietary starch level and calcium salts of palm fatty acids on individual fatty acids of the lamb Longissimus dorsi muscle.

Item	SCAGP			<i>p</i> Value
	Starch	0	30	
Caproic (C6:0), %	220	0.21	0.14	0.959
	420	0.18	0.30	0.042
	<i>p</i> Value	0.550	0.041	
Heptadecenoic (C17:1), %	220	0.17	0.18	0.400
	420	0.21	0.18	<0.001
	<i>p</i> Value	<0.001	0.978	
Arachidic (C20:0), %	220	0.70	0.72	0.400
	420	0.82	0.73	<0.001
	<i>p</i> Value	<0.001	0.978	
Eicosenoic (C20:1), %	220	0.25	0.43	<0.001
	420	0.41	0.52	0.006
	<i>p</i> Value	<0.001	<0.001	
Nervonic (C24:1), %	220	0.13	0.11	0.596
	420	0.15	0.21	<0.001
	<i>p</i> Value	0.145	<0.001	
Erucic (C22:1n9), %	220	0.20	0.14	<0.001
	420	0.09	0.11	0.809
	<i>p</i> Value	<0.001	0.809	
Eicosatrienoic (C20:3n3), %	220	0.23	0.11	<0.001
	420	0.35	0.33	0.848
	<i>p</i> Value	<0.001	<0.001	
EPA (C20:5n3) ¹ , %	220	0.21	0.17	<0.001
	420	0.32	0.35	0.017
	<i>p</i> Value	<0.001	<0.001	

¹ Eicosapentaenoic Acid.

CSPFA supplementation also altered overall lipid profiles (Table 7). Diets containing CSPFA showed higher total SFA, AI, TI, and n-6/n-3 ratio, and lower MUFA and h: H ratio ($p < 0.05$). In contrast, increasing dietary starch reduced the n-6/n-3 ratio ($p = 0.004$), indicating a favorable enrichment of n-3 PUFA under high-fermentable energy conditions.

Collectively, these results demonstrate that dietary starch levels and CSPFA supplementation jointly shape the deposition of bioactive fatty acids and determine the nutritional quality of lamb meat.

Dietary starch concentration significantly influenced the Δ^9 -desaturase 14 index ($p = 0.040$; Table 8), which increased with a high-starch diet (57.6 vs. 19.6). No effects

were observed for Δ^9 -desaturase 16 or Δ^9 -desaturase 18 ($p > 0.05$). CSPFA supplementation did not significantly influence the desaturase index. These results indicate that higher starch availability modestly enhanced desaturase activity for shorter-chain fatty acids, which is consistent with the greater deposition of unsaturated lipids observed in the high-starch treatments.

Table 7. Lipid profile and health indices of lamb Longissimus dorsi muscle as affected by dietary starch and calcium salts of palm fatty acids.

Item	Starch (S), g/kg		CSPFA (FA), g/kg		SEM	p Value		
	220	420	0	30		S	FA	SxFA
SFA ¹	48.74	45.02	44.47	49.28	1.00	0.221	0.005	0.228
MUFA ²	39.32	40.38	41.43	38.29	0.97	0.492	0.048	0.219
PUFA ³	12.36	12.82	12.99	12.19	0.15	0.021	<0.001	0.027
Atherogenicity index	0.49	0.49	0.43	0.55	0.02	0.868	<0.001	0.258
Thrombogenicity index	0.93	0.90	0.79	1.04	0.04	0.659	<0.001	0.210
h:H ⁴	2.21	2.16	2.50	1.86	0.11	0.756	0.003	0.281
n-6/n-3 ratio	5.10	4.17	4.29	4.98	0.19	0.004	0.021	0.252
Cholesterol (mg/100g)	58.87	58.44	59.15	58.16	2.46	0.937	0.859	0.837

¹Saturated fatty acids, ²Monounsaturated fatty acids, ³Polyunsaturated fatty acids, ⁴Hypocholesterolemic:hypercholesterolemic ratio.

Table 8. Desaturase activity indices in the Longissimus dorsi muscle of lambs fed diets with different starch levels and calcium salts of palm fatty acids.

Item	Starch (S), g/kg		CSPFA (FA), g/kg		SEM	p Value		
	220	420	0	30		S	FA	SxFA
Δ^9 -desaturase 14	19.59	57.57	40.58	36.58	8.89	0.040	0.816	0.945
Δ^9 -desaturase 16	27.71	15.23	36.20	6.75	7.51	0.389	0.054	0.644
Δ^9 -desaturase 18	60.43	42.18	52.54	50.07	5.94	0.154	0.842	0.959

4.4. Discussion

4.4.1. Carcass Traits

The absence of effects of dietary starch level or CSPFA supplementation on slaughter weight and carcass yield is physiologically coherent. Rumén-protected fats, such as CSPFA, bypass microbial lipolysis and biohydrogenation, increasing the post-ruminal flow of long-chain fatty acids for absorption. Because calcium salts of fatty acids (CSFA) resist ruminal hydrolysis, they allow unsaturated fatty acids to reach the jejunum for absorption, thereby modifying circulating lipid pools [34]. This energy is primarily incorporated into adipose tissue without necessarily stimulating lean tissue accretion, which explains why the carcass yield remained unchanged in the present study despite the increased dietary energy density from CSPFA supplementation at 30 g/kg DM.

This neutral response aligns with the findings for similar fat sources. [35] reported that neither palm oil-based calcium salts nor a blend containing palm, cottonseed, and soybean oils improved carcass yield in cattle. However, this effect appears to depend on the fatty acid source. In contrast to the present results, [31] it was observed that calcium salts of soybean fatty acids (35 g/kg DM) increased slaughter body weight (45.5 vs. 39.1–42.8 kg) and hot carcass yield (46.5% vs. 41.5–42.1%) compared with whole soybean or corn germ. This discrepancy suggests that unsaturated-rich salts (soybean-based) may enhance carcass deposition, whereas palm oil-based salts, dominated by palmitic acid (C16:0), preferentially direct energy toward non-carcass adipose depots.

Therefore, the lack of effect on carcass yield in the present study reinforces the interpretation that protected fats, particularly those from palm sources, are more likely to redistribute lipid partitioning to specific body depots than to enhance overall carcass accretion. However, changes in lipid deposition may occur in specific depots rather than uniformly across all carcass tissues.

Intramuscular fat deposition is primarily regulated by key lipogenic enzymes (LPL, DGAT, GPAT, and ACC) and local lipolytic control (HSL), which respond more directly to the availability of circulating fatty acids than to total energy intake [36]. In contrast, subcutaneous and visceral depots, due to their greater volumetric capacity, may respond more strongly to energy balance and finishing duration [37]. This differential metabolic response likely explains why lipid supplementation can alter fat distribution and meat composition without significantly affecting carcass yield [37]. Indeed, a recent meta-analysis supports this, showing increased intramuscular fat with no consistent effect on carcass yield [38].

4.4.2. *Fat Depots and Meat Quality*

Calcium salts of palm fatty acids supplementation increased perirenal fat, subcutaneous fat thickness, and renal fat scores, consistent with the known ability of long-chain fatty acid salts to partially escape ruminal biohydrogenation and enhance post-ruminal absorption [31,35]. Once absorbed, these fatty acids are incorporated into lipoproteins, thereby increasing the lipid supply for adipose deposition [39]. The preferential response of the perirenal and subcutaneous depots is consistent with their greater capacity for lipid sequestration when circulating substrate levels are elevated.

The depot-specific response is likely due to differences in lipid-handling capacity and enzyme regulation. Increased flux of preformed fatty acids may enhance uptake via lipoprotein lipase and esterification pathways (via GPAT and DGAT), while high-energy

diets reduce oxidation and further promote fat deposition [36]. Subcutaneous fat depots are particularly responsive to endocrine and nutritional cues; high-concentrate diets may suppress sirtuin pathways, favoring lipogenesis [37].

Chilling loss was not affected by starch concentration or CSPFA supplementation, indicating that cooling dynamics were largely independent of the dietary treatments. However, CSPFA increased subcutaneous fat thickness, which may have slightly reduced heat transfer during cooling. Similar effects have been reported in carcasses with thicker fat layers, where slower heat dissipation can prolong the period above the evaporative threshold and potentially increase moisture loss under specific chilling conditions [34]. However, in the present study, this mechanism did not result in measurable differences in chilling loss, indicating that the insulation effect was limited to carcass temperature at 24 hours and did not affect overall evaporative loss. Therefore, the effect of dietary fat sources on carcass cooling appears to be limited to thermal dissipation rather than actual moisture evaporation.

This is reinforced by the interaction between starch and CSPFA: in the absence of CSPFA, high-starch diets led to lower 24 h carcass temperatures; however, this cooling advantage was neutralized by CSPFA, likely due to increased fat cover. Notably, loin eye area remained unaffected, suggesting CSPFA-derived energy was preferentially partitioned to adipose tissue rather than muscle hypertrophy [36,40]. Despite these changes, meat pH values at 30 minutes and 24 hours remained within physiological ranges (30 min: 6.54–6.56; 24 h: 5.62–5.67), indicating glycolytic metabolism and meat quality were not compromised.

4.4.3. *Fatty Acid Profile and Desaturase Activity*

A significant starch $\times$ CSPFA interaction was detected for eight fatty acids: C6:0, C17:0, C20:0, C20:1, C22:1n9, C24:1, C20:3n3, and EPA (C20:5n3). In low-starch diets, CSPFA reduced the content of odd- and long-chain fatty acids, whereas in high-starch diets, it increased the same fatty acids, demonstrating that carbohydrate availability modulates lipid metabolism and tissue fatty acid composition [6,13].

Long-chain PUFA responses also depended on the starch level. CSPFA reduced C20:3n3 and EPA under low-starch diets, but increased them under high-starch conditions. This response was accompanied by higher Δ^9 -desaturase 14 activity in lambs fed the high-starch diet, indicating enhanced enzymatic conversion of saturated to monounsaturated fatty acids. This effect is consistent with previous findings that carbohydrate-rich diets stimulate stearoyl-CoA desaturase (SCD1) expression and Δ^9 -desaturase activity in

lipogenic tissues [22,41,42]. In addition, greater availability of fermentable starch can modify the ruminal hydrogen balance and biohydrogenation, favoring unsaturated intermediates and improving PUFA transfer to tissues [43,44]. Together, these mechanisms explain the greater proportion of unsaturated lipids observed in high-starch diets and the starch-dependent response of n-3 PUFA (e.g., EPA) to CSPFA.

In contrast, low-starch diets may intensify ruminal biohydrogenation and reduce PUFA transfer to the muscles [12,45]. High-starch diets also increased CLA isomers and DHA while reducing C18:0, indicating shifts in ruminal biohydrogenation pathways driven by higher fermentable energy [46]. CSPFA supplementation increased palmitic (C16:0) and palmitoleic (C16:1) acids, but decreased C17:0, C20:0, oleic (C18:1n9c), and CLA isomers. The increase in C16:0 reflects the high palmitic acid content of palm oil [35]. Reductions in CLA and DHA levels have also been observed. Although DHA has been consistently linked to cardioprotective effects through anti-inflammatory and lipid-lowering mechanisms [47,48], the effects of CLA remain controversial, with human studies reporting neutral or adverse outcomes depending on the isomer and dose [49]. These outcomes may result from competitive incorporation or partial downregulation of desaturase gene expression [12,36,50].

Collectively, these results indicate that starch concentration and CSPFA supplementation act synergistically to modulate desaturase activity, ruminal biohydrogenation, and intramuscular fatty acid deposition, ultimately affecting the nutritional quality of lamb meat lipids.

4.4.4. *Health-Related Lipid Indices*

From a human health standpoint, CSPFA supplementation deteriorated meat lipid quality, increasing SFA and reducing both MUFA and PUFA. These shifts translated into higher AI and TI values and a lower h:H ratio, consistent with increased cardiovascular risk [30,51]. The main contributor to this decline was the increase in palmitic acid, a hypercholesterolemic fatty acid that may elevate LDL-C levels and trigger pro-inflammatory pathways [52–54].

It is important to stress that these negative health-related effects are not universal to all rumen-protected fats but are strongly dependent on the supplement composition. Palm-oil-based CSPFA typically contains ~44–45% palmitic acid (C16:0), a hypercholesterolemic SFA, which explains the deterioration in health-related indices observed here. In contrast, calcium salts derived from sunflower oil provide ~60–70% oleic acid (C18:1n9), while flax-based salts supply ~50% α -linolenic acid (C18:3n3). These

unsaturated-rich profiles have been associated with improved AI, TI, h: H ratios, and lower n-6/n-3 values in lamb meat [37,55]. Thus, the type of rumen-protected fat is likely to be decisive in determining whether the nutritional quality of meat is improved or compromised.

4.4.5. Study Limitations, Practical Implications, and Final Synthesis

This study had several limitations that should be acknowledged. First, no direct measurements of ruminal fermentation, plasma lipids, or gene expression were conducted, limiting mechanistic interpretation. Second, only one CSPFA dose was tested, preventing assessment of dose–response relationships. Third, the experimental duration was relatively short, and long-term effects on carcass traits and lipid metabolism remain unclear. Finally, all animals belonged to a single breed, which may limit the applicability of the findings across breeds. Future studies should incorporate longitudinal designs, molecular analyses, and alternative fat sources to refine feeding strategies to improve both animal performance and meat nutritional quality.

From an agro-industrial standpoint, palm oil–derived calcium salts of fatty acids (CSPFA) are widely used in ruminant nutrition due to their low cost, broad availability, and oxidative stability, making them a practical source of rumen-protected energy [56,57]. However, the predominance of palmitic acid (C16:0) in these supplements poses a challenge to the nutritional quality of meat, as demonstrated in the present study. While palm oil utilization supports the sustainability and economic efficiency of the feed industry, its extensive use in animal diets warrants careful evaluation, given the potential negative implications for consumer health associated with its higher saturated fatty acid content.

4.5. Conclusions

This study demonstrated that supplementation with calcium salts of palm fatty acids (CSPFA) predominantly altered the fatty acid composition of lamb meat, increasing the proportion of saturated fatty acids and worsening lipid health indices, particularly under low-starch conditions. These effects were mainly driven by the fatty acid profile of the palm oil source itself rather than by the fermentable energy level.

Dietary starch concentration modulated specific responses, such as the content of long-chain n-3 PUFA (EPA) and total PUFA, indicating that fermentable energy can partially influence post-ruminal lipid metabolism. However, these effects were secondary to the consistent impact of CSPFA supplementation on the overall lipid quality.

Collectively, these results indicate that palm-oil-based rumen-protected fats increase carcass adiposity without improving meat nutritional quality. These findings suggest that further research should evaluate rumen-protected lipid sources with higher PUFA contents as potential alternatives to enhance both the production efficiency and nutritional value of lamb meat.

References

1. Van Le, H.; Nguyen, D.V.; Vu Nguyen, Q.; Malau-Aduli, B.S.; Nichols, P.D.; Malau-Aduli, A.E.O. Fatty Acid Profiles of Muscle, Liver, Heart and Kidney of Australian Prime Lambs Fed Different Polyunsaturated Fatty Acids Enriched Pellets in a Feedlot System. *Sci Rep* **2019**, *9*, 1238, doi:10.1038/s41598-018-37956-y.
2. Prates, J.A.M. The Role of Meat Lipids in Nutrition and Health: Balancing Benefits and Risks. *Nutrients* **2025**, *17*, 350, doi:10.3390/nu17020350.
3. Ponnampalam, E.; Priyashantha, H.; Vidanarachchi, J.; Kiani, A.; Holman, B. Effects of Nutritional Factors on Fat Content, Fatty Acid Composition, and Sensorial Properties of Meat and Milk from Domesticated Ruminants: An Overview. *Animals* **2024**, *14*, 840, doi:10.3390/ani14060840.
4. Orzuna-Orzuna, J.F.; Hernández-García, P.A.; Chay-Canul, A.J.; Díaz Galván, C.; Razo Ortiz, P.B. Microalgae as a Dietary Additive for Lambs: A Meta-Analysis on Growth Performance, Meat Quality, and Meat Fatty Acid Profile. *Small Ruminant Research* **2023**, *227*, 107072, doi:10.1016/j.smallrumres.2023.107072.
5. Zhang, M.; Zhang, Z.; Zhang, X.; Lu, C.; Yang, W.; Xie, X.; Xin, H.; Lu, X.; Ni, M.; Yang, X.; et al. Effects of Dietary *Clostridium Butyricum* and Rumen Protected Fat on Meat Quality, Oxidative Stability, and Chemical Composition of Finishing Goats. *J Anim Sci Biotechnol* **2024**, *15*, 3, doi:10.1186/s40104-023-00972-8.
6. Toral, P.G.; Monahan, F.J.; Hervás, G.; Frutos, P.; Moloney, A.P. Review: Modulating Ruminant Lipid Metabolism to Improve the Fatty Acid Composition of Meat and Milk. Challenges and Opportunities. *Animal* **2018**, *12*, s272–s281, doi:10.1017/S1751731118001994.
7. Wang, X.; Martin, G.B.; Wen, Q.; Liu, S.; Li, Y.; Shi, B.; Guo, X.; Zhao, Y.; Guo, Y.; Yan, S. Palm Oil Protects α -Linolenic Acid from Rumen Biohydrogenation and Muscle Oxidation in Cashmere Goat Kids. *J Anim Sci Biotechnol* **2020**, *11*, 100, doi:10.1186/s40104-020-00502-w.
8. Lashkari, S.; Bonefeld Petersen, M.; Krogh Jensen, S. Rumen Biohydrogenation of Linoleic and Linolenic Acids Is Reduced When Esterified to Phospholipids or Steroids. *Food Sci Nutr* **2020**, *8*, 79–87, doi:10.1002/fsn3.1252.
9. Frank, E.; Livshitz, L.; Portnick, Y.; Kamer, H.; Alon, T.; Moallem, U. The Effects of High-Fat Diets from Calcium Salts of Palm Oil on Milk Yields, Rumen Environment, and

- Digestibility of High-Yielding Dairy Cows Fed Low-Forage Diet. *Animals* **2022**, *12*, 2081, doi:10.3390/ani12162081.
10. Shpirer, J.; Livshits, L.; Kamer, H.; Alon, T.; Portnik, Y.; Moallem, U. Effects of the Palmitic-to-Oleic Ratio in the Form of Calcium Salts of Fatty Acids on the Production and Digestibility in High-Yielding Dairy Cows. *J Dairy Sci* **2024**, *107*, 6785–6796, doi:10.3168/jds.2023-24382.
 11. Oyebade, A.; Lifshitz, L.; Lehrer, H.; Jacoby, S.; Portnick, Y.; Moallem, U. Saturated Fat Supplemented in the Form of Triglycerides Decreased Digestibility and Reduced Performance of Dairy Cows as Compared to Calcium Salt of Fatty Acids. *Animal* **2020**, *14*, 973–982, doi:10.1017/S1751731119002465.
 12. Costa, M.; Alves, S.P.; Francisco, A.; Almeida, J.; Alfaia, C.M.; Martins, S. V.; Prates, J.A.M.; Santos-Silva, J.; Doran, O.; Bessa, R.J.B. The Reduction of Starch in Finishing Diets Supplemented with Oil Does Not Prevent the Accumulation of Trans-10 18:1 in Lamb Meat1. *J Anim Sci* **2017**, *95*, 3745–3761, doi:10.2527/jas.2017.1578.
 13. Francisco, A.; Alves, S.P.; Portugal, P.V.; Dentinho, M.T.; Jerónimo, E.; Sengo, S.; Almeida, J.; Bressan, M.C.; Pires, V.M.R.; Alfaia, C.M.; et al. Effects of Dietary Inclusion of Citrus Pulp and Rockrose Soft Stems and Leaves on Lamb Meat Quality and Fatty Acid Composition. *animal* **2018**, *12*, 872–881, doi:10.1017/S1751731117002269.
 14. Pereira, E.S.; Teixeira, I.A.M.A.; Azevêdo, J.A.G.; Santos, S.A. *Exigências Nutricionais de Caprinos e Ovinos – BR-Caprinos & Ovinos*; Editora Scienza: São Carlos, 2024;
 15. da Cruz, C.H.; Santos, S.A.; de Carvalho, G.G.P.; Azevedo, J.A.G.; Detmann, E.; Valadares Filho, S. de C.; Mariz, L.D.S.; Pereira, E.S.; Nicory, I.M.C.; Tosto, M.S.L.; et al. Estimating Digestible Nutrients in Diets for Small Ruminants Fed with Tropical Forages. *Livest Sci* **2021**, *249*, 104532, doi:10.1016/j.livsci.2021.104532.
 16. AOAC Association of Official Analytical Chemists. *Official Methods of Analysis, 20th ed. Arlington, VA: Journal - Association of Official Analytical Chemists*. **2016**.
 17. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *J Dairy Sci* **1991**, *74*, 3583–3597, doi:10.3168/jds.S0022-0302(91)78551-2.
 18. Dische, Z. General Color Reactions. *Methods in carbohydrate chemistry* **1962**, *1*, 478–512.
 19. Tebbe, A.W.; Faulkner, M.J.; Weiss, W.P. Effect of Partitioning the Nonfiber Carbohydrate Fraction and Neutral Detergent Fiber Method on Digestibility of Carbohydrates by Dairy Cows. *J Dairy Sci* **2017**, *100*, 6218–6228, doi:10.3168/jds.2017-12719.
 20. Brasil Regulamento Da Inspeção Industrial e Sanitária de Produtos de Origem Animal – RIISPOA 2017.
 21. Cezar, M.F.; Sousa, W.H. *Carcças Ovinas e Caprinas: Obtenção, Avaliação e Classificação*; Editora Agropecuária Tropical: Uberaba, 2007;
 22. Vasta, V.; Priolo, A.; Scerra, M.; Hallett, K.G.; Wood, J.D.; Doran, O. $\Delta 9$ Desaturase Protein Expression and Fatty Acid Composition of Longissimus Dorsi Muscle in Lambs

- Fed Green Herbage or Concentrate with or without Added Tannins. *Meat Sci* **2009**, *82*, 357–364, doi:10.1016/j.meatsci.2009.02.007.
23. Wood, J.D.; Enser, M.; Fisher, A.V.; Nute, G.R.; Sheard, P.R.; Richardson, R.I.; Hughes, S.I.; Whittington, F.M. Fat Deposition, Fatty Acid Composition and Meat Quality: A Review. *Meat Sci* **2008**, *78*, 343–358, doi:10.1016/j.meatsci.2007.07.019.
 24. Bligh, E.G.; Dyer, W.J. A RAPID METHOD OF TOTAL LIPID EXTRACTION AND PURIFICATION. *Can J Biochem Physiol* **1959**, *37*, 911–917, doi:10.1139/o59-099.
 25. Bannon, C.D.; Craske, J.D.; Hai, N.T.; Harper, N.L.; O'Rourke, K.L. Analysis of Fatty Acid Methyl Esters with High Accuracy and Reliability. *J Chromatogr A* **1982**, *247*, 63–69, doi:10.1016/S0021-9673(00)84856-6.
 26. O'Fallon, J. V.; Busboom, J.R.; Nelson, M.L.; Gaskins, C.T. A Direct Method for Fatty Acid Methyl Ester Synthesis: Application to Wet Meat Tissues, Oils, and Feedstuffs. *J Anim Sci* **2007**, *85*, 1511–1521, doi:10.2527/jas.2006-491.
 27. Visentainer, J.V.; Franco, M.R.B. *Ácidos Graxos Em Óleos e Gorduras: Identificação e Quantificação*; Varela, 2006; ISBN 8585519991.
 28. Bichi, E.; Toral, P.G.; Hervás, G.; Frutos, P.; Gómez-Cortés, P.; Juárez, M.; de la Fuente, M.A. Inhibition of $\Delta 9$ -Desaturase Activity with Sterculic Acid: Effect on the Endogenous Synthesis of Cis-9 18:1 and Cis-9, Trans-11 18:2 in Dairy Sheep. *J Dairy Sci* **2012**, *95*, 5242–5252, doi:10.3168/jds.2012-5349.
 29. Malau-Aduli, A.E.O.; Siebert, B.D.; Bottema, C.D.K.; Pitchford, W.S. A Comparison of the Fatty Acid Composition of Triacylglycerols in Adipose Tissue from Limousin and Jersey Cattle. *Aust J Agric Res* **1997**, *48*, 715–722, doi:10.1071/A96083.
 30. Ulbricht, T.L.V.; Southgate, D.A.T. Coronary Heart Disease: Seven Dietary Factors. *The Lancet* **1991**, *338*, 985–992, doi:10.1016/0140-6736(91)91846-M.
 31. Alba, H.D.R.; Freitas Júnior, J.E. de; Leite, L.C.; Azevêdo, J.A.G.; Santos, S.A.; Pina, D.S.; Cirne, L.G.A.; Rodrigues, C.S.; Silva, W.P.; Lima, V.G.O.; et al. Protected or Unprotected Fat Addition for Feedlot Lambs: Feeding Behavior, Carcass Traits, and Meat Quality. *Animals* **2021**, *11*, 328, doi:10.3390/ani11020328.
 32. Saldanha, T.; Mazalli, M.R.; Bragagnolo, N. Avaliação Comparativa Entre Dois Métodos Para Determinação Do Colesterol Em Carnes e Leite. *Ciência e Tecnologia de Alimentos* **2004**, *24*, 109–113, doi:10.1590/S0101-20612004000100020.
 33. SAS - Statistical Analysis System, Version 9.4, SAS Institute Inc., Cary, NC 2013.
 34. Pewan, S.B.; Otto, J.R.; Kinobe, R.T.; Adegboye, O.A.; Malau-Aduli, A.E.O. Nutritional Enhancement of Health Beneficial Omega-3 Long-Chain Polyunsaturated Fatty Acids in the Muscle, Liver, Kidney, and Heart of Tattykeel Australian White MARGRA Lambs Fed Pellets Fortified with Omega-3 Oil in a Feedlot System. *Biology (Basel)* **2021**, *10*, 912, doi:10.3390/biology10090912.
 35. Colombo, E.; Cooke, R.F.; Mackey, S.; Pickett, A.; Batista, L.F.; Pohler, K.; Sousa, O.; Cappelozza, B.; Brandão, A.P. 36 Productive and Physiological Responses of Feedlot

Cattle Receiving Different Sources of Ca Salts of Fatty Acids in the Finishing Diet. *J Anim Sci* **2023**, *101*, 36–37, doi:10.1093/jas/skad068.042.

36. Guerrero-Bárcena, M.; Domínguez-Vara, I.A.; Morales-Almaraz, E.; Sánchez-Torres, J.E.; Bórquez-Gastelum, J.L.; Hernández-Ramírez, D.; Trujillo-Gutiérrez, D.; Rodríguez-Gaxiola, M.A.; Pinos-Rodríguez, J.M.; Velázquez-Garduño, G.; et al. Effect of Zilpaterol Hydrochloride and Zinc Methionine on Growth, Carcass Traits, Meat Quality, Fatty Acid Profile and Gene Expression in Longissimus Dorsi Muscle of Sheep in Intensive Fattening. *Agriculture* **2023**, *13*, 684, doi:10.3390/agriculture13030684.
37. Gao, C.; Gao, D.; Zhang, Q.; Wang, Y.; Gao, A. Performance, Meat Quality, Intramuscular Fatty Acid Profile, Rumen Characteristics and Serum Parameters of Lambs Fed Microencapsulated or Conventional Linseed Oil. *Czech Journal of Animal Science* **2022**, *67*, 365–373, doi:10.17221/77/2022-CJAS.
38. Santos Torres, R. de N.; Ghedini, C.P.; Chardulo, L.A.L.; Baldassini, W.A.; Curi, R.A.; Pereira, G.L.; Schoonmaker, J.P.; Almeida, M.T.C.; Costa, C.; Neto, O.R.M. Potential of Different Strategies to Increase Intramuscular Fat Deposition in Sheep: A Meta-Analysis Study. *Small Ruminant Research* **2024**, *234*, 107258, doi:10.1016/j.smallrumres.2024.107258.
39. Ramos-Méndez, J.L.; Estrada-Angulo, A.; Rodríguez-Gaxiola, M.A.; Gaxiola-Camacho, S.M.; Chaidez-Álvarez, C.; Manriquez-Núñez, O.M.; Barreras, A.; Zinn, R.A.; Soto-Alcalá, J.; Plascencia, A. Grease Trap Waste (Griddle Grease) as a Feed Ingredient for Finishing Lambs: Growth Performance, Dietary Energetics, and Carcass Characteristics. *Can J Anim Sci* **2021**, *101*, 257–262, doi:10.1139/cjas-2020-0102.
40. Quadros, D.G.; Kerth, C.R. Replacing Cottonseed Meal and Sorghum with Dried Distillers' Grains with Solubles Enhances the Growth Performance, Carcass Traits, and Meat Quality of Feedlot Lambs. *Transl Anim Sci* **2022**, *6*, doi:10.1093/tas/txac040.
41. Miyazaki, M.; Dobrzyn, A.; Man, W.C.; Chu, K.; Sampath, H.; Kim, H.-J.; Ntambi, J.M. Stearoyl-CoA Desaturase 1 Gene Expression Is Necessary for Fructose-Mediated Induction of Lipogenic Gene Expression by Sterol Regulatory Element-Binding Protein-1c-Dependent and -Independent Mechanisms. *Journal of Biological Chemistry* **2004**, *279*, 25164–25171, doi:10.1074/jbc.M402781200.
42. Oliveira, M.A.; Alves, S.P.; Santos-Silva, J.; Bessa, R.J.B. Effect of Dietary Starch Level and Its Rumen Degradability on Lamb Meat Fatty Acid Composition. *Meat Sci* **2017**, *123*, 166–172, doi:10.1016/j.meatsci.2016.10.001.
43. Bougouin, A.; Martin, C.; Doreau, M.; Ferlay, A. Effects of Starch-Rich or Lipid-Supplemented Diets That Induce Milk Fat Depression on Rumen Biohydrogenation of Fatty Acids and Methanogenesis in Lactating Dairy Cows. *Animal* **2019**, *13*, 1421–1431, doi:10.1017/S1751731118003154.
44. Ungerfeld, E.M. Metabolic Hydrogen Flows in Rumen Fermentation: Principles and Possibilities of Interventions. *Front Microbiol* **2020**, *11*, doi:10.3389/fmicb.2020.00589.
45. Carreño, D.; Toral, P.G.; Pinloche, E.; Belenguer, A.; Yáñez-Ruiz, D.R.; Hervás, G.; McEwan, N.R.; Newbold, C.J.; Frutos, P. Rumen Bacterial Community Responses to DPA,

- EPA and DHA in Cattle and Sheep: A Comparative in Vitro Study. *Sci Rep* **2019**, *9*, 11857, doi:10.1038/s41598-019-48294-y.
46. Castro, D.P.V.; Pimentel, P.R.S.; dos Santos, N.J.A.; da Silva Júnior, J.M.; Virginio Júnior, G.F.; de Andrade, E.A.; Barbosa, A.M.; Pereira, E.S.; Ribeiro, C.V.D.M.; Bezerra, L.R.; et al. Dietary Effect of Palm Kernel Oil Inclusion in Feeding Finishing Lambs on Meat Quality. *Animals* **2022**, *12*, 3242, doi:10.3390/ani12233242.
 47. Manso, T.; Gallardo, B.; Lavín, P.; Ruiz Mantecón, Á.; Cejudo, C.; Gómez-Cortés, P.; de la Fuente, M.Á. Enrichment of Ewe's Milk with Dietary n-3 Fatty Acids from Palm, Linseed and Algae Oils in Isoenergetic Rations. *Animals* **2022**, *12*, 1716, doi:10.3390/ani12131716.
 48. Dannenberger, D.; Eggert, A.; Kalbe, C.; Woitalla, A.; Schwudke, D. Are n -3 PUFAs from Microalgae Incorporated into Membrane and Storage Lipids in Pig Muscle Tissues?—A Lipidomic Approach. *ACS Omega* **2022**, *7*, 24785–24794, doi:10.1021/acsomega.2c02476.
 49. Omachi, D.O.; Aryee, A.N.A.; Onuh, J.O. Functional Lipids and Cardiovascular Disease Reduction: A Concise Review. *Nutrients* **2024**, *16*, 2453, doi:10.3390/nu16152453.
 50. Urrutia, O.; Mendizabal, J.A.; Alfonso, L.; Soret, B.; Insausti, K.; Arana, A. Adipose Tissue Modification through Feeding Strategies and Their Implication on Adipogenesis and Adipose Tissue Metabolism in Ruminants. *Int J Mol Sci* **2020**, *21*, 3183, doi:10.3390/ijms21093183.
 51. Dal Bosco, A.; Cavallo, M.; Menchetti, L.; Angelucci, E.; Cartoni Mancinelli, A.; Vaudo, G.; Marconi, S.; Camilli, E.; Galli, F.; Castellini, C.; et al. The Healthy Fatty Index Allows for Deeper Insights into the Lipid Composition of Foods of Animal Origin When Compared with the Atherogenic and Thrombogenicity Indexes. *Foods* **2024**, *13*, 1568, doi:10.3390/foods13101568.
 52. Jackson, K.H.; Harris, W.S.; Belury, M.A.; Kris-Etherton, P.M.; Calder, P.C. Beneficial Effects of Linoleic Acid on Cardiometabolic Health: An Update. *Lipids Health Dis* **2024**, *23*, 296, doi:10.1186/s12944-024-02246-2.
 53. Davinelli, S.; Intrieri, M.; Corbi, G.; Scapagnini, G. Metabolic Indices of Polyunsaturated Fatty Acids: Current Evidence, Research Controversies, and Clinical Utility. *Crit Rev Food Sci Nutr* **2021**, *61*, 259–274, doi:10.1080/10408398.2020.1724871.
 54. Vela-Vásquez, D.A.; Sifuentes-Rincón, A.M.; Delgado-Enciso, I.; Ordaz-Pichardo, C.; Arellano-Vera, W.; Treviño-Alvarado, V. Effect of Consuming Beef with Varying Fatty Acid Compositions as a Major Source of Protein in Volunteers under a Personalized Nutritional Program. *Nutrients* **2022**, *14*, 3711, doi:10.3390/nu14183711.
 55. Marino, R.; Caroprese, M.; della Malva, A.; Santillo, A.; Sevi, A.; Albenzio, M. Role of Whole Linseed and Sunflower Seed on the Nutritional and Organoleptic Properties of Podolian × Limousine Meat. *Ital J Anim Sci* **2024**, *23*, 868–879, doi:10.1080/1828051X.2024.2359588.

56. Pramana, A.; Kurnia, D.; Firmanda, A.; Rossi, E.; AR, N.H.; Putri, V.J. Using Palm Oil Residue for Food Nutrition and Quality: From Palm Fatty Acid Distillate to Vitamin E toward Sustainability. *J Sci Food Agric* **2025**, *105*, 4728–4740, doi:10.1002/jsfa.13878.
57. Barkah, N.N.; Despal; Retnani, Y.; Putri, N.O.H.; Rahmayuni, S.; Metania, R.D.N. Comparative Analysis of The Physical Properties and Energy Content of Rumen-Protected Fats from Crude Palm Oil for Dairy Nutrition. *IOP Conf Ser Earth Environ Sci* **2025**, *1484*, 012014, doi:10.1088/1755-1315/1484/1/012014.

5. CONCLUSÃO GERAL

A utilização de sais de cálcio de ácidos graxos de palma (SCAGP) em dietas para cordeiros em confinamento configura-se como uma estratégia nutricional altamente eficaz para a otimização do desempenho produtivo. A inclusão deste lipídio protegido atua de forma sinérgica com dietas que apresentam alta disponibilidade de amido, favorecendo significativamente o metabolismo nitrogenado e a eficiência no aproveitamento dos nutrientes. Além de maximizar a deposição de gordura na carcaça, garantindo um acabamento de qualidade superior, a suplementação com SCAGP preserva a integridade fisiológica dos animais, consolidando-se como uma ferramenta tecnológica indispensável para sistemas intensivos que visam o ganho de peso acelerado e a máxima performance em regimes de alto grão.