**JAVIER ALEXANDER BETHANCOURT GARCIA**

# DESEMPENHO, QUALIDADE DA CARNE E EXPRESSÃO DE GENES ENVOLVIDOS NO METABOLISMO LIPÍDICO DE NOVILHOS NELORE RECEBENDO MONENSINA E/OU ÓXIDO DE MAGNÉSIO

**LAVRAS-MG**

**2024**

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Zootecnia, área de concentração em Produção e Nutrição de Ruminantes, para a obtenção do título de Doutor.

Prof. Dr. Marcio Machado Ladeira

Orientador

Prof. Dr. Mateus Pies Gionbelli

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**LAVRAS-MG**

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# PERFORMANCE, MEAT QUALITY AND EXPRESSION OF GENES INVOLVED IN LIPID METABOLISM OF NELORE STEERS RECEIVING MONENSIN AND/OR MAGNESIUM OXIDE

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Zootecnia, área de concentração em Produção e Nutrição de Ruminantes, para a obtenção do título de Doutor.

APROVADA em 28 de fevereiro de 2024.

Dr. Erick Batista – DZO/UFLA

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Dr. Mario Chizzotti – DZO/UFV

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Prof. Dr. Marcio Machado Ladeira

Orientador

**LAVRAS-MG**

**2024**

*A Meus pais, por me ensinarem os valores da vida, por sempre acreditarem em mim e me apoiarem em meu crescimento profissional...*

*À minha irmã e meus sobrinhos, por serem as pessoas que sempre me deram energia para continuar...*

*Aos meus padrinhos Franki Ivan Garcia e Tilsaura Quintero Carrasco (in memoriam).*

*DEDICO*

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*“El científico encuentra su recompensa en lo que Henri Poincare llama el placer de la comprensión, y no en las posibilidades de aplicación que cualquier descubrimiento pueda conllevar”*

*(Albert Einstein)*

## RESUMO

A inclusão de aditivos nas dietas de confinamento de bovinos tem sido uma estratégia para melhorar o desempenho através de mecanismos relacionados ao controle de acidose ruminal. Uma fonte de MgO foi desenvolvida para ter alta ação sobre o pH ruminal. Este experimento avaliou o efeito de um blend de diferentes fontes de óxido de magnésio associado ou não à monensina sobre o desempenho, características de carcaça, qualidade da carne, expressão de genes envolvidos na regulação do turnover proteico e no metabolismo lipídico. Oitenta e quatro novilhos Nelore castrados com 367,3 ± 37,9 kg de peso corporal inicial foram distribuídos em um delineamento inteiramente casualizado em esquema fatorial 2 × 2 com os seguintes tratamentos: CON – sem aditivos; MG - pHix-Up® a 0,50% de matéria seca (MS) e 0% de monensina sódica; MON - monensina até 20 mg/kg e 0% pHix-Up®; e MON+MG - combinação de monensina × pHix-Up®. Os animais receberam durante o período experimental (100 dias) uma dieta basal contendo 20% fibra insolúvel em detergente neutro (FDN) e 52,5% amido. O peso corporal foi registrado nos dias 0, 13, 39, 70 e 100 para medidas de desempenho. Amostras de sangue foram coletadas nos dias 0, 13 e 70 para análise de D-Lactato. No dia 70 foram realizadas biópsias de fígado e de músculo (*Longissimus thoracis)* de um animal por baia para analise de análise de expressão gênica. Os bovinos abatidos em frigorifico comercial e amostras de músculo e do epitélio ruminal foram coletadas para análise de expressão gênica e histologia, respectivamente. Além disso, foram coletadas amostras de carne (*Longissimus thoracis*) para análises qualitativas. Não houve efeito do tratamento MON+MG foi detectado ($P \geq 0,22$) para desempenho, características de carcaça, comportamento alimentar, pH, cor e perdas por cocção. Contudo este tratamento aumentou as concentrações de C18:1c9 e ácidos graxos monoinsaturados e tendeu a aumentar a maciez da carne. O MG aumentou ($P < 0,01$) o consumo de MS (CMS), o ganho médio diário (GMD), a expressão do gene *Propionyl-CoA Carboxylase Alpha Subunit* (*PCCA*), a concentração de D-lactato e tendeu aumentar o peso da carcaça quente assim como as perdas por descongelamento. Não houve efeitos dos tratamentos ($P \geq 0,10$) sobre o epitélio ruminal. A MON aumentou a eficiência alimentar e seleção de partículas longas, mas sem efeito no CMS, GMD e a qualidade da carne. Em conclusão, a inclusão de MG melhora o desempenho dos novilhos em dietas de confinamento com alto teor de amido, mas o MG associado ao MON tende a melhorar as características qualitativas da carne dos novilhos.



**Palavras-chave**: alcalinização; alternativas antibióticas; amido; confinamento; óxido de magnésio.

## ABSTRACT

The inclusion of additives in cattle confinement diets has been a strategy to improve performance through mechanisms related to the control of rumen acidosis. A source of MgO was developed to have a high effect on ruminal pH. This experiment evaluated the effect of a blend of different sources of magnesium oxide associated or not with monensin on performance, carcass characteristics, meat quality, expression of genes involved in the regulation of protein turnover and lipid metabolism. Eighty-four castrated Nelore steers with 367.3 ± 37.9 kg of initial body weight were distributed in a completely randomized design in a 2 × 2 factorial scheme with the following treatments: CON – without additives; MG - pHix-Up® at 0.50% dry matter (DM) and 0% sodium monensin; MON - monensin up to 20 mg/kg and 0% pHix-Up®; and MON+MG - the combination of monensin × pHix-Up®. During the experimental period (100 days), the animals received a basal diet containing 20% neutral detergent insoluble fiber (NDF) and 52.5% starch. Body weight was recorded on days 0, 13, 39, 70 and 100 for performance measures. Blood samples were collected on days 0, 13 and 70 for D-Lactate analysis. On day 70, liver and muscle biopsies (*Longissimus thoracis*) were performed from one animal per pen for gene expression analysis. Cattle slaughtered in a commercial slaughterhouse and muscle and rumen epithelium samples were collected for gene expression and histology analysis, respectively. In addition, meat samples (*Longissimus thoracis*) were collected for qualitative analysis. No effect of the MON+MG treatment was detected ($P \geq 0.22$) for performance, carcass characteristics, feeding behavior, pH, color and cooking losses. However, this treatment increased the concentrations of C18:1c9 and monounsaturated fatty acids and tended to increase the tenderness of the meat. MG increased ($P < 0.01$) DM intake (CMS), average daily gain (ADG), expression of the *Propionyl-CoA Carboxylase Alpha Subunit* (*PCCA*) gene, D-lactate concentration and tended to increase hot carcass weight as well as thawing losses. There were no effects of treatments ($P \geq 0.10$) on the ruminal epithelium. MON increased feed efficiency and selection of long particles, but without effect on DMI, ADG and meat quality. In conclusion, the inclusion of MG improves the performance of steers in feedlot diets with high starch content, but MG associated with MON tends to improve the qualitative characteristics of the steers' meat.



**Key-words**: alkalizing; antibiotic alternatives; starch; feedlot; magnesium oxide.

## IMPACTOS SOCIAIS, TECNOLÓGICOS, ECONÔMICOS E CULTURAIS

A alta inclusão de carboidratos de rápida fermentação, principalmente amido, em dietas de confinamento pode levar ao aumento da fermentação ruminal e ao acúmulo de ácidos orgânicos. Isto pode posteriormente reduzir o pH ruminal e alterar a microbiota ruminal, impactando negativamente os padrões de ingestão e ruminação e, consequentemente, o desempenho animal. O objetivo desta investigação foi avaliar o impacto de uma mistura de óxido de magnésio associada ou não à monensina no desempenho, no consumo de ração, no comportamento alimentar, nas características da carcaça, na morfometria do epitélio ruminal, na expressão de genes envolvidos na regulação do turnover protéico no músculo *Longissimus dorsi* (LD) e a expressão de genes envolvidos na lipogênese em machos Nelore alimentados com dietas ricas em amido. Este estudo confirmou que não existe interação substancial entre o óxido de magnésio e a monensina em termos dos seus efeitos combinados nos parâmetros estudados. Os resultados indicam que a incorporação de uma mistura de óxido de magnésio produz resultados positivos em termos de consumo de ração (0,6 kg a mais de matéria seca por dia do que aqueles alimentados com dietas sem este aditivo), o que poderia estar potencialmente ligado a uma melhoria no ambiente ruminal. Isto, por sua vez, se traduz em aumento do peso corporal final (13,5 kg a mais para novilhos alimentados com dietas com mistura de óxido de magnésio), maior ganho médio diário (~9,4% em confinamento total) e maior acúmulo de nutrientes. Por outro lado, a inclusão de óxido de magnésio na dieta não teve impacto no comportamento alimentar ou na morfologia da papila. A inclusão de monensina na dieta não melhorou o desempenho nem o mérito de carcaça dos novilhos. No entanto, impactou o comportamento alimentar e levou a uma melhor eficiência alimentar. Assim, este estudo destaca a importância da mistura de óxido de magnésio como uma alternativa segura e interessante à monensina para ser utilizada em dietas ricas em energia.

## SOCIAL, TECHNOLOGICAL, ECONOMIC AND CULTURAL IMPACTS

The high inclusion of rapidly fermenting carbohydrates, mainly starch, in confinement diets can lead to increased ruminal fermentation and the accumulation of organic acids. This can subsequently reduce rumen pH and alter the rumen microbiota, negatively impacting intake and rumination patterns and, consequently, animal performance. The objective of this investigation was to evaluate the impact of a mixture of magnesium oxide associated or not with monensin on performance, feed consumption, feeding behavior, carcass characteristics, morphometry of the ruminal epithelium, the expression of genes involved in the regulation of

protein turnover in the *Longissimus dorsi* muscle and the expression of genes involved in lipogenesis in Nelore males fed starch-rich diets. This study confirmed that there is no substantial interaction between magnesium oxide and monensin in terms of their combined effects on the parameters studied. The results indicate that the incorporation of a mixture of magnesium oxide produces positive results in terms of feed intake (0.6 kg more dry matter per day than those fed diets without this additive), which could potentially be linked to an improvement in the ruminal environment. This, in turn, translates into increased final body weight (13.5 kg more for steers fed magnesium oxide blend diets), greater average daily gain (~9.4% in total confinement) and greater nutrient accumulation. On the other hand, the inclusion of magnesium oxide in the diet had no impact on eating behavior or papilla morphology. The inclusion of monensin in the diet did not improve the performance or carcass merit of the steers. However, it impacted feeding behavior, led to better feed efficiency and supplemented animals. Thus, this study highlights the importance of the magnesium oxide mixture as a safe and interesting alternative to monensin to be used in energy-rich diets.

# EFEITOS DA MONENSINA E/OU UMA MISTURA DE DIFERENTES FONTES DE MAGNÉSIO (pHix-Up) EM BOVINOS DE CORTE ALIMENTADOS COM ALTO AMIDO


## INTRODUÇÃO

Dietas com alto amido maximizam o **desempenho** e a **eficiência alimentar.** No entanto, reduzem o pH ruminal e aumentam o risco potencial de distúrbios como a **acidose.** Neste sentido, o uso de **aditivos** é de imensa importância para manter um ambiente ruminal estável, prevenir **distúrbios digestivos,** melhorar a utilização de nutrientes e também o **desempenho animal**

## OBJETIVO

Avaliar o efeito de uma mistura de diferentes fontes de óxido de magnésio associada ou não à monensina, sobre o desempenho, características de carcaça, qualidade da carne e expressão de genes envolvidos no metabolismo lipídico

**Oitenta e quatro** (367,3 kg)

Dieta basal contendo 52.5% de amido

## TRATAMENTOS

- Controle (CON)
- Monensina (MON)
- pHix-up (MG)
- MON + MG

## RSULTADOS com MG

1. + 5% de consumo de MS

2. + 9.3% de ganho médio diário

3. + 8 kg de peso de carcaça quente

4. + 10.4% de área muscular do *Longissimus thoracis*

5. + 7.2% de área muscular do *longissimus Thoracis* por kg de peso de carcaça

6. + 21.5% de espessura de gordura

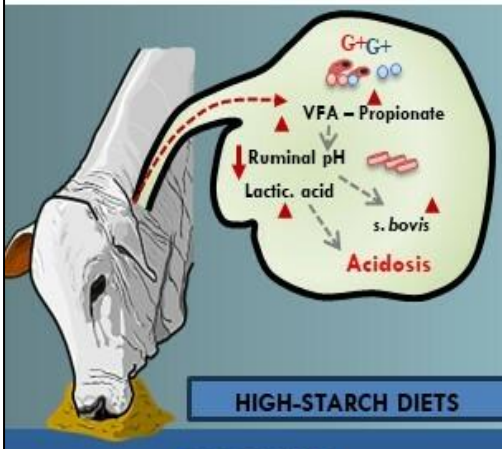
7. A associação de aditivos melhorou a maciez da carne

8. Aumento da expressão do mRNA para o gene PCCA – que possui papel chave na gliconeogênese

## CONCLUSÃO

A inclusão de MG em dietas de terminação com alta densidade energética **melhora o desempenho** e as **características da carcaça,** além de aumentar a expressão do gene que ***PCCA,*** envolvido na gliconeogênese. A associação entre MON + MG melhora a **maciez da carne.**

# EFFECTS OF MONENSIN AND/OR A MIXTURE OF DIFFERENT MAGNESIUM SOURCES (PHIX-UP) IN BEEF CATTLE FED HIGH-STARCH DIETS




HIGH-STARCH DIETS

## INTRODUCTION

High-starch diets maximize **performance** and **feed efficiency.** However, these diets reduce ruminal pH and increase the potential risk of disorders such as **acidosis.** In this sense, the use of additives is of immense importance to maintain a stable ruminal environment, prevent **digestive disorders,** improve nutrient utilization, and also **animal performance**

## OBJECTIVES

**Evaluate the effect of a mixture of different sources of magnesium oxide associated or not with monensin on performance, carcass characteristics, meat quality, and expression of genes involved in lipid metabolism**

Eighty-four (367.3 kg)

Basal diet containing 52.5% starch

## TREATMENTS

- Control (CON)
- Monensin (MON)
- pHix-up (MG)
- MON + MG

## RESULTS WITH MG

1. + 5% Dry matter intake

2. + 9.3% average daily gain

3. + 8 kg of hot carcass weight

4. + 10.4% of *Longissimus thoracis* muscle area

5. + 7.2% of *Longissimus thoracis* muscle area per kilogram of carcass weight

6. + 21.5% of fat thickness

7. The associated use of additives improved meat tenderness

8. Increased expression of mRNA for the PCCA gene - which plays a key role in gluconeogenesis

## CONCLUSION

The inclusion of MG in high-energy-density finishing diets improves **performance** and **carcass characteristics,** as well as increases the expression of the **PCCA gene** involved in gluconeogenesis. The association between MON + MG enhances **meat tenderness**

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### PRIMEIRO CAPÍTULO



### SEGUNDO CAPÍTULO – ARTIGO 1



### TERCEIRO CAPÍTULO – ARTIGO 2

**SUMÁRIO**

# PRIMEIRO CAPÍTULO

## 1. INTRODUÇÃO GERAL

Com o aumento da demanda por produtos de origem animal, os sistemas intensivos como os confinamentos têm se destacado como uma alternativa estratégica cada vez mais adotada na pecuária. O número de animais abatidos provenientes de sistemas de confinamento tem crescido significativamente, passando de 9,00% em 2010 para 18,20% em 2023, representando um aumento considerável na composição do abate nacional (ABIEC, 2023).

O sucesso dos confinamentos está atrelado a capacidade de maximizar o consumo de energia liquida para ganho e, consequentemente, reduzir o tempo de engorda. Porém, para atender tais resultados torna-se necessário a inclusão de ingredientes na dieta capazes de aumentar a densidade energética (OWENS & SODERLUND, 2006; CAETANO et al., 2015). Nesse sentido, as dietas de terminação dos confinamentos brasileiros têm passado por diferentes ajustes, especialmente em características físico-químicas, como a redução da proporção de alimentos volumosos e o aumento da proporção de concentrados, contendo carboidratos de fermentação mais rápida, como o amido. Além disso, há uma maior utilização de subprodutos, como DDG, WDG, entre outros (SILVESTRE & MILLEN, 2021).

No entanto, quando a inclusão de carboidratos de rápida fermentação na dieta é abrupta e sem um período prévio de adaptação, os animais se tornam mais susceptíveis a distúrbios digestivos, como acidose (OWENS et al., 1998; LADEIRA et al., 2014). A acidose se caracteriza pela depressão do pH ruminal que, consequentemente, pode ter impactos negativos na síntese se ácidos graxos na carne, devido a mudanças na rota da biohidrogenação, produzindo isômeros do ácido linoleico conjugado que interferem na expressão de genes lipogênicos no músculo esquelético (TEIXEIRA et al., 2017). Nesse sentido, uma forma de diminuir os riscos de acidose ruminal e ao mesmo tempo facilitar a utilização de dietas ricas em energia consiste no uso de aditivos dietéticos incorporados à dieta (BROWN et al., 2006). Estudos com a inclusão de aditivos em dietas com alta densidade energética tem apresentados efeitos positivos, tais como o tamponamento do pH ruminal e melhora da digestibilidade da fibra, bem como aumento do desempenho animal e de caraterísticas de carcaça (SCHELLING, 1984; ERICKSON

et al., 2003; COLOMBO et al., 2021; CECONI et al., 2022; NASCIMENTO et al., 2024).

A monensina sódica é um aditivo antibiótico amplamente utilizado nas dietas de confinamento brasileiro bem como nos Estados Unidos (SAMUELSON et al., 2016; SILVESTRE & MILLEN, 2021). Contudo, por ser um antibiótico, diversas opiniões públicas se opõem dessa classe de aditivos na alimentação animal, motivadas pelo princípio de precaução (RUSSELL & HOULIHAN, 2003; CAMERON & MCALLISTER, 2016; THOMAS et al., 2017). Nesse sentido, a exploração de alternativas viáveis e mais seguras para os consumidores em alternativa aos antibióticos exploraram a inclusão de agentes neutralizantes, como o óxido de magnésio nas dietas ricas em amido, que tem se mostrado efetivo em controlar os efeitos negativos da acidose ruminal (BACH et al., 2018; COLOMBO et al., 2021; BACH et al., 2023; NASCIMENTO et al., 2024).

Diante disso, o presente trabalho teve como objetivo avaliar a utilização de um blend de diferentes fontes de óxido de magnésio como um aditivo natural com ou sem adição de monensina sobre o desempenho, as características de carcaça, e a expressão de genes envolvidos na lipogênese em bovinos alimentados com dietas ricas em amido.

## 2. REFERENCIAL TEÓRICO

### 2.1. **Dietas com alto teor de amido**

O rebanho bovino Brasileiro é estimado em 202,78 milhões de cabeças (ABIEC, 2023), tornando o Brasil o segundo maior produtor de bovinos do mundo. Nos últimos anos, o número de animais confinados no país aumentou, com uma estimativa de 7,62 milhões de cabeças (ABIEC, 2023), representando 18,02% dos abates totais. Em comparação com 2010, em que representava apenas 9% dos abates totais, houve um crescimento significativo. No Brasil, a raça Nelore é a predominante nos sistemas de produção por sua capacidade para se adaptar as condições climáticas com sucesso em comparação com raças Europeias.

Com base em uma pesquisa realizada com nutricionistas de confinamentos brasileiros, SILVESTRE & MILLEN (2021) observaram uma redução na inclusão de alimentos volumosos nas dietas de confinamento nos últimos dois anos, passando de

20,6% para 16,8%. Além disso, quase 97% dos consultores relataram o uso de dietas compostas por mais de 70% de concentrado.

O uso frequente de grãos de cereais para aumentar a densidade energética da dieta é fundamental para melhorar a fermentação ruminal, a digestibilidade da matéria orgânica e consequentemente melhorar o desempenho animal, sendo o milho (*Zea mays*) e o sorgo (*Sorghum bicolor*) as principais fontes utilizadas nas dietas ao redor do mundo (OWENS et al., 1998; SILVESTRE & MILLEN (2021). O milho e o sorgo são compostos por aproximadamente 72% de amido (HUNTINGTON, 1997), porém apresentam diferentes taxa de degradação do amido, sendo esta maior para o milho (ROONEY & PFLUGFELDER, 1986; OWENS & SODERLUND, 2006).

Quimicamente, o amido é constituído por dois tipos de polímero de glicose, a amilose e amilopectina (FRENCH, 1972). A amilose é um polímero linear com ligações glicosídicas α – 1,4 entre as moléculas de glucose, enquanto que a amilopectina é um polímero mais longo e altamente ramificado com ligações α – 1-4 e α – 1-6 no ponto de ramificação a cada 20-25 moléculas de glicose (ROONEY & PFLUGFELDER, 1986). As moléculas de amilose e amilopectina representam de 98% a 99% de um grânulo de amido e seus pesos moleculares dependem do estágio de maturidade e origem botânica da planta (TESTER et al., 2004).

As moléculas amilose e amilopectina são mantidas unidas por ligações de hidrogênio, resultando em grânulos de amido com estrutura altamente organizada (ROONEY & PFLUGFELDER, 1986). Os grânulos de amido estão interligados e envolvidos por uma camada ou matriz proteica, cujas características são atribuídas à baixa degradabilidade ruminal (OWENS & SODERLUND, 2006). Essa matriz proteica é classificada como prolamina, a qual contém quatro subclasses (α, β, γ, δ), que apresentam ligações por pontes de hidrogênio dentro dos grânulos, precisando serem quebradas para melhorar a sua capacidade de hidratação, deixando o amido disponível para digestão enzimática (ROONEY & PFLUGFELDER, 1986).

Inicialmente, o alimento ingerido pelos ruminantes passa pelos pré-estômagos. O rúmen é o principal local de degradação do amido, sendo apresentadas taxas de digestão entre 70% a 80% (OWENS & SODERLUND, 2006). O processo de degradação ruminal é realizado a partir de diferentes enzimas microbianas, tais como as α-amilases, as isoamilases, glucoamilases e as β – amilases (Maltase) (HARMON et al., 2004). No entanto, trabalhos pontuais na literatura demostram que a taxa de digestão do amido

dependente de fatores como o método de processamento, vitreosidade, entre outros (OWENS & SODERLUND, 2006).

Embora o rúmen seja o local principal de degradação do alimento, quando são fornecidas dietas altamente concentradas à base de grãos de cereais, de 4% a 60% do amido da dieta consegue escapar da fermentação ruminal para o intestino delgado, onde aproximadamente 35 a 60% da quantidade que chega pode ser digerida (HARMON et al., 2004). Entretanto, trabalhos na literatura reportaram que a digestão e absorção do amido no intestino delgado parece ser limitado pelas secreções de α-glicohidrolase (OWENS et al., 1986; HUNTINGTON, 1997; BRAKE & SWANSON, 2018). Por outro lado, um dos indicadores frequentemente utilizado para determinar a digestibilidade do amido no trato gastrointestinal total é a concentração de amido nas fezes, uma vez que este parâmetro possui alta correlação com a digestibilidade, podendo demostrar que existe uma alteração no local da digestão (OWENS et al., 2016; ZINN et al., 2007).

Após a degradação da molécula de amido, ocorre o processo de fermentação ruminal que é realizado por bactérias, tendo como resultado principal a maltose e glicose. Os microrganismos fermentam rapidamente a glicose até piruvato através da via da glicólise, o qual posteriormente é convertido em ácidos graxos voláteis (AGCC) os quais são utilizados como fonte de energia pelo hospedeiro. No caso dos carboidratos não fibrosos (CNF), esse processo é realizado por bactérias amilolíticas, sendo as principais as *Streptococcus bovis, Ruminobacter fibrisolvens, Prevotella ruminicola, Butirivibrio fibrisolvens, Succinomonas amylolytica, Selenomonas ruminantion, Eubacterium ruminantion* e *Clostridium spp* (PETRI et al., 2013; RUSSELL & RYCHLIK, 2001).

Os AGCC produzidos durante a fermentação diferem de acordo com o tipo de dieta fornecida ao animal. No caso de dietas com maior inclusão de amido, as bactérias aminolíticas são as mais ativas e consequentemente, a digestão e fermentação por essas comunidades de bactérias resultam em maior produção de propionato. O propionato é o precursor primário da glicose e é mais eficiente energeticamente em função da produção e utilização de todos os hidrogênios produzidos no metabolismo ruminal em comparação com os outros AGCC (HUNTINGTON, 1997).

Ao longo dos anos, as pesquisas têm explorado estratégias para melhorar a disponibilidade de amido dos cereais utilizados nas dietas de bovinos. Uma dessas estratégias envolve a utilização de métodos de processamento para quebrar a matriz

proteica, o que aumenta a área exposta à ação das enzimas dos microrganismos. Isso possibilita uma maior digestão do amido e melhora a capacidade fermentativa do rúmen, resultando em uma maior síntese proteica e produção de AGCC (ALLEN et al., 2009). Nesse sentido, a escolha de um método de processamento adequado aumenta a disponibilidade do amido no rúmen (OWENS & SODERLUND, 2006).

O método de processamento mais utilizado no Brasil é a moagem fina (SILVESTRE & MILLEN, 2021), em virtude do interesse dos nutricionistas em melhorar a digestibilidade do amido e o desempenho de bovinos de corte (PASSINI et al., 2004). Por outro lado, ZINN et al. (2002) reportam que a adequada floculação dos grãos de milho incrementa em aproximadamente 15% o teor de energia líquida de manutenção e 18% o teor de energia líquida de ganho, quando comparada com a moagem grosseira do milho ou a laminação a seco. Além disso, trabalhos realizados no Brasil, verificaram que o uso de silagem de milho reidratado aumenta em 13,9% a eficiência alimentar em bovinos confinados em comparação com o milho moído (CAETANO et al., 2015)

A classe de milho e suas caraterísticas também influenciam na digestibilidade e aproveitamento do amido. Entre as classes de milho destacam-se o dentado, o duro, os farináceos, o pipoca e o doce (CORREA et al., 2002; ZINN et al., 2002). No Brasil, mais de 78% do milho produzido é do tipo duro (Flint -*Zea mays ssp*.) (CORREA et al., 2002), caracterizado por um endosperma vítreo que ocupa quase todo o seu volume, com uma baixa proporção de endosperma farináceo, tornando-o menos suscetível à degradação ruminal e exigindo métodos de processamento (PINTO & MILLEN, 2019).

## 2.2. **Acidose ruminal**

O interesse na utilização de dietas com limitada inclusão de volumoso vem crescendo nos últimos anos. Estudos realizados por SILVESTRE & MILLEN (2021) relatam que a inclusão de volumoso nas dietas de engorda nos confinamentos brasileiros é inferior a 16% e tem sido substituída por CNF de rápida fermentação, principalmente amido. Essa substituição tem um efeito positivo no desempenho devido à maior produção de AGCC, que são uma fonte de energia importante. No entanto, a inclusão elevada de CNF na dieta pode ter efeitos negativos na digestibilidade da fibra, resultando na diminuição do pH e aumento da pressão parcial de hidrogênio ($H_2$) no rúmen, o que pode levar a distúrbios digestivos como acidose metabólica, timpanismo, dentre outros (Enemark, 2008; NASEM, 2016).

No caso da acidose, STEDMAN (1982) a definiu como a diminuição das bases no fluído corporal em relação ao grau de acidez (íons de hidrogênio), processo que está relacionado diretamente com o pH ruminal. No ambiente anaeróbico do rúmen e no ceco, a digestão microbiana transforma os carboidratos da dieta em glicose, e posteriormente, ocorre a fermentação dessa glicose em AGCC. O lactato é absorvido pela parede do rúmen e do ceco e metabolizado no fígado, fornecendo energia ao ruminante (OWENS & SODERLUND, 2006; ZINN et al., 2002).

Se os produtos finais da fermentação ruminal (ácidos graxos orgânicos), que basicamente são determinados pelo tipo de dieta fornecida, não excedem a capacidade de absorção, o pH ruminal é próximo da neutralidade (6,2 – 6,5). Nesse sentido, um pH ruminal próximo da neutralidade é caraterístico de dietas com elevada inclusão de volumoso com níveis adequados de fibra fisicamente efetiva na dieta, permitindo estimular a atividade de ruminação resultando em maior secreção de saliva, sendo essa uma das maiores fontes de tamponamento no rúmen (HOLTSHAUSEN et al., 2013; PETRI et al., 2013).

Por outro lado, quando os ruminantes consomem grandes quantidades de carboidratos rapidamente fermentáveis, a hidrólise do amido provoca aumento de populações de microrganismos produtores de lactado, especialmente o *Streptococcus bovis*. Tal fato, em conjunto com a baixa absorção de AGCC via parede ruminal, causada pelo aumento da osmolaridade, diminui a capacidade tamponamento do bicarbonato, provocando redução drástica do pH ruminal, e consequente um quadro de acidose ruminal. Além da acidose, podem ocorrer lesões na parede do rúmen, causando ruminite, devido a invasão e colonização de microrganismos ruminais oportunistas como *Actinomyces pyogenes* ou *Fusobacterium necrophorum* (RUSSELL & HINO, 1985). A partir do quadro de ruminite, bactérias do gênero *F. necrophorum* podem atravessar o epitélio, atingindo à corrente sanguínea e posteriormente alojando-se no fígado, o que leva a presença de abcesso hepático (BROWN et al., 2006). A acidose clínica é caraterizada por valores de pH abaixo de 5,0 ou 5,2, enquanto que valores de 5,2 ou 5,5 é caraterística de acidose subclínica (Figura 1) (SCHWARTZKOPF-GENSWEIN et al., 2003). Apesar da acidose subclínica ocorrer de forma mais frequente, essa se caracteriza pela ausência de sintomas clínicos da doença pelos animais.

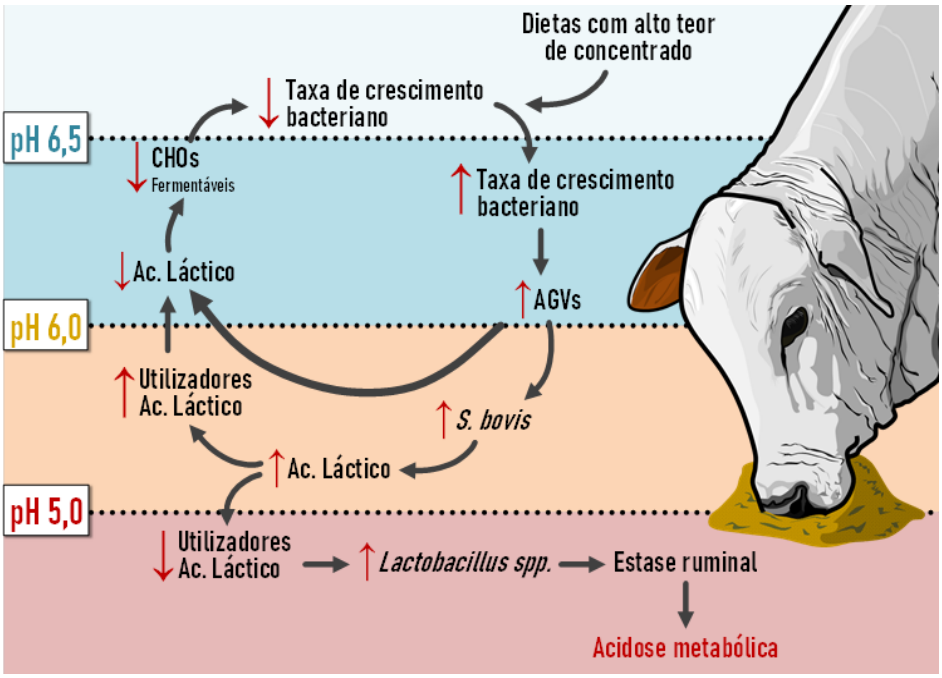


**Figura 1**. Consequências metabólicas do consumo de ração de bovinos em terminação em confinamento sobre o pH ruminal e as populações microbianas do rúmen (SCHWARTZKOPF-GENSWEIN et al., 2003). Imagem elaborada por Germán Darío Ramírez Zamudio.

Embora existam bactérias como *Megasphera elsdenii* e *Selenomonas ruminantium* que utilizam lactato, as mesmas são sensíveis a valores de pH < 5,5 (OWENS et al., 1998). Por outro lado, a redução de pH abaixo de 5,2 inibe o crescimento das bactérias *S. bovis*, porém é um ambiente favorável para que bactérias do gênero *Lactobacillus* se proliferem e continuem produzindo ácido láctico (RUSSELL & HINO, 1985). Diversos eventos estão associados à queda no pH ruminal, tais como: a redução da motilidade ruminal, aumento na variação no CMS, diminuição das refeições, redução na ruminação e presença de fezes com consistência líquida, sendo essas situações associadas com a acidose clínica e subclínica (OWENS et al., 1998; SCHWARTZKOPF-GENSWEIN et al., 2003). Nesse sentido, para evitar a ocorrência dessa desordem metabólica é necessário um período de adaptação a uma dieta com pouca fibra e grande quantidade de amido, a qual permite que a população microbiana se adapte ao novo substrato e aumente de forma menos drástica a população de *S. bovis* no rúmen reduzindo o acúmulo de ácido láctico (RUSSELL & HINO, 1985).

Atualmente, no sistema de confinamento de bovinos de corte o desafio é equilibrar os níveis de CNF com FDN vindo da forragem, sendo uma estratégia para

minimizar os distúrbios metabólicos. Nesse sentido, a FDN da forragem é historicamente considerada a principal contribuinte para manter o pH ruminal adequado (ALLEN, 1997), aumentar a atividade mastigatória (GALYEAN & HUBBERT, 2014) e melhorar a secreção de saliva (ZEBELI et al., 2012). Tal fato auxilia o tamponamento ruminal, auxiliando na manutenção da saúde da microbiota ruminal (YANG & BEAUCHEMIN, 2009), e contribuindo para maximizar a ingestão de energia. Em geral, a concentração de FDN da forragem mostra uma associação negativa com a concentração de CNF e o conteúdo energético da dieta (MERTENS, 1997). Dessa forma, informações precisas sobre a eficácia da alimentação das fontes de forragem utilizadas nas dietas é um aspecto chave da nutrição de bovinos de corte (GOULART et al., 2020).

Um conceito relevante para formulação de dietas com alta concentração de carboidratos de rápida fermentação é o de FDN fisicamente efetiva (FDNfe). Tal conceito foi introduzido por Mertens (1997), e refere-se ao tamanho das partículas da dieta necessário para manter a fisiologia normal do ruminante, ou seja, a fração capaz de estimular a atividade mastigatória, e manter um ambiente ruminal saudável (ALLEN, 1997). O conceito de FDNfe integra aspectos físicos dos alimentos da dieta, relacionados principalmente com o tamanho de partícula. O FDNfe consiste em multiplicar o teor de FDN no alimento pelo pef, o qual pode variar entre de 0 a 1, sendo que 0 corresponde a FDN que não é capaz de estimular a atividade mastigatória e 1 a FDN que possui grande capacidade de estimular a atividade mastigatória. As recomendações para estimativa do fator de efetividade foram baseadas em um conjunto de três peneiras denominado "*Pen State Particle Separator*" (PSPS), com os crivos de 19,0; 8,0 e 1,18 mm, embora existam diferentes propostas para se obter a efetividade da FDN (LAMMERS et al., 1996; KONONOFF et al., 2003; ZEBELI et al., 2012). Por ser mais prática e de menor custo, a metodologia proposta por KONONOFF et al. (2003) tem sido adotada nos modelos nutricionais (NRC, 2001; NASEM, 2016) que recomendaram a proporção do alimento retido acima do crivo de 1,18 mm. Esse tamanho foi considerando como o tamanho mínimo para que um alimento passasse do rúmen-retículo para o omaso, sendo assim uma medida para predição da resposta em pH ruminal. Nesse caso, foi assumido um FDN constante em cada peneira. No entanto, o NASEM (2016) sugeriu realizar uma correção com a substituição da peneira de 1,18 mm pela de 4 mm de crivo, pois estudos mostram que algumas partículas de maior tamanho estavam presentes nas digestas omasais, ou seja, o orifício reticulo omasal

pode variar de tamanho entre diferentes categorias animal. Uma alternativa descrita na literatura como medida da efetividade da fibra é a distribuição de partículas do alimento com base na matéria natural (NRC, 2001), assumindo-se que tanto o teor de MS quanto o de FDN são constantes nas peneiras.

No entanto, os dados na literatura são controversos ao comparar o CMS, com os parâmetros de FDN total, FDN dos volumosos (FDNv) e da FDNfe. Estudo realizados por Defoor et al. (2002) demostram que os parâmetros de FDNv e FDNfe podem ser utilizados na tomada de decisão sobre o tipo de volumoso utilizado na dieta, ao conseguir explicar o CMS em 69,9%, enquanto que a FDNv e FDNfe explicaram 92% e 93,1%, respectivamente. Trabalhos realizados por Fox & Tedeschi (2002) recomendaram o uso de 7 a 10% de FDNfe para dietas com alto teor de concentrado para manter o pH do rúmen acima de 5,7. Alhadas et al. (2021), sugerem no mínimo de 6,14 a 10,2%% FDNfe na MS.

### 2.2.1. **Absorção de AGCC pelo epitélio ruminal**

Os ruminantes possuem a capacidade de obter energia de carboidratos complexos como os carboidratos rapidamente fermentáveis, produzindo AGCC, sendo esses os ácidos acético, propiônico e butírico. Esses AGCC representam 95% de todos os ácidos de fermentação, variando de 45 a 70%, 15 a 40% e 5 a 20%, respectivamente (PENNER et al., 2009; UDÉN, 2011). Estima-se que os AGCC forneçam até 75% da energia metabolizável total para os bovinos (BERGMAN, 1990). Considerando esses resultados, não é surpreendente que as dietas com elevadas concentrações de CNF (principalmente amido), promovam melhores resultados produtivos em períodos curtos de tempo (OBA & ALLEN, 2003). No entanto, Steele et al. (2011) manifestam que dietas com grandes quantidades CNF está, de fato, associado ao diferencial na expressão de genes envolvidos no crescimento epitelial e estrutural, pois podem comprometer a integridade estrutural do epitélio ruminal e intestinal. Estudos recentes mostram que níveis crescentes de amido na dieta (> 45%) aumentam a expressão da proteína inibidora da *elastase leucocitária*, associada à resposta inflamatória, a qual pode ocasionar efeitos significativos no epitélio cecal (ROCHA et al., 2022).

Os ácidos graxos voláteis mencionados anteriormente são ácidos fracos, pois apresentam um pKa de ~ 4.8 (LEONHARD-MAREK et al., 2010). No caso do fluído ruminal com pH variando de 5,8 a 6,8, entre 90 a 99% desses ácidos se dissociarão no rúmen sendo liberado um próton ($H^+$), o qual será tamponado ou eliminado. A

concentração de ácido de fermentação no fluído ruminal varia entre 60 e150 mmol/L (BERGMAN, 1990), sendo essa quantidade influenciada pelas características da dieta.

Embora, a ingestão de energia determine a quantidade absoluta de AGCC para suprir as exigências de energia do animal, não há relação entre a ingestão de energia (PENNER et al., 2009), ou as taxas de produção de AGCC (PETERS et al., 1990) e a passagem fracionada de AGCC para o omaso. Por essas razões que absorção de AGCC para a homeostase do pH ruminal não pode ser reduzida a uma única consideração quantitativa das taxas de absorção de AGCC (ASCHENBACH et al., 2009). Um exemplo evidente é quando a taxa de produção de AGCC ultrapassa a capacidade de neutralizar prótons, resultando em alterações no pH ruminal e podendo levar a distúrbios metabólicos (ALLEN, 1997; ASCHENBACH et al., 2009), o que compromete a produtividade do animal.

A maior parte da produção de AGCC ocorre principalmente no rúmen, o qual é um ambiente com baixo pH e de grande volume (STORM & KRISTENSEN, 2010). ASCHENBACH et al. (2009) reportam que entre 50% a 85% dos AGCC produzidos a partir da fermentação ruminal são absorvidos diretamente através da parede reticulo-ruminal, sendo que apenas 15 a 50% passam para o pós-rúmen. Porém, essa capacidade de absorção está relacionada à área de superfície das papilas, fato que se apresenta como um desafio o qual é exposto ao epitélio ruminal (DIJKSTRA et al., 1993). Assim, destaca-se a importância do epitélio ruminal como um componente-chave, com uma contribuição significativa na redução dos ácidos ruminais e da carga de prótons (PETERS et al., 1990).

No rúmen não há nenhuma área sem papilas, sendo que as partes mais densas estão localizadas nas regiões ventrais dos sacos ruminais. No entanto, para compreender a absorção de AGCC, é importante considerar o arranjo histológico do epitélio ruminal. O tecido do epitélio ruminal é complexo e consiste em quatro estratos, formados por várias camadas de células estruturais altamente vascularizadas, cuja função é proteger esses estratos da abrasão física. Além disso, essas camadas possuem atividade metabólica, permitindo a absorção e metabolismo dos ácidos graxos de cadeia curta (STEVEN & MARSHALL, 1970).

A camada mais externa do epitélio ruminal é o estrato córneo, composto por células cuja função é proteger os estratos subjacentes da abrasão física. No entanto, sua contribuição como barreira ou fator de promoção para a absorção de AGCC é limitada (STEVEN & MARSHALL, 1970). A próxima camada é o estrato granuloso,

caracterizado por células cada vez mais queratinizadas e com poucas organelas intracelulares. Embora o estrato granuloso seja o principal local para as junções de células fechadas, ele também atua como barreira física para garantir a absorção de AGCC, enquanto impede a passagem de moléculas indesejadas. A camada seguinte é o estrato espinhoso, composto por células metabolicamente ativas com junções comunicantes que facilitam a comunicação célula-célula e a troca de íons. Por fim, a camada mais interna é o estrato basal, caracterizado por células altamente ativas. Esta é a camada onde ocorre a divisão celular, sendo necessária para a produção de novas células que amadurecem e se diferenciam enquanto migram em direção ao estrato córneo (ASCHENBACH et al., 2009; PENNER et al., 2009).

Estudos prévios se concentram em dois mecanismos pelos quais os AGCC são absorvidos através da parede ruminal: (**1**) absorção de AGCC não dissociados, que podem ser absorvidos por difusão passiva através do epitélio ruminal (ALLEN, 1997; GÄBEL et al., 2002); ou (**2**) absorção por difusão passiva dependente de bicarbonato (ASCHENBACH et al., 2009) (Figura 2). O primeiro mecanismo, em baixo pH, envolve a captação por via difusiva com AGCC acoplado a um próton. O influxo é regulado por um microclima apical de pH dentro do estrato córneo. A transferência transepitelial passiva, ou seja, sem gasto de energia, dos AGV não dissociados para o sangue contribui diretamente para a estabilização do pH intraruminal, pois os prótons são eliminados junto com os AGCC (ASCHENBACH et al., 2011). Assim é importante reconhecer que à medida que o pH diminui, a proporção de AGCC no estado não dissociado aumenta e que apenas os AGCC não dissociados são permeáveis para atravessar a bicamada lipídica das células (GÄBEL et al., 2002).

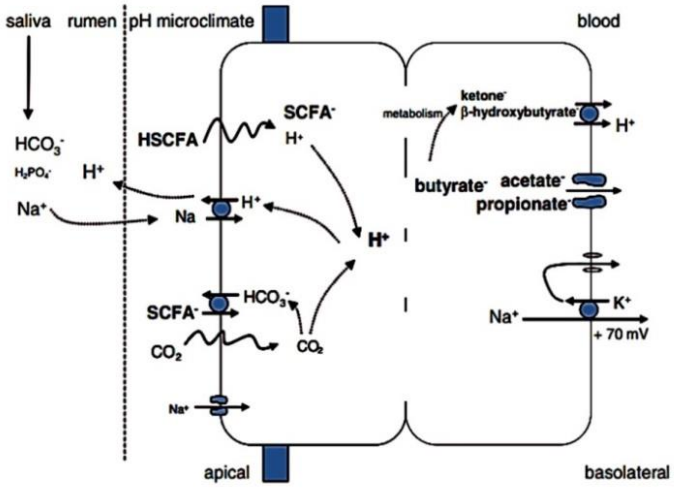


**Figura 2**. Vias de absorção de AGCC através da membrana apical do epitélio ruminal.

O segundo mecanismo requer proteínas de transporte e se aplica aos ânions dissociados, sendo que absorção do acetato principalmente, dependente em maior parte de bicarbonato. Uma vez que o ânion do ácido é absorvido, é necessário para a manutenção do equilíbrio de cargas (cátions / ânions), um processo que é realizado através do cotransporte com bicarbonato (ASCHENBACH et al., 2009; PENNER et al., 2009). Esse bicarbonato é secretado pela parede do rúmen e pode ser proveniente do sangue ou formado novamente pelas células do epitélio ruminal através da conversão de $CO_2$, um processo catalisado pela enzima citoplasmática anidrase carbônica (GÄBEL et al., 2002).

Ambas as vias são eletroneutras e transferem um próton para o citosol, o qual é removido em troca da captação apical de Na via troca $Na^+$ / $H^+$. Como o AGCC se dissocia rapidamente no pH quase neutro do citosol (pKE4,8), o efluxo basolateral não pode ocorrer por meio da difusão de AGCC, mas deve envolver proteínas de transporte específicas (LEONHARD-MAREK et al., 2010). No caso do $CO_2$, a solubilidade de equilíbrio é determinada a partir da pressão parcial de $CO_2$ na fase gasosa que pode ser eructado ou absorvido, e assim essa remoção de $CO_2$ aumenta a eficiência do tampão de bicarbonato (KOHN & DUNLAP, 1998).

Os mecanismos de absorção desempenham papel fundamental na estabilização do pH ruminal. Curiosamente, estudos realizados por PENNER et al. (2009) relatam diferenças nas vias de absorção de AGCC com base no tipo de AGCC e, portanto, a contribuição relativa para a estabilização do pH ruminal também pode diferir. Um exemplo seria a secreção de bicarbonato e a absorção de acetato que estão acopladas, porém o acetato não é tão lipofílico quanto o butirato e, portanto, as vias mediadas por proteínas contribuem substancialmente para sua absorção (ASCHENBACH et al., 2009).

## 2.3. **Aditivos**

A manipulação da microbiota ruminal é considerada ferramenta fundamental para melhorar a eficiência da produção animal (RUSSEL & STROBEL, 1989). Diversos produtos têm sido utilizados visando auxiliar a digestibilidade da fibra, diminuir o risco de distúrbios metabólicos, melhorar a eficiência e diminuir as perdas energéticas. A perda de energia metabolizável na forma de metano representa em média 12%, podendo essas perdas serem diminuídas pelo uso de aditivos (BROWN et al.,

2006). Por essa razão, a utilização de aditivos nas dietas tem sido cada vez mais estudada.

Durante anos, os antibióticos têm sido utilizados para a prevenção de doenças como a acidose e lesões persistentes, como paraqueratose ou ruminite (BROWN et al., 2006), além de melhorar o desempenho animal. Isso é possível através da exclusão de microrganismos que competem no trato gastrointestinal. Embora os resultados fossem anteriormente positivos para os produtores, devido ao uso descontrolado dos antibióticos nos sistemas de produção, verificou-se que tanto os patógenos como os microrganismos favoráveis para o animal eram eliminados. Isso causou resistência bacteriana aos antibióticos e possíveis problemas na saúde humana e no bem-estar animal (RUSSELL & HOULIHAN, 2003).

Conforme definido pela legislação brasileira, um aditivo é uma substância, microrganismo ou produto formulado, adicionado intencionalmente aos produtos que normalmente não são utilizados como ingredientes, tenham ou não valor nutritivo. Seu propósito é melhorar as características dos produtos destinados à alimentação animal ou dos produtos animais, melhorar o desempenho dos animais saudáveis ou atender às necessidades nutricionais. Esses aditivos são classificados como tecnológicos, sensoriais, nutricionais e zootécnicos. Por outro lado, a Autoridade Europeia de Segurança Alimentar (EFSA) define os aditivos como substâncias, microrganismos ou preparados, que não sejam matérias para a alimentação animal ou pré-misturas, que sejam intencionalmente acrescentados aos alimentos para animais ou à água, nomeadamente a fim de desempenharem pelo menos uma das funções mencionadas na legislação vigente, sendo classificados em cinco grupos: (**1**) Aditivos tecnológicos: que inclui conservantes, antioxidantes, emulsificantes, estabilizantes, reguladores da acidez, entre outros; (**2**) Aditivos sensoriais, que ao incluí-los aos alimentos melhoram ou modificam as propriedades organolépticas ou as características visuais, tais como corantes e palatabilizantes; (**3**) Aditivos nutricionais, referindo-se as vitaminas, microminerais, aminoácidos e ureia; (**4**) Aditivos zootécnicos: qualquer implemento utilizado para favorecimento do desempenho animal, tais como enzimas, equilibradores da flora intestinal (probióticos, prebióticos, simbióticos, ácidos orgânicos e nutracêuticos) e promotores de crescimento e/ou eficiência alimentar (ionóforos e os hormônios, principalmente); e (**5**) Aditivos anticoccidianos.

### 2.3.1. **Ionóforos**

Os ionóforos são um grupo distinto de antibióticos produzidos principalmente por bactérias do gênero *Streptomyces spp.* (BERGEN & BATES, 1984; ROCHA et al., 2022). Eles têm sido amplamente utilizados como complemento na alimentação dos bovinos desde 1975 (GOODRICH et al., 1984). Devido à preocupação dos consumidores sobre o uso de antibióticos e também aos possíveis níveis de resistência nos produtos de origem animal (RUSSELL & HOULIHAN, 2003) a União Europeia, que representa mais de 27 países, em 2006 proibiu o uso de antibióticos na alimentação dos bovinos, levando a indústria da carne a buscar novas alternativas.

A monensina é o ionóforo mais utilizado no mundo, sendo comercializada por várias indústrias. A principal função da monensina é aumentar a energia metabolizável da dieta, melhorar a eficiência alimentar e prevenir e controlar a coccidiose (FDA, 2006; NASEM, 2016). Já no Brasil, estudos mostram que 86% dos especialistas encarregados do manejo nutricional de bovinos em confinamentos, utilizam a monensina sódica como aditivo alimentar primário, com nível de inclusão recomendado de 24,6 mg por kg (base MS) (SILVESTRE & MILLEN, 2021).

### 2.3.2. **Modo de ação da monensina**

A monensina é um composto ionóforo de poliéter carboxílico que altera o movimento dos íons através das membranas microbianas do rúmen (ELLIS et al., 2012; DUFFIELD; MERRILL; BAGG, 2012). Através de um mecanismo transportador de membrana, a monensina inibe a hidrólise de ATP e a oxidação de substratos seletivos (RUSSEL & STROBEL, 1989), alterando consequentemente as proporções de ácidos graxos voláteis (RICHARDSON et al., 1976).

Essa alteração do movimento de íons inibe o crescimento das bactérias gram-positivas no rúmen, pois os ionóforos inserem canais iônicos na membrana plasmática da célula, permitindo que as moléculas penetrem na bicamada lipídica que protege a bactéria. Isso interrompe o gradiente de concentração de íons. Apesar das concentrações de K no meio extracelular serem maiores do que no interior celular (RUSSEL & STROBEL, 1989), a molécula de monensina provoca a formação do complexo monensina-catíon, resultando em um rápido efluxo de K+ para fora da célula, o que leva ao acúmulo de H+ dentro da célula e à rápida diminuição do pH intracelular (CALLAWAY et al., 1999; RUSSEL & STROBEL, 1989).

Como um mecanismo a célula se defende para tal situação e mantém os níveis de pH normal, exportando $H^+$ via bomba de prótons.

No entanto, esse mecanismo requer a utilização de ATP, resultando em um gasto de energia, porém sem potencial para aumentar o pH. Neste momento, o metabolismo celular provoca a expulsão de H+, permitindo o influxo de Na+, o que eventualmente leva à incapacidade do microrganismo se multiplicar e à degradação da célula, resultando em morte (RUSSEL & STROBEL, 1989) (Figura 3). Tal processo impacta diretamente o metabolismo energético, devido à competição entre bactérias gram-positivas e gram-negativas. Isso favorece as últimas, pois possuem uma camada lipídica externa na membrana celular que impede a penetração dos ionóforos (SCHELLING, 1984).

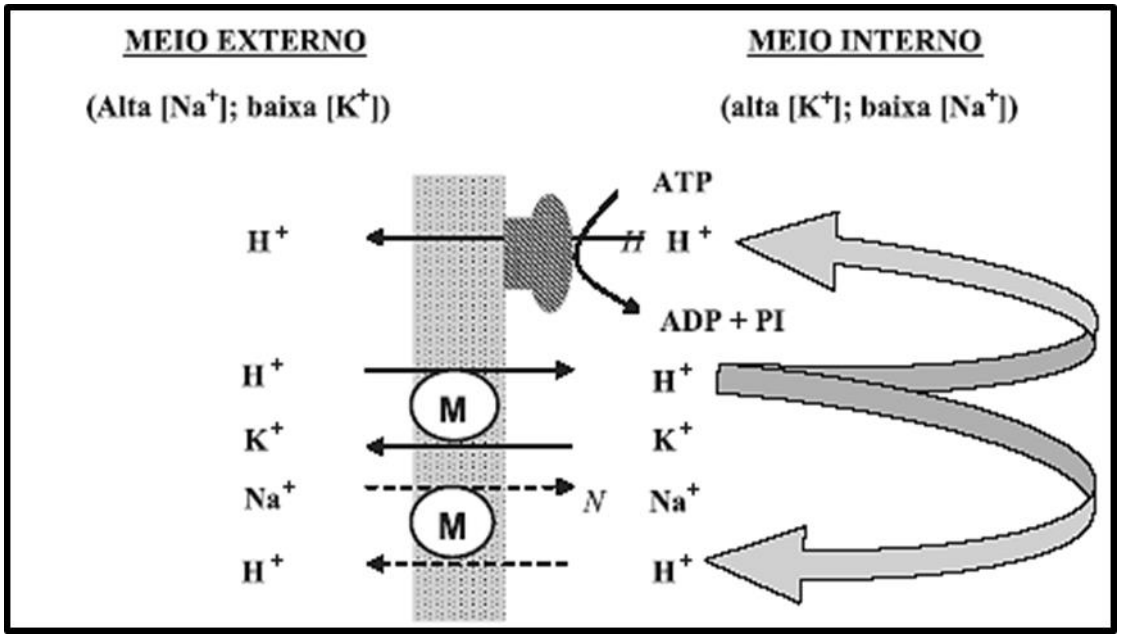


**Figura 3.** Diagrama esquemático mostrando os efeitos hipotéticos da monensina sobre o fluxo de íons em bactérias gram-positivas Streptococcus bovis (RUSSEL & STROBEL, 1989).

A diminuição das bactérias gram-positivas permite mudanças na proporção de AGVs produzidos, como de propionato e succinato em comparação com o acetato e lactato (BERGEN & BATES, 1984; RICHARDSON et al., 1976). Por outro lado, a produção de acetato leva à produção de compostos secundários, que são substratos para a metanogênese. O propionato é mais eficiente que o acetato, pois entra no ciclo do ácido tricarboxílico como succinil-CoA (4C) sem ter perdas de carbono por mol de

glicose, enquanto que o acetato entra no ciclo como acetil-CoA resultando em perdas de dois carbonos por mol de glicose.

### 2.3.3. **Impacto da monensina em dietas de confinamento**

Os efeitos verificados da monensina nas dietas com alta densidade energética e baixa inclusão de fibras consistem em mudanças na microbiota ruminal e nas rotas de fermentação. É importante destacar que 75 a 85% da energia dos alimentos é convertida em AGVs. A energia disponível a partir do metabolismo dos componentes dietéticos é utilizada pelo animal para funções fisiológicas, tais como crescimento, mantença e ganho, sendo o restante perdida na forma de metano e calor (JOHNSON & JOHNSON, 1995; WOLIN et al., 1997).

O NASEM (2016) sugere redução no consumo predito em 3% quando a monensina é utilizada na dieta. Esse conceito corrobora com evidências disponíveis na meta-análise realizada por Duffield et al. (2012), em que os autores avaliaram o uso de moensina em dietas de terminação disponíveis em 64 estudos. Os autores relataram que o uso da monensina diminuiu o CMS em 3,1%, aumentou o GMD em 2,5% e melhorou a eficiência alimentar em 1,3%. Consistente com esse resultado, CECONI et al. (2022) avaliando o uso da monensina, observaram uma diminuição de 4,3% no CMS e uma melhoria de 9,4% na eficiência alimentar quando foi utilizada uma combinação de monensina e virginiamicina. Erickson et al. (2003) relataram que o uso da monensina em dietas de alta densidade energética ajudou a controlar variações no CMS, a qual é a principal condição verificada em quadros de acidose subclínica. Esses resultados verificados em relação aos efeitos da monensina sobre o consumo, e consequente sobre o desempenho, podem estar relacionados à diminuição da degradação de proteínas e aminoácidos no rúmen (TEDESCHI et al., 2003). A monensina inibe o crescimento de baterias gram-positiva *Peptostreptococcu*s e *Clostridium*, as quais são produtoras de nitrogênio e amônio (YANG & RUSSELL, 1993), assim como de microrganismos que utilizam o amônio produzido como fonte de energia (RUSSELL & HOULIHAN, 2003). Assim, o NASEM (2016) sugere um aumento mais moderado de 2,3% no valor de energia metabolizável da dieta quando a monensina é fornecida. Consequentemente, maior fluxo de proteínas e aminoácidos passa para o intestino, sítio em que estes são mais eficientemente aproveitados pelo animal.

## 2.4. **Caraterização do óxido de magnésio**

O óxido de magnésio produzido a partir da magnesita deve ser aquecido (calcinado) para oxidar o carbonato de Mg ($MgCO_3$) em óxido de Mg (JITTAKHOT et al., 2004). Devido a sua alta biodisponibilidade, que varia de 28 a 50%, o óxido de magnésio é uma das fontes mais utilizada na alimentação animal (TSIPLAKOU et al., 2017; MARTENS et al., 2018). Porém, a sua disponibilidade depende de caraterísticas físicas e químicas como o tamanho da partícula (TSIPLAKOU et al., 2017), fonte de origem, temperatura de calcinação (magnesitas calcinadas a temperaturas abaixo de 650°C estão pouco disponíveis para ruminantes, enquanto que aquelas calcinadas a temperaturas iguais ou maiores que 800°C são altamente disponíveis (VAN RAVENSWAAY et al., 1992; TSIPLAKOU et al., 2017).

Estima-se que MgO de boa qualidade, para ser utilizado como suplemento dietético animal, deve ter biodisponibilidade próxima a 50% (MARTENS et al., 2018). Por isso, recomenda-se avaliar sua qualidade antes do uso prático. Nesse sentido, Goff et al. (2018), indicam que um teste simples seria colocar 3 g de uma fonte de MgO em um recipiente, adicionando lentamente 40 mL de ácido acético a 5% (vinagre branco). Posteriormente, os autores recomendam tampar o recipiente e agitar por 15 segundos, deixando posteriormente a mistura descansar. Posteriormente, a mistura deve ser novamente agitada por 15 minutos e o pH aferido aos 30 minutos. O vinagre sozinho tem um pH de 2,6 a 2,8. As melhores fontes de MgO elevarão o pH até 8,2 e as piores para apenas 3,8. No entanto, dada a variabilidade entre as fontes de óxido de magnésio disponíveis no mercado e o fato de que uma única fonte de óxido de magnésio pode não ser suficiente para induzir efeitos significativos no pH ruminal, torna-se imperativo empregar um processo industrial específico e escolher meticulosamente as fontes mais adequadas de óxido de magnésio (MARTENS et al., 2018).

### 2.4.1. **O óxido de magnésio como aditivo alcalinizante**

De acordo com STAPLES & LOUGH (1989) um agente alcalinizante é qualquer substância que em solução aquosa aumenta o pH da solução.

O principal alcalinizante utilizado na nutrição de ruminantes é o óxido de magnésio, que tem a capacidade de aumentar o pH ruminal e cecal (resultados

compilados publicados na Tabela 1), promovendo uma maior produção de AGCC na forma não protonada. Isso facilita a absorção dos ácidos graxos voláteis (AGVs), uma vez que um próton é removido juntamente com o AGV. Entretanto, como o pH do rúmen é normalmente maior que o pKa dos AGVs, a grande maioria dos AGVs se encontram na forma dissociada, necessitando de proteínas transportadoras. Nesse sentido, o possível benefício do uso de óxido de magnésio na absorção de AGVs dissociados é o seu maior aporte de cátions para a absorção através da parede ruminal do que os ânions dos AGV. Como descrito anteriormente, para manter o equilíbrio de cargas, é necessária uma maior absorção de cátions ou uma maior excreção de ânions para dentro do rúmen (GÄBEL et al., 2002; ASCHENBACH et al., 2009).

Para demonstrar os efeitos do óxido de magnésio sobre o pH ruminal e as concentrações de AGCC, exemplos relevantes podem ser os seguintes: ao incluir óxido de magnésio como alcalinizante, o pH está em 6,0, enquanto sem a presença de algum agente neutralizante, o pH ruminal está em torno de 5,6. Sabendo que o pKa é o -log da constante de acidez de determinado ácido, e que o valor para o ácido propiônico é de 4,88, logo, nesse pH, metade do ácido propiônico estaria na sua forma protonada e metade na sua forma não protonada (propionato). Utilizando a fórmula da equação de Henderson-Hasselbach, pH = pKa + log ([A-] / [HA]) temos que na primeira situação a concentração de ácido na sua forma não protonada é 13,2 vezes maior que da sua forma protonada, e na segunda situação essa relação cai para apenas 5,2 vezes.

Outra teoria que pode explicar a capacidade do produto de neutralizar os ácidos produzidos na fermentação ruminal é o aumento da taxa de passagem. De acordo com RUSSELL & CHOW (1993) os agentes neutralizantes possuem efeito na taxa de renovação da fase líquida, o que contribui para maior lavagem de pequenas partículas de amido, reduzindo a produção de ácidos orgânicos no rúmen. HARRISON et al. (1975) ao infundirem tampões no rúmen verificaram aumentos nas taxas de diluição da fase líquida, sendo acompanhado pelo aumento no fluxo de amido e diminuição na proporção de propionato de acetato no rúmen. Esses resultados são semelhantes aos indicados por HU & MURPHY (2005) que notaram também mudança na relação entre acetato e propionato. De forma consistente, em um estudo realizado por RAZZAGHI et al. (2021) os autores reportaram aumento da proporção de acetato para propionato ruminal com inclusão dietética de 8 g / kg de uma mistura tampão à base de magnésio e cálcio.

Os resultados mencionados acima evidenciam um efeito sobre a taxa de renovação da fase líquida, o que pode estar associado ao consumo de água, produção de saliva e a melhora nos ambientes ruminal e intestinal (RUSSELL & CHOW, 1993). Estudos realizados por ROGERS et al. (1979) e TEBBE et al. (2018) relataram um aumento do consumo de água, o que pode influenciar positivamente o CMS e aumentar a taxa de passagem da fase líquida, o que diminuiria a concentração ruminal de propionato e melhoraria a absorção de nutrientes no intestino. WHEELER & NOLLER (1977) defendem o poder da adição de agentes neutralizantes para criar um ambiente intestinal e um pH mais favorável para a digestão de amido no intestino delgado. O pH ideal de ação para a amilase pancreática é por volta de 6,9, enquanto para a maltase intestinal é de 5,8 (RUSSELL & CHOW, 1993). No entanto, o trabalho de OWEN et al. (1986) demostrou que não existe limitação na digestão de amido no intestino delgado quando são utilizados aditivos neutralizantes. Embora estudos demostrem o efeito da capacidade de neutralização de ácidos através do aumento da taxa de passagem, é preciso ressaltar que esse fluxo de amido do rúmen para o duodeno depende do tipo de processamento utilizado, tamanho de partícula e tipo de amido.

Dados recentes de um estudo que avaliou os efeitos da suplementação com um blend de óxido de magnésio, indicaram que seu uso promoveu melhorias na manutenção de um pH ruminal consistente sob um desafio nutricional em vacas leiteiras (BACH et al., 2022; BACH et al., 2018). Isto, por sua vez, estava ligado à prevenção da queda do consumo de MS e da produção de leite. Em outro estudo (Colombo et al., 2021), em que se avaliou a inclusão do mesmo blend de óxido de magnésio em diferentes níveis (0%, 0,25%, 0,50% ou 0,75% da dieta) para bovinos confinados alimentados com dieta de terminação à base de milho, foi verificado que a adição do blend de óxido de magnésio na dieta levou a um aumento linear no pH ruminal. Porém não foram observados efeitos sobre o desempenho animal. Nascimento et al., 2024, também relataram que o pH ruminal aumentou linearmente com o nível de inclusão do óxido de magnésio na dieta (0%, 0,25%, 0,50% da dieta) em touros canulados no rúmen e alimentados com dietas de alta energia. Assim, o impacto consistente do uso de óxido de magnésio no pH ruminal foi confirmado.

**Tabela 1**. Efeitos de óxido de magnésio sobre o pH ruminal e fecal.

| Categoria | Relação V:C | Dose % | pH ruminal | pH Fecal | Fonte |
|---|---|---|---|---|---|
| Vacas | 10:90 | 0.4 | 6.22 | ---- | EMERY et al. (1965) |
| Vacas | 40:60 | 0.8% | 6.46 | ---- | ERDMAN et al. (1982) |
| Novilhos | 15:85 | 0.5 | 6.3 | 6.8 | PEIRCE et al. (1983) |
| Vacas | 25:75 | 0.5 | 6.75 | 6.36 | THOMAS et al. (1984) |
| Vacas | 50:50 | 0.4, 0.8 | 6.23; 6.47 | 6.14; 6.34 | TEH et al., (1985) |
| Vacas | 40:60 | 0.4 | 6.38 | ----- | XIN et al., (1989) |
| Novilhas Angus | 20:80 | 0.50 | ---- | 7.18 | CHRISTIANSEN & WEBB, (1990) |
| Vacas | 34:66 | 0.4 | 5.93 | ---- | BACH et al. (2018) |
| Vacas | 40:60 | 0.4 | 5.79 | ------- | RAZZAGHI et al., (2021) |
| Novilhos | 6:94 | 0.25, 0.50, 0.75 | 5.1; 5.2; 5.4 | ----- | COLOMBO et al., (2022) |
| Vacas | 40:60 | 0.25 | 6.61 | 6.50 | BACH et al., (2023) |

## 2.5. Expressão de genes envolvidos no metabolismo lipídico

As características da carcaça, como a gordura intramuscular e o marmoreio, estão principalmente ligadas a fatores complexos como os ambientais, a alimentação e também estão sujeitas a alterações genéticas que, em conjunto com outros fatores, ocasionam mudanças fisiológicas (LADEIRA et al., 2018). Animais em confinamento recebem um grande enfoque para aumentar o teor de gordura na carcaça, tanto a gordura subcutânea quanto o marmoreio. De acordo com OWENS et al. (1995), o processo de deposição de gordura ocorre quando o consumo de energia líquida é superior às

exigências, principalmente após a maturidade, quando a taxa de crescimento muscular diminui e a taxa de deposição de tecido adiposo aumenta.

De acordo com LADEIRA et al. (2018), o conteúdo de gordura intramuscular é resultado da síntese, captação e degradação muscular, e os resultados da expressão gênica mostram que esses mecanismos atuam de forma semelhante. A síntese de gordura depende da incorporação de triglicerídeos aos adipócitos, que podem ser oriundos da absorção de ácidos graxos da dieta ou da síntese *de novo*. A síntese *de novo* depende do acetil-CoA, proveniente do acetato gerado na fermentação ruminal, ou da glicose oriunda da gliconeogênese (PETHICK et al., 2004).

A expressão de genes envolvidos no metabolismo lipídico é regulada principalmente por dois fatores de transcrição mestres, que são o Proliferador de Peroxissomas (*PPAR*) (LEMAY & HWANG, 2006) e os reguladores de esterol ligados a proteínas (*SREBP*) (LADEIRA et al., 2016; XU et al., 2001). O *PPAR*, possui três isoformas - α, γ e β. O *PPAR*-α, é um regulador do metabolismo lipídico e da homeostase energética, e está relacionado diretamente na oxidação de ácidos graxos via β-oxidação, estando relacionado a genes como a *carnitina palmitoiltransferase* 1A (*CPT1A*) e *carnitina palmitoiltransferase* 2 (*CPT2*) (BIONAZ et al., 2013). Por outro lado, o *PPAR*- γ é considerado um regulador transcricional chave de genes que controlam o metabolismo energético, adipogênese no músculo, manutenção do estado diferenciado e sensibilidade à insulina (ROSEN & MACDOUGALD, 2006). Por sua vez, o *PPAR-β*, menos estudado, tem função no catabolismo de ácidos graxos no músculo esquelético e regula a captação de glicose, assim como a proliferação de células satélites musculares e a regeneração muscular pós-natal (ANGIONE et al., 2011; OSORIO & MOISA, 2019).

Outro fator de transcrição importante é o *SREBP* que também está associado com a regulação de genes que promovem a lipogênese e adipogênese (XU et al., 2001; LADEIRA et al., 2018), atuando de forma especifica na síntese de ácidos graxos, principalmente os que codificam as enzimas acetil-CoA carboxilase (*ACC*) e ácido graxo sintetase (*FAS*). Assim como o *PPAR*, o *SREBP* possui três isoformas (1a, 1c e 2). O *SREBP-1c*, codificado pelo gene *SREBF1*, atua principalmente na regulação da expressão de genes lipogênicos nos animais, incluindo *ACACA*, *FASN* e *SCD1* (SHIMANO et al., 1997). Por outro lado, a isoforma *SREBP-2* possui mais influência nos genes colesterogênicos (EBERLÉ et al., 2004).

De maneira geral, independentemente do substrato utilizado (acetato ou glicose) a síntese de *novo* de ácidos graxos inicia-se pela presença de acetil-coA no citosol. A enzima acetil-CoA carboxilase catalisa a transformação da molécula de acetil-CoA em malonil-CoA. Imediatamente, um complexo multienzimático chamado de ácido graxo sintetase (codificado pelo gene *FASN*) adiciona moléculas de acetil-CoA à molécula de malonil-CoA sintetizada, em um processo de múltiplas reações em série. Isso resulta na formação de um ácido graxo saturado de cadeia longa, como o ácido palmítico (C16:0) (WARD et al., 2010; LADEIRA et al., 2016). Após a formação do ácido palmítico, sua estrutura pode sofrer alongamento ou instauração pela ação da enzima estearoil-CoA dessaturase (SCD1), codificada pelo gene *SCD1* (DUCKETT et al., 2009; LADEIRA et al., 2016).

No caso de dietas com elevadas concentrações de amido, que fornecem grandes quantidades de propionato e, portanto, de glicose, isso favorece a deposição de gordura intramuscular (SMITH et al., 1984). No entanto, elevados teores de carboidratos de rápida fermentação tornam os animais mais susceptíveis a distúrbios digestivos, principalmente a acidose ruminal, causando uma queda no pH ruminal. Assim, a redução do pH ruminal tem um impacto negativo na síntese de gordura no músculo, devido a alterações nas vias de biohidrogenação ruminal que interferem a expressão de genes lipogênicos no músculo esquelético (BAUMAN & GRIINARI, 2003; TEIXEIRA et al., 2017; LADEIRA et al., 2018).

Durante o processo normal de biohidrogenação, o ácido linoleico (C18:2, cis-9, cis-12) é primeiro isomerizado para a forma ácido linoleico conjugado (CLA) (C18:2, cis-9, trans-11). Esse ácido graxo é rapidamente hidrogenado, formando ácido vacênico (C18:1, trans-11), que, consequentemente, leva à formação de ácido esteárico (C18:0), em uma etapa que requer hidrogenação (BAUMAN & GRIINARI, 2003; QIU et al., 2004).

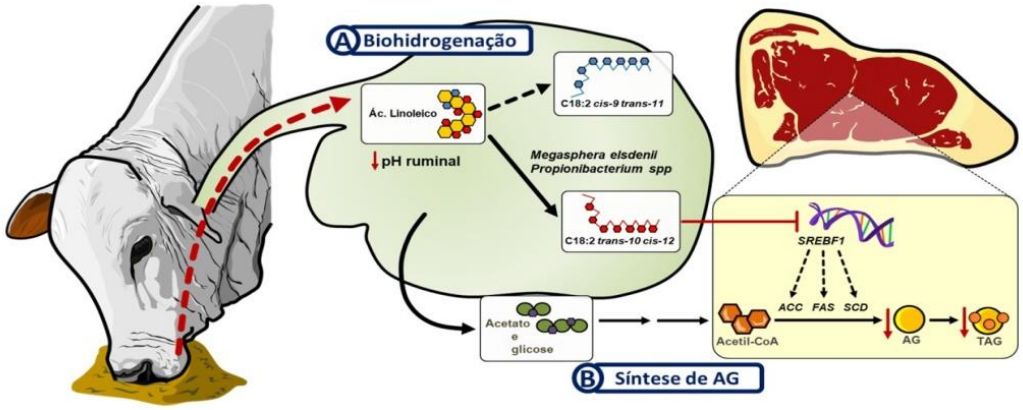


**Figura 4.** Efeito da dieta sobre pH ruminal e expressão de genes envolvidos no mestabolismo lipídico. Adaptado de Ladeira et al. (2018). Imagem elaborada por Germán Darío Ramírez Zamudio.

Embora o pH ruminal seja uma variável de extrema importância devido a seu efeito na biohidrogenação de ácidos graxos insaturados, ao se avaliar a efetividade dos aditivos como a monensina e um agente neutralizante em dietas de terminação, parece não existir um consenso na literatura. OLIVEIRA et al. (2014), relataram que a inclusão de monensina em dietas de terminação não mostrou efeito na expressão de genes lipogênicos, como *PPARA*, proteína de ligação ao elemento regulador de esterol (*SREBP*-1C), *ACACA* e *SCD* no músculo *longissimus thoracis* de touros jovens Red North. Em um estudo realizado por OGUNADE et al. (2018), que integrou análises metagenômicas e metabolômicas, os autores demonstraram um aumento da abundância de genes funcionais envolvidos no metabolismo lipídico e uma regulação positiva da via do metabolismo do ácido linoleico em novilhos alimentados com monensina. LADEIRA et al. (2014) avaliando o perfil de ácidos graxos e as características qualitativas da carne de tourinhos alimentados com grão de soja moído ou gordura ruminal protegida de óleo de soja, com ou sem monensina não observaram efeito no perfil de ácidos graxos ou nos aspectos qualitativos do músculo *longissimus dorsi*.

Infelizmente, há poucos trabalhos na literatura avaliando o uso de agentes neutralizantes em bovinos de corte alimentados com dietas de alta densidade energética. No entanto, um possível efeito do óxido de magnésio como agente neutralizante em dietas de terminação, caracterizadas pelo alto teor de amido e alta degradação ruminal, é o aumento do pH ruminal. Isso reduz o acúmulo e a absorção de AG intermediários da

biohidrogenação de lipídeos insaturados, principalmente C18:2, trans-10, cis-12, capaz de inibir a síntese *de novo*.

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## SEGUNDO CAPÍTULO – ARTIGO

# Effects of monensin and a blend of magnesium oxide on performance, feeding behavior, and rumen morphometrics of beef cattle fed high-starch diets

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Lay Summary: The high inclusion of rapidly fermenting carbohydrates, primarily starch, in feedlot diets can lead to an increase in rumen fermentation and the accumulation of organic acids. This can subsequently reduce rumen pH and alter the rumen microbiota, negatively impacting intake and rumination patterns, and consequently, animal performance. The objective of this investigation was to assess how a blend of magnesium oxide, in combination or not with monensin, influenced the productive, ruminal, and nutritional aspects of Nellore steers fed high-energy diets. This study confirmed that there is no substantial interaction between magnesium oxide and monensin in terms of their combined effects on the studied parameters. The results indicate that incorporating a blend of magnesium oxide yields positive outcomes in terms of feed intake, which could potentially be linked to an enhancement in the ruminal environment. This, in turn, translates to increased body weight and higher average daily gain. In addition, the inclusion of magnesium oxide in the diet did not have any impact on feeding behavior or papilla morphology. The monensin inclusion in the diet did not enhance the steers' performance or carcass merit. However, it did impact the feeding behavior and led to improved feed efficiency. Thus, this study highlights that magnesium oxide blend is a safe and interesting alternative to monensin to be used in feedlot energy-rich diets.

Teaser text: Employing a magnesium oxide blend for finishing beef cattle enhances feed intake, animal performance, and carcass characteristics, making it a promising substitute for ionophores additives.

**Abstract**

This study aimed to evaluate the effects of a blend of different sources of magnesium oxide associate or not with monensin, on productive, ruminal, and nutritional parameters and the expression of genes involved in the regulation of protein turnover in the *Longissimus dorsi* (LD) muscle of steers fed high-energy diets. Eighty-four Nellore steers with an initial body weight (BW) of 367.3 ± 37.9 kg were allocated to one of 28 pens, with three steers per pen. Each pen was considered an experimental unit, and using a completely randomized design with a 2 × 2 factorial arrangement, the following treatments were assigned to each pen: (1) Control (CON) – basal diet without additive inclusion; (2) Magnesium oxide blend (MG) – basal diet plus a magnesium based product (pHix-up®, Timab Magnesium, Dinard, France) provided at 0.50% of dry matter (DM); (3) Monensin (MON) – basal diet plus 25 mg/ kg of DM of sodium monensin (Rumensin, Elanco Animal Health, Greenfield, IN); and (4) MG association with MON – basal diet plus MG + MON, at the same doses of the individual treatments. The experimental period lasted 100 days. Blood samples were collected on days 0, 13, and 70 to determine D-lactate levels. Daily feed intake was recorded, and animal ingestive behavior was visually observed on days 66 and 67. On day 70, skeletal muscle tissue samples were obtained through biopsy for gene expression analysis. At the end of experimental period, carcass ultrasonography was conducted. Subsequently, the steers were slaughtered, and rumen epithelium samples were collected for morphometric analysis. Steers fed MG-containing diets consumed approximately 0.6 kg more dry matter per day than those fed diets without this additive ($P = 0.01$; 11.3 vs. 11.9 kg/day). The inclusion of MG in the diet increased ($P = 0.02$) the average daily gain (ADG) and final BW by ~9.4% and 2.7%, respectively. There was a greater *Longissimus* muscle area (LMA) and LMA per 100 kg of body weight (BW) ($P \leq 0.03$) for steers fed diets

with MG. Steers fed MON exhibited reduced mRNA expression of the Atrogin-1 and mTOR compared to steers fed diets containing MG + MON (MON × MG: $P \leq 0.04$). Steers fed MON had 6.9% higher feed efficiency ($P = 0.02$) and spent 11% more time per day on feeding activities ($P = 0.04$; 170 vs. 190 min/day). Papillae width was lower for CON compared to other treatments (MON × MG: $P = 0.02$). Magnesium oxide blend inclusion into concentrate-rich diets boosts feed intake and leads to improved feedlot performance and carcass characteristics, offering a potential strategy as an alternative to monensin.

Key-Words: alkalizing additives, antibiotic alternatives, ionophores, feedlot, magnesium.

**Abbreviations:**

ADG, average daily gain;

Atrogin-1, Atrogin 1;

BW, body weight;

DMI, dry matter intake;

eIf4E, eukaryotic translation initiation factor 4 E;

HWC, hot carcass weight;

G:F, feed efficiency;

GSK3B, glycogen synthase kinase 3β;

LMA, *longissimus* muscle area;

mTOR, Mammalian Target of Rapamycin;

MuRF1, muscle ring finger 1;

SBW, initial body weight obtained in fasting;

SFT, subcutaneous fat thickness;

p7056k, ribosomal protein S6 kinase;

RML, rump muscle length;

## Introduction

The inclusion of high starch content in feedlot diets has become an increasingly common practice aimed at maximizing growth performance and feed efficiency (Crawford et al., 2022). However, energy-dense diets achieved through high amounts of fast-fermenting carbohydrates can lead to the accumulation of organic acids, resulting in the development of digestive disorders (Boerman et al., 2015). Consequently, the depression of ruminal pH increases the risk of metabolic disorders, such as ruminal acidosis and bloating, which in turn amplifies the inflammatory response due to damage of the rumen epithelium and cecum, ultimately affecting animal productivity (Owens et al., 1998; Nagaraja and Lechtenberg, 2007). Therefore, the adoption of nutritional technologies is necessary to facilitate the utilization of high-energy diets, with the goal of optimizing nutrient utilization while concurrently diminishing the risk of metabolic disorders (Tedeschi et al., 2003; Bach et al., 2018).

Due to the multiple benefits of sodium monensin on rumen fermentation, animal performance, and feed efficiency (Duffield et al., 2012) this additive is extensively used in Brazilian feedlots (Silvestre and Millen, 2021), as well as in the United States (Samuelson et al., 2016). Nevertheless, public opinion stands opposed to the use of antibiotics in animal nutrition, driven by the precautionary principle. There has been a rise in antibiotic-resistant bacterial strains detected in animal-derived products, leading specific countries to take measures in response to this concern (Russell and Houlihan, 2003; Cameron and McAllister, 2016). Based on this, magnesium-derived products, like magnesium oxide, emerge as a viable and human-safe alternative to replace ionophores and other antibiotics commonly employed as growth promoters and pH modulators.

Nonetheless, given the variability among magnesium oxide sources and the fact that a single source of magnesium oxide may not suffice to induce significant effects on

ruminal pH, it becomes imperative to employ a specific industrial process and meticulously choose the most suitable sources of magnesium oxide in order to enhance its neutralizing effect of acids in the rumen. In light of this, the inclusion of a magnesium oxide blend in high-energy diets has been demonstrated to effectively mitigate reductions in ruminal pH in dairy cattle (Bach et al., 2018), resulting in favorable effects on dry matter intake and milk production. Similarly, in beef cattle fed corn-based finishing diets, the inclusion of a magnesium oxide mixture improved ruminal pH stability in a dose-dependent manner, but did not promote significant effects on PC, GMD or PQ (Colombo et al., 2022). Additionally, it tended to led to a reduction in the plasma concentration of haptoglobin, an acute phase protein used as a biomarker of systemic inflammation in cattle (Colombo et al., 2022). Nevertheless, it is still necessary to further investigate the potential effects of using magnesium oxide, either alone or with monensin, on Zebu animals, which constitute the primary genetic resource for animal production in tropical regions, such as Brazil. Thus, additional research is needed to conduct a comprehensive assessment of the potential impacts of magnesium oxide blend, whether associated or not with monensin in beef cattle.

Hence, the aim of this study was to assess the impact of a magnesium oxide blend associated or not with monensin on performance, feed intake, feeding behavior, carcass traits,rumen epithelium morphometry and the expression of genes involved in the regulation of protein turnover in the *Longissimus dorsi* (LD) muscle of Nellore males fed high-starch diets. Our hypothesis was that replacing sodium monensin with a blend of diverse magnesium oxide sources would maintain an appropriate ruminal pH and yield beneficial effects on the performance, feeding pattern, ruminal histological profile and upregulate the expression of of genes involved in protein synthesis and degradation of steers, providing a safe and promising alternative for future use in animal

nutrition.

## Materials and methods

### Animal welfare

This study was performed in the feedlot of the Beef Cattle Experimentation Unit of the Universidade Federal de Lavras (Lavras, Minas Gerais, Brazil). All the procedures and handling of animals described was conducted in accordance with the guidelines of the Ethics Committee for Animal Experimentation of the Universidade Federal de Lavras (Protocol n° 043/2019).

### Experimental management

Eighty-four immunocastrated Nellore steers with an initial body weight (BW) of 367.3 ± 37.9 kg were distributed into 28 pens (3 steers/pen), following a completely randomized design with a 2 × 2 factorial arrangement. There were seven pens per treatment, and each pen was considered an experimental unit. Each pen (4 m × 10 m) was equipped with an individual feeder and a collective water bin, which in turn was shared between two adjacent pens. The experimental period extended over a duration of 100 days. Prior the beginning of experimental period, steers were acclimated to the facilities. One day prior to the beginning of the experimental period, all animals received their initial immunization (Bopriva®, Pfizer Animal Health, Australia). The second immunization was administered 39 days after the first vaccination. Steers were also vaccinated against respiratory diseases (parainfluenza-3 virus and bovine respiratory syncytial virus), dewormed using ivermectin, and properly identified.

At the beginning of experimental period, the pens were randomly assigned to the following treatments: (1) Control (CON) – basal diet without additive inclusion; (2) Magnesium oxide blend (MG) – basal diet plus a magnesium based product (pHix-up®,

Timab Magnesium, Dinard, France) containing magnesium oxide (81%), calcium oxide (5.5%), and other natural components, which was provided at the level of 0.50% of dry matter (DM); (3) Monensin (MON) – basal diet plus 25 mg/ kg of DM of sodium monensin (Rumensin, Elanco Animal Health, Greenfield, IN); and (4) MG association with MON – basal diet plus MG + MON, at the same doses of the individual treatments. The basal diet consisted of corn silage, ground corn, rehydrated corn grain silage, soybean meal, urea, and a commercial mineral mixture (Table 1). The rehydrated corn silage was produced at the Universidade Federal de Lavras. Briefly, after grinding the dry corn grain until it reached a particle size of 5 mm, it was rehydrated or reconstituted with enough water to raise the moisture content of the dry grain to values around 28% to 35%. Bacterial inoculants (*Lactobacillus Buchneri*) were also introduced into the material. Afterwards, the silo was compacted and sealed for future use. The storage duration of the material before subsequent use was approximately 70 days after the ensiling process. The diet was formulated according to the Nutrient Requirements of Zebu and Crossbred Cattle – BR- CORTE 3.0 (Valadares Filho et al., 2016). Additionally, the animals were acclimated to the corn-based finishing diet over a period of 13 days, following a step-up protocol. From day 1 to 4, their diet consisted of 33.3% of the finishing diet. Subsequently, from day 5 to 8, their intake was raised to 55.6%, and from day 9 to 13, they were provided with 77.8% of the finishing diet. During the finishing phase, the diet was provided ad libitum with two daily feedings, at 0700 a.m. (supply of 60% of the daily amount of diet) and at 0400p.m. (supply of 40% of the daily amount of diet). The amount of diet offered was monitored to prevent the accumulation of leftovers exceeding 3%. Feed offered and refusals were weighed daily and sampled once a week for chemical analysis and adjustment of the dry matter intake (DMI). Samples were weighed, identified, and then dried at 55°C in a forced ventilation oven

for ~72 hours. Subsequently, composite samples were separated into three periods (13-39, 40-70, and 71-100 of feedlot days) and stored in a room temperature until analysis. Individual DMI was calculated based on the daily records of feed provided and the weight of refusals the following day, which was then divided among the three animals in each pen.

**Body weight**

The body weight of the steers was recorded at the beginning and end of the adaptation period, and at days 39, 70 and 100 of feedlot using a digital balance (Toledo, model MGR 3000, Brazil). Weighing was conducted with a 16-hour interval of water and feed restriction at the beginning of the adaptation period and at the end of experimental period (days 1 and 100 of the feedlot phase). The average daily gain of the steers for the entire period was determined by calculating the difference between the initial and final weights of the animals and then dividing that difference by the duration of the period.

At the end of the experimental period, ultrasonography was performed on the right side of each animal using an Aloka 500-V machine (Corometrics Medical Systems, Wallingford, CT) equipped with a 3.5-MHz, 17.2-cm linear array transducer. The following measurements were performed: *longissimus muscle* area (LMA, $cm^2$), subcutaneous fat thickness of the *longissimus muscle* (SFT, mm), rump muscle length (RML, cm), and rump fat thickness (cm). The LMA and SFT images were captured on the *longissimus thoracis* muscle between the 12th and 13th ribs. The RML and rump fat measurements were taken at the junction of the biceps femoris and gluteus medius, situated between the ischium and ileum, and aligned parallel to the vertebral column. The images were recorded on a portable computer and subsequently analyzed using the

Image J software (National Institutes of Health, Bethesda, Maryland, USA).

**Gene expression analysis in skeletal muscle**

On day 70 of the experiment, a skeletal muscle biopsy was conducted for gene expression analysis. Tissue samples were collected from one animal per pen. To perform the biopsy, the lumbar region was shaved, and a subcutaneous local anesthetic (lidocaine hydrochloride HCl, 20 mg/mL, total volume 6 mL) was administered. The biopsy site was disinfected with iodophor. Afterward, a one cm incision was made with a scalpel and a sterile Bergstrom biopsy needle was used to collect a sample (~1 g) of *longissimus thoracis* muscle tissue from the 13th rib (on the right side). Later, an antibiotic spray was administered to the incision site. Continuous monitoring of all steers was conducted for 48 hours following the biopsy. Immediately after each biopsy, the muscle samples were placed in cryovials and swiftly frozen them in liquid nitrogen. At the end of the biopsy procedure, samples were stored under -80ºC for subsequent gene expression analysis.

Total RNA was isolated from frozen muscle and liver tissue using QIAzol reagent (QIAGEN, Valencia, CA) and treated with DNA-free DNase (Invitrogen) following the manufacturer's protocols. To assess the integrity and quality of the RNA samples, electrophoresis was performed on 1% agarose gel (w/v) stained with GelRed Nucleic Acid Gel Stain (Biotium, Hayward, CA, USA), visualized using an E-Gel® Imager camera hood (Life Technologies, Neve Yamin, Israel). The quantity (ng/µL) and quality (measured by 260/280-nm and 260/230-nm optical density ratios) of the RNA were determined using a nanospectrophotometer (DeNovix DS-11, Wilmington, DE, USA). Subsequently, cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) in accordance

with the manufacturer's guidelines. Following this process, the samples were stored at -20 °C. For qPCR analysis, an Eppendorf Realplex thermocycler (Eppendorf, Hamburg, Germany) was employed with the SYBR Green detection system (Applied Biosystems, USA). Each gene under investigation was analyzed using distinct cDNAs (10 ng/μL) from a pool of 14 biological replicates, each with two technical replicates. The primer sequences (Table 2) were developed based on sequences available in the GenBank public database. Reference genes were tested, and the best individual gene or combination of endogenous controls was chosen using the web-based tool RefFinder. This tool selected the β-actin and and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the most reliable reference genes for gene expression analyses in bovine liver, (Coelho, 2018). The design process utilized the Oligo Perfect™ Design software (Primer Quest Tool). Subsequently, the primer sequences were analysed through Oligo Analyzer 3.1 and Premier Bosoft software. Once the primer sequences were designed, they were procured through commercial synthesis (Invitrogen, Carlsbad, CA, USA). The relative expression levels were determined using the method outlined by Pfaffl (2001), which takes into account the CT values adjusted for the amplification efficiency specific to each primer pair.

**D-lactate concentration in the blood**

Blood samples were collected 6 hours after the morning feeding on days 0, 13, and 70 of the experimental period through jugular vein puncture using Vacutainer tubes (Becton Dickinson, Franklin Lakes, USA) without anticoagulant for D-lactate determination. The samples were promptly placed on ice after collection, then centrifuged at 3000 × g for 20 minutes at 4°C. The collected plasma was subsequently frozen at -20°C until analysis. Plasma lactate concentration was determined using the D-Lactate Colorimetric Assay Kit (MAK058-1KT, Sigma-Aldrich Brasil Ltda, São Paulo,

Brazil) as per the manufacturer's instructions. Briefly, in this analysis, D-Lactate undergoes targeted oxidation via D-Lactate hydrogenase, resulting in the production of a corresponding colorimetric byproduct spectrophotometrically detected at 450 nm.

**Feeding behavior**

Visual observation of feeding behavior was performed on day 66 and 67 of the experimental period. The feeding behavior evaluation involved a continuous monitoring over a 48-hour period, spanning both day and night, across two consecutive days. This monitoring was conducted by human observers, with a minimum of five observers per hour. To assess the activities of individual animals, each observer was responsible for evaluating a specific group of animals. Care was taken to position observers in a way that did not disrupt the natural behavior of the animals they were observing. During nighttime observations, artificial lighting within the facility was deliberately turned off, and flashlights were used to aid in the assessment of feeding behavior. The following activities were documented for each animal: number of meals per day, the duration of time spent on eating, as well as the time dedicated to ruminating and periods of idleness, all measured in minutes. These activities were monitored at 5-minute intervals (throughout the entire 48-hour evaluation period). Afterward, the duration of each activity was calculated in terms of minutes per day by dividing the obtained value by 2880 (which corresponds to the total minutes in a span of two days). To determine the number of meals per day, the frequency with which each animal visited the trough for meals was monitored at each 5-minute interval. Meal size was timed and recorded for each animal. To determine the animal behavior parameters of the pen, the averages obtained from the 3 animals that made up the pen were used.

**Particle size and sorting**

Feed offered and orts samples were collected at days 0, 30, 45, 60 and 80 of feedlot phase to determine particle-size distribution. To achieve this objective, the Penn State Particle Size Separator was employed, which consisted of screens with apertures of 19 mm (long), 8 mm (medium), and 4 mm (short), along with a fine pan. Briefly, the samples were weighed, and subsequently, on a flat surface, the sieves containing the sample were shaken horizontally in one direction 5 times, with the sieves being rotated 1/4 turn after each series of 5 shakes. Later, the material removed from each of the sieves and from the bottom pan was weighed again to determine the proportion retained in each of them (Heinrichs and Kononoff, 1996), determining the percentage of long, medium, short, and fine particles for each sample.

Feed sorting was determined by assessing the real intake of each fraction of the Penn State Particle Separator in relation to the predicted consumption of that fraction, following the method outlined by Leonardi and Armentano (2003). The actual intake of each specific fraction was computed as the disparity between the quantity of that fraction in the provided diet and the quantity in the orts. In contrast, the expected intake for each individual fraction was computed by multiplying the intake of the total diet by the proportion of that fraction within the offered diet. Values reaching 1 indicate no sorting, values below 1 suggest selective refusals (sorting against), while values exceeding 1 imply preferential consumption (sorting for) (Devries et al., 2014).

**Fecal pH and starch**

Fresh fecal samples were collected in the morning (7:00 a.m.) using the spot technique from each animal at days 35 and 69 of the feedlot phase for the determination of fecal pH and starch concentration. Samples from the three animals in each pen were proportionally combined to create a daily composite sample for that pen. The fecal pH

was promptly determined. To achieve this, 50 g of feces was diluted in 100 mL of water and mixed thoroughly using a stirring rod. Subsequently, fecal pH was measured using a previously calibrated portable pH meter (model HI 208; SP Labor, São Paulo, Brazil). The remaining fecal content was stored at -20°C and later utilized for starch analyses.

**Chemical analysis of feedstuffs and fecal samples**

All chemical analyses were conducted at the Animal Nutrition Laboratory within the Department of Animal Science at the Universidade Federal de Lavras (UFLA). Prior to analysis, the feed ingredients, refusals, and fecal samples were submitted to a drying process in a forced-air oven at 65°C until a consistent weight was achieved. Subsequently, these samples were finely ground using 1 mm sieve through a Wiley mill (Wiley® TE- 680, Philadelphia, PA, USA) and then stored at ambient temperature. Moisture content (method No G-003/1), ash content (M-001/1), nitrogen content (CP; N-001/1), ether extract (EE; G-004/1), and ash- and protein- free neutral detergent fiber (NDFap; N- 002/1), were determined in accordance with the analytical protocols outlined by the Brazilian National Institute of Science and Technology (INCT-CA) guidelines (Detmann et al., 2012). Non-fibrous carbohydrates (NFC) were calculated using the formula proposed by Detmann and Valadares Filho (2010): NFC = [100 – (% CP + % NDFap + % EE + % ash)]. Starch analysis was also conducted following the recommended INCT-CA procedures (method No G-007/1). Measurements were taken using a UV/visible spectrophotometer set to 630 nm.

**Slaughter and carcass assessment**

At the end of experimental period, the steers were slaughtered at a commercial slaughterhouse located in Campo Belo (MG, Brazil), which was situated 57 km away from the experimental feedlot. All procedures conducted within the slaughterhouse

adhered to humane slaughter practices and complied with the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 1997). The animals were rendered unconscious using the captive bolt technique, and subsequently, they underwent the bleeding and dressing procedures. The hot carcass weight (HCW) was recorded after removing the head, hide, feet, and viscera. Following the HCW recording, the carcasses were stored in a refrigerated room at 4°C for 24 hours. The carcass yield was then calculated by dividing the HCW by the final body weight and multiplying the result by 100.

**Ruminal histological profile**

During the slaughter process, 1 $cm^2$ samples of rumen tissue (from 2 steers per pen) were collected from the cranial-ventral sac and immediately immersed in a buffered 10% formalin solution for 48 hours to assess papillae morphology. The rumen tissue samples were submitted to dehydration through a series of alcohol solutions with concentrations of 70%, 80%, 85%, 90%, 95%, and 100% ethanol, followed by clarification using two rounds of xylene. The tissue was then embedded in paraffin blocks and sliced into 3 μm thick sections using a rotary microtome (model MRP-09, Lupetec, Cidade, SP, Brazil). Three slides per sample were generated and promptly stained with hematoxylin and eosin, following the method described by Pluske et al. (1996). Histological images were captured at 4× magnification using a bright-field Olympus CX31 microscope equipped with an Olympus BX51 camera (Olympus Corp., Tokyo, Japan). Papillae derived from four images were analyzed utilizing ImageJ software (National Institutes of Health, Bethesda, Maryland, USA).

Sampling and measurements of the rumen papillae were consistently performed by the same observer. The length and width of papillae, as well as the thickness of

papillae epithelium and keratin thickness, were measured following the protocol by Lesmeister et al. (2004) with modifications suggested by Mirzaei-Alamouti et al. (2016) (Supplementary Figure 1).

**Statistical analysis**

The data were analyzed using a 2 × 2 factorial arrangement, considering the use of monensin, magnesium oxide blend, and their interactions as fixed effects. The pens were considered as a random effect. The following statistical model was employed:

$$Yij = \mu + Mi + Pj + (MP)ij + \varepsilon ij$$

Where:

$Yij$ = observed measurement

$\mu$ = overall mean

$Mi$ = fixed effect of monensin

$Pj$ = fixed effect of magnesium oxide blend

$MPij$ = interaction between the effects of M and P

$\varepsilon ij$ = the random error associated with Yij, following a normal distribution with mean 0 and variance $\sigma e2$, expressed as $\varepsilon ij \sim N(0, \sigma e2)$.

All measurements were subjected to analysis using PROC GLM (SAS Inst., Inc., Cary, NC). Initial shrunk body weight (SBW) served as a covariate for feedlot performance variables and carcass characteristics, while DMI was used as a covariate for plasma D-lactate concentration. Statistical significance was determined when $P \leq 0.05$, and trends were discussed when $0.05 < P \leq 0.10$. One steer from the CON treatment experienced muscle trauma two days before slaughter. As a result, all carcass

measurements for this animal at the slaughterhouse were excluded from the analyses.

## RESULTS

### Feedlot performance and carcass measurements

There was no MON × MG interaction for performance data, ultrasound measurements, or carcass traits ($P \geq 0.19$; Table 3). Animals fed diets containing MON had similar ($P \geq 0.21$) body weight, muscle measurements, fat deposition, and carcass yield compared to those that did not receive MON. The inclusion of MG in the diet increased the ADG ($P = 0.02$) by ~ 43% during the adaptation period and ~ 9.4% in the whole feedlot, while the final SBW were 13.5 kg greater for steers fed diets with the magnesium oxide blend ($P = 0.02$; 498 vs. 511.5 kg for steer fed diets without or with MG, respectively). The use of MG in finishing diets promoted approximately 10% and 7.5% additional LMA and LMA per 100 kg of BW ($P \leq 0.03$) compared to animals fed without this additive (Table 3). There was also a trend ($P = 0.09$) towards increased subcutaneous fat thickness with use of MG in the diet (2.94 vs. 3.55 mm for steer fed diets without or with MG, respectively). Despite the absence of effects of MG use in the diet on carcass yield ($P = 0.75$), there was a trend ($P = 0.08$) towards a ~7% higher hot carcass weight for animals receiving MG in their diet (Table 3).

### Gene expression in skeletal muscle

There was an interaction between MON and MG for the expression of the Atrogin-1 and mTOR genes ($P \leq 0.04$; Table 4). Animals receiving MON exhibited reduced mRNA expression of the Atrogin-1 and mTOR compared to steers fed diets containing MG + MON. No effects of MON or MG inclusion were observed on the other evaluated genes ($P \geq 0.11$).

### Feed intake, ingestive behavior and particle sorting

Steers fed MG diets ingested ~0.6 additional kg of DM per day than steers fed diets without this additive (P = 0.01; 11.3 vs. 11.9 kg/day for steer fed diets without or with MG, respectively; Table 5). Additionally, the inclusion of MG resulted in a ~3.7% higher DMI per kg of SBW (P = 0.03). On the other hand, effects of the use of MG in finishing diets on feed efficiency were only observed during the adaptation period (P < 0.001; Table 5), but not during the whole feedlot. The inclusion of MON in the diet did not affect DMI (P = 0.18), but it tended (P = 0.10) to decrease the DMI per kg of SBW (2.59 vs. 2.57 for steers fed diets without and with MON, respectively). In contrast, the inclusion of MON in the diet promoted approximately 6.9% higher feed efficiency compared to diets without MON (P = 0.02; Table 5) during the whole feedlot. There was no MON × MG interaction for any of the evaluated ingestive behavior parameters (P ≥ 0.09; Table 5). Additionally, no effect of including MG in the diet was observed on the animals' feeding behavior pattern (P ≥ 0.10). Animals fed diets containing MON spent 11% more time eating (P = 0.04; without MON = 170 vs. with MON = 190 min/day) than animals without MON in their diet. The time spent eating relative to DMI was also higher (P = 0.02) for animals receiving MON. There was a tendency for more meals per day (P = 0.09) and greater DMI per meal (P = 0.10) for steers fed MON (Table 5). Although no effects of MG use or interaction between MON × MG on particle sorting were observed (P ≥ 0.53), there was a greater selection for medium-sized particles (P = 0.05) and a tendency for greater selection for long particles (P = 0.08) in animals with MON inclusion in the diet.

**Concentration of starch in feces and fecal pH**

The inclusion of MON in finishing diets tended (P = 0.08; Table 6) to increase the starch concentration in feces by approximately 7.3%. Fecal pH at 35 and 69 days in the feedlot was not affected (P ≥ 0.09) by the studied nutritional plans (Table 6).

**Papillae Morphology**

There was a MON × MG interaction for papillae width (P = 0.02), in which CON animals had an unfavorable response compared to the other treatments that used additives in the diet (Table 7). Papillae surface area, papillae height, and keratinized layer thickness were not influenced by the treatments applied (P ≥ 0.41; Table 7).

**D-lactate concentration in the blood**

Steers from fed MG diets had greater concentration of D-lactate concentration (*P* = 0.001) after the adaptation period (day 13), and tended to increase by ~19.2% in D-lactate concentrations in the final period (Figure 1).

**DISCUSSION**

Consistent with our hypothesis, the use of the magnesium oxide blend was able to improve the average daily gain of steers fed high-energy diets, which resulted in a higher SBW at the end of the experimental period. Additionally, including MG in the diet increased the loin eye area of the steers and tended to improve hot carcass weight. Therefore, a consistent effect of its use on animal performance and on nutrient accretion, was observed. However, it is important to say that MgO was not enough to maintain the increase in efficiency caused by Monensin, which is its classic effect.

Such an effect may be associated with several factors. The first of them is that the use of MG increased dry matter intake. Therefore, the greater feed intake for steers fed magnesium oxide blend provided a greater supply of nutrients and energy for metabolic processes, including tissue synthesis, contributing to the increase in BW measurements. Additionally, it is important to highlight that magnesium acts as a significant intracellular cation, functioning as a vital cofactor in enzymatic reactions that are essential for all major metabolic pathways (Goff et al., 2018). It also plays a pivotal

role in fundamental homeostatic processes, including glycolysis and energy-driven membrane transport, cyclic-AMP synthesis, and genetic information transmission (NASEM, 2016). Therefore, considering the importance of magnesium in metabolic functions, this may have reflected in an improvement in overall organism functioning, consequently enhancing animal performance. Nevertheless, the improved performance observed in animals fed the magnesium oxide blend contrasts with the results reported by Colombo et al. (2022), where the inclusion of the same magnesium oxide blend in the diet did not promote any effects on BW, ADG, HCW or on LMA of beef heifers and steers compared to animals fed without MG. While these conflicting responses require further investigation, the differences in these outcomes may potentially be associated with factors such as animal category, breed characteristics, and the dietary composition employed in each of the studies.

Despite the higher intake observed in animals fed MG in their diet, no substantial effects on ingestive behavior were observed. Only a tendency towards greater DMI per meal was verified in animals fed diets with the MG inclusion. Although it is beyond the scope of this study to evaluate the ruminal pH values based on the treatments used, the increased feed intake observed in steers fed the MG blend may be associated with an improvement in the ruminal pH. Under a potential nutritional challenge scenario, such as under high-grain diets, there is an organic acids accumulation in the rumen, including volatile fatty acids (VFA) and lactic acid, which reflects an imbalance between microbial production, microbial utilization, and ruminal absorption of organic acids (Nagaraja and Titgemeyer, 2007). As a result, this situation can cause a decline in fiber digestibility, elevate the concentration of VFAs (particularly propionate), and induce alterations in rumen osmolality, ultimately resulting in a decreased feed intake (Allen, 2000). Previous studies with dairy (Bach et al., 2018) and

beef (Colombo et al., 2022) cattle demonstrated the magnesium oxide blend capacity to improve ruminal pH stability. Nevertheless, this condition did not necessarily enhance the feed intake. By the other hand, in a supplementary metabolism study performed by our lab (Nascimento et al., 2024 – unpublished data), the magnesium oxide blend was administered at varying doses (0, 2.5, or 5.0 g/kg of dry matter) to Nellore bulls following the same experimental diet as utilized in this study. It was observed that increasing MG supplementation levels in the diet had a linearly positive effect on ruminal pH, with the 5.0 g/kg of dry matter dosage (the same as tested in this trial) yielding the most favorable intake response, which is in line with the findings obtained.

The primary factors influencing the availability of magnesium are the potassium (K) concentration and the source of magnesium (Tebbe et al., 2018). In this sense, monensin is a feed supplement employed to enhance feed efficiency by depleting intracellular potassium in cells and selectively modifying the microbial population within the rumen (Bergen and Bates, 1984). Based on this, ionophores such as monensin have been proposed to substantially enhance $Mg^{2+}$ digestion, as evidenced by their ability to reduce ruminal $K^{+}$ concentrations, which in turn indicates a potential reduction in the inhibitory effect of $K^{+}$ on $Mg^{2+}$ transport (Martens et al., 2018). Nevertheless, this study confirmed that there is no substantial interaction between magnesium oxide and monensin in terms of their combined effects on the studied parameters for steer fed high-energy diets.

The inclusion of MON in the diet did not impact the body weight, ADG, nutrient accretion (ultrasonic carcass measurements) or carcass parameters. Additionally, although substantial effects on genes associated with protein turnover in skeletal muscle tissue have not been observed, was verified that steers fed diets containing MG + MON exhibited higher expression of the gene encoding *mammalian target of rapamycin*

(mTOR) compared to those fed exclusively with MON. The mTOR pathway is a highly regulated intracellular signaling cascade that is essential for the protein synthesis. It acts as a metabolic sensor, integrating several upstream signals related to nutritional status, nutrient availability, and cellular growth factors and stress (Lee et al., 2006). When stimulated, mTOR activates the phosphorylation of key targets to stimulate messenger RNA translation into proteins (Fonseca et al., 2014), resulting in increased protein synthesis. So, these findings demonstrate the presence of associative effects between the use of MON and MG at the molecular level, indicating potential unfavorable effects in terms of protein synthesis when animals are exclusively fed with MON compared to animals fed MG + MON. Additionally, steers fed MON showed lower mRNA expression of Atrogin-1, a gene associated with the protein degradation process, compared to those fed MON + MG. This observation suggests the existence of an adaptive molecular mechanism at play, potentially aimed at mitigating damage within the mTOR pathway. This, to some extent, could explain the lack of performance effects arising from the MON × MG interaction.

While no significant effects of MON use on animal DMI were detected, there was a trend indicating a reduction in DMI when expressed in kg of DM per kg of SBW in steers fed MON. Furthermore, steers fed MON had enhanced feed efficiency compared to those without MON in their diet. Monensin is well-known for its ability to reduce feed intake while simultaneously enhancing the feed efficiency. In a meta-analysis conducted by Duffield et al. (2012), based on data from studies involving growing and finishing beef cattle, it was verified that MON led to a decrease in DMI and an improvement in both ADG and feed efficiency. Monensin has the potential to enhance the efficiency of feed utilization by reshaping rumen fermentation processes, consequently augmenting the energy available for productive purposes. Monensin alters

the rumen fermentation dynamics by selectively inhibiting gram-positive bacteria. This alteration stimulates the proliferation of gram-negative bacteria, resulting in an elevated production of propionate in the rumen (Ogunade et al., 2018). Consequently, this heightened propionate production further amplifies hepatic gluconeogenesis. Monensin also reduces methane production by impeding the proliferation of bacteria responsible for providing substrates (hydrogen and formate) required for methanogenesis (Bergen and Bates, 1984). Therefore, this change in the ruminal environment leads to an improvement in the overall energy status of ruminant animals, which explains the favorable effect observed in feed efficiency due to the MON use.

Dietary additives have the capacity to modify feeding behavior, which can be associated with factors such as palatability, nutrient concentration, metabolic variables, pH levels, or osmolality in bodily fluids and organs (González et al., 2012). These changes can act as feedback signals that impact the onset and the end of meals through the central nervous system. In this sense, monensin is well-known to promotes an increase in propionate flow for gluconeogenesis in the liver, which, in turn, sends feedback signals to the brain, triggering behavioral mechanisms to end the meal (Allen et al., 2009). Consequently, it can induce a hypophagic effect, resulting in smaller meals and reduced daily feed intake (Allen et al., 2005). Therefore, the feeding behavior of monensin- supplemented animals was characterized by more frequent and smaller meals, consumed at a slower rate compared to animals fed diets without MON (Erickson et al., 2003). This condition led to more stable ruminal fermentation patterns throughout the day, reduced variability, and a smaller post-prandial drop in ruminal fluid pH in monensin-fed animals (González et al., 2012). In the present study, steers fed MON diets exhibited increased eating time compared to those without MON, spent more time eating relative to DMI and tended to have more daily meals and to consumed

larger amounts per meal. Hence, these results align only partially with the findings reported in the scientific literature regarding the use of MON on ingestive behaviour. Additionally, there was also a greater selection for medium-sized particles and a tendency for greater selection for long particles in animals with MON inclusion in the diet. Thus, this mechanism may have occurred as an alternative to avoid metabolic disturbances in an attempt to increase physically effective fiber intake, to stimulating the chewing activity, saliva flow, and ruminal pH (Rigueiro et al., 2023). Conversely, in a study examining the effects of MON, both alone and in combination with virginiamycin or zinc bacitracin on feedlot young bulls, Oliveira Junior et al. (2023) also found that animals fed with MON displayed a preference for consuming larger and medium particles (>19 and >8 mm, respectively).

The ruminal epithelium plays a pivotal role in the uptake of short-chain fatty acids (SCFA) via the layered squamous rumen lining. Hence, in response to a challenging nutritional scenario (e.g., diets high in concentrates), the papillae projecting from the rumen wall may enlarge to maximize the surface area for SCFA absorption (Steele et al., 2011). This phenomenon is primarily governed by the action of butyrate, which, in turn, promotes cellular proliferation while inhibiting apoptosis (Skata et al., 1978; Mentschel et al., 2001). In addition to butyrate, propionate also seems to act as a stimulator of papillary growth (Vair et al., 1960). Nonetheless, if the normal rumen fermentation process is disturbed, the SCFA and lactate accumulation within the rumen may result in damage to the rumen epithelium. In this context, an excessive increase in cellular proliferation and abnormal cellular differentiation may manifest within the stratified squamous epithelium of the rumen wall, resulting in the development of parakeratosis (Steele et al., 2012). Additionally, this impairment can compromise the protective role of the ruminal epithelium, potentially allowing the passage of pathogenic

606 substances from the rumen into the bloodstream (Monteiro and Faciola, 2020). In the
607 current study, no effects of the use of MG on papilla morphology were observed. This
608 suggests that this dietary additive did not induce significant histological changes that
609 could either enhance or impair the absorption of acids by the rumen epithelium. By the
610 other hand, steers fed MON exhibited increased papilla width compared to the CON
611 group, which may possibly be related to greater stimulation via propionate on ruminal
612 epithelium by MON. In line with this, Pereira et al. (2014) observed alterations in the
613 histology of rumen papillae in feedlot Nellore cattle when using MON. These responses
614 were dose-dependent, with doses of 9 or 36 ppm of MON resulting in improved
615 development of the rumen epithelium.

616 Elevated concentrations of D-lactate in the bloodstream serve as a critical
617 indicator of the severity of ruminal acidosis. In the ruminal environment, both L-lactate
618 and D-lactate are generated. When there is an abundance of rapidly fermenting
619 carbohydrates and a subsequent decrease in pH due to the accumulation of VFAs in the
620 rumen, this environment notably promotes the proliferation *Streptococcus bovis*, which
621 is recognized as a lactate-producing bacteria (L-lactate) (Valente et al., 2017). This
622 scenario further exacerbates the ruminal pH drop, facilitating the growth of pH-resistant
623 bacteria like *Lactobacillus* spp., which are efficient producers of D-lactate (Valente et
624 al., 2017). Therefore, when the pH drops sharply, there is an increase in D-lactate in the
625 blood, which characterizes a condition of metabolic acidosis. In this investigation,
626 animals that received MG as a dietary supplement exhibited higher levels of D-lactate in
627 their blood compared to those fed without MG during the adaptation period. However,
628 it's crucial to interpret these findings cautiously, as the observed average values do not
629 indicate the metabolic acidosis incidence (Grude et al., 1999). Drawing a parallel with
630 our metabolic study employing the same MG blend and diet for cannulated beef cattle

(Nascimento et al., 2024 – *unpublished data*), the ruminal pH ranged from 6.05 to 6.28 during experimental period. Thus, these pH values suggest that the dietary challenge applied might not have been excessively severe.

In addition, MON-fed steers had a greater starch concentration in their feces. This finding contradicted expectations, as previous research with steers indicated that monensin reduces starch digestion in the rumen while increasing starch digestion in the intestine (Muntifering et al., 1981). It is also plausible that the observed alterations in ingestive behavior, in response to potential changes in rumen acid-base balance, could significantly augment the outflow of starch to later segments of the gastrointestinal tract (Zinn et al., 1998; Swanson et al., 2002). Although a greater amount of starch was detected in the feces, no difference in fecal pH was observed, suggesting that fermentation conditions in the post-ruminal section remained unaltered (Owens et al., 1998; Erickson et al., 2003; González et al., 2012).

## Conclusions

It is concluded that the use of a magnesium oxide mixture in diets with a high concentrate content can be an alternative during the adaptation period, improving weight gain, dry matter consumption and food efficiency. Collectively, the results demonstrate that there are no substantial effects in terms of consumption, feed efficiency, ingestive behavior and papilla morphology due to the combined use of MON + MG.

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**Disclosures**

The authors declare no conflict of interest.

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862 **TABLES**

863 **Table 1.** Ingredients and chemical composition of experimental diets.

| Item | Treatments[1] |
|---|---|
| *Ingredients, % of DM* | |
| Corn shredlage | 23.0 |
| Silage of rehydrated corn grain | 10.9 |
| Ground corn | 52.1 |
| Soybean mean | 7.00 |
| Soybean hulls | 4.00 |
| Urea | 1.05 |
| Ammonium Sulfate | 0.11 |
| Salt (NaCl) | 0.35 |
| Calcareous | 0.70 |
| Dicalcium phosphate | 0.30 |
| Mineral nucleus[2] | 0.035 |
| Rumensin 200 (monensin) | 0.01 |
| pHix-up | 0,50 |
| Inert material | 0.51 |
| *Chemical composition, % of DM* | |
| Dry matter | 71.37 |
| Metabolizable energy[3], Mcal/kg DM | 2.81 |
| Net energy for maintenance, Mcal/kg | 1.87 |
| Net energy for gain, Mcal/kg | 1.23 |
| Total digestible nutrients | 77.8 |
| Neutral detergent fiber | 20.2 |
| Crude protein | 13.3 |
| Ether extract | 3.41 |
| Starch | 52.5 |
| Organic matter | 95.3 |

864 [1] Treatments: CON = no MON with 0% MgOB inclusion; MgOB = no MON with 0.50% MgOB
865 inclusion (pHix-Up, Timab, Dinard, France), MON = 0.0125% MON (Rumensin-200; Elanco Animal
866 Health) with 0% MgOB, MON×MgOB = 0.0125% MON with 0.50% MgOB, [2]Mineral nucleus: Inclusion
867 level = 0.1% of the concentrate (1 kg per ton). Assurance levels per kilogram of product: 1.9 g I; 0.56 g
868 Se; 1.50 g Co; 267.2 g Zn; 172 g Mn; 60 g Cu; 350 g Fe. [3] ME = TDN (g/kg DM) × 4.4 × 0.82 (Carvalho
869 et al., 2019).

870 **Table 2.** List of primers sets used to quantify mRNA expression of bovine genes by quantitative real-time PCR (RT-qPCR)

| Gene name | Gene abbreviation | Access number | Primer | Function |
|---|---|---|---|---|
| Muscle ring finger 1 | *MuRF1* | NM_001046155.1 | **F** GGACAGATGAGGAAGAGGA<br>**R** CCTCATCATCGCCTTACTGG | Protein degradation |
| Atrogin 1 | *Atrogin-1* | NM_001046155.1 | **F** CCTTGAAGACCAGCAAAACA<br>**R** AGACTTGCCGACTCTTTGGA | Protein degradation |
| Ribosomal protein S6 kinase | *p7056k* | AY396564.1 | **F** TTGAACCAAAAATCCGATCC<br>**R** AGCACCTCTTCCCCAGAAA | Protein synthesis |
| Glycogen synthase kinase 3β | *GSK3B* | NM_001101310.1 | **F** GCCCAGAACCACCTCCTTT<br>**R** TGCTGCCATCTTTGTCTCTG | Protein synthesis |
| Eukaryotic translation initiation factor 4 E | *eIf4E* | NM_174310 | **F** AAACCACCCCTACTCCGAAT<br>**R** TGCCCATCTGTTCTGTAAAGG | Protein synthesis |
| Mammalian Target of Rapamycin | *mTOR* | XM_015475105.1 | **F** GTCATGGAGGACACGGATTAG<br>**R** GGACCAGTGAGGTAATGAGATG | Protein synthesis |
| Insulin like growth factor 1 receptor | *IGFR1* | HQ703508.1 | **F** TGCGGTTCTGTTGATAGTGG<br>**R** TGGAGTGCTGTATGCCTCTG | Energetic metabolism |
| Actin beta | *β-actin* | NM_173979.3 | **F** GTCCACCTTCCAGCAGATGT<br>**R** CAGTCCGCCTAGAAGCATTT | Endogenous Control |

871 **Table 3.** Effects of monensin (MON) and magnesium oxide blend (MG) on
872 performance, nutrient accretion and carcass traits of steers fed high-energy diets.

| Item | Treatments[1] | | | | SEM | *P-value* | | |
|---|---|---|---|---|---|---|---|---|
| | CON | MON | MG | MON + MG | | MON | MG | MON × MG |
| *Feedlot Performance (adaptation)* | | | | | | | | |
| Initial SBW, *kg* | 365 | 367 | 370 | 367 | 14.9 | 0.86 | 0.98 | 0.85 |
| Final SBW, *kg* | 395 | 390 | 402 | 404 | 2.81 | <0.01 | 0.61 | 0.23 |
| ADG, *kg/day* | 1.64 | 1.36 | 2.07 | 2.19 | 0.15 | 0.59 | <0.01 | 0.20 |
| *Feedlot Performance* | | | | | | | | |
| Initial BW, *kg* | 365 | 367 | 370 | 367 | 14.9 | 0.86 | 0.98 | 0.85 |
| Initial SBW, *kg* | 349 | 347 | 351 | 349 | 13.4 | 0.87 | 0.92 | 0.96 |
| Final SBW, *kg* | 493 | 503 | 511 | 512 | 5.58 | 0.33 | 0.02 | 0.39 |
| ADG, *kg/day* | 1.43 | 1.54 | 1.62 | 1.63 | 0.05 | 0.32 | 0.02 | 0.38 |
| *Ultrasonic carcass measurements* | | | | | | | | |
| LMA, $cm^2$ | 79.6 | 86.3 | 91.4 | 91.2 | 2.69 | 0.24 | <0.01 | 0.21 |
| LMA/100 kg SBW, $cm^2$ | 16.2 | 17.1 | 17.9 | 17.9 | 0.53 | 0.42 | 0.03 | 0.38 |
| RML, *cm* | 9.87 | 9.48 | 9.92 | 10.03 | 0.185 | 0.45 | 0.11 | 0.19 |
| SFT, *mm* | 3.04 | 2.83 | 3.73 | 3.36 | 0.358 | 0.43 | 0.09 | 0.82 |
| Rump fat, *mm* | 4.91 | 5.19 | 4.87 | 5.64 | 0.479 | 0.27 | 0.68 | 0.62 |
| *Slaughterhouse carcass measurements* | | | | | | | | |
| HCW, *kg* | 270 | 276 | 278 | 282 | 3.83 | 0.21 | 0.08 | 0.95 |
| Carcass Yield, *%* | 54.9 | 54.8 | 54.3 | 55.2 | 0.40 | 0.36 | 0.75 | 0.23 |

873 [1] Treatments: CON = diets without additives; MON = basal diet plus 25 mg/ kg of DM of sodium
874 monensin (Rumensin, Elanco Animal Health, Greenfield, IN); MG = basal diet plus a magnesium oxide
875 blend product (pHix-up®, Timab Magnesium, Dinard, France) provided at 0.50% of dry matter; MON +
876 MG = basal diet plus MG + MON, at the same doses of the individual treatments.
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886 **Table 4.** Effects of monensin (MON) and magnesium oxide blend (MG) on relative
887 gene expression in the *longissimus thoracis* muscles of steers fed high-energy diets.

| Item | Treatment | | | | SEM | *P-value* | | |
|---|---|---|---|---|---|---|---|---|
| | CON | MON | MG | MON + MG | | MON | MG | MON × MG |
| *MURF1* | 1.00 | 0.76 | 0.88 | 0.83 | 0.13 | 0.32 | 0.86 | 0.48 |
| *Atrogin1* | 1.00[ab] | 0.67[b] | 0.91[ab] | 1.28[a] | 0.15 | 0.86 | 0.11 | 0.04 |
| *mTOR* | 1.00[ab] | 0.74[b] | 0.99[ab] | 1.04[a] | 0.05 | 0.11 | 0.03 | 0.03 |
| *P7056K* | 1.00 | 0.78 | 0.89 | 0.72 | 0.17 | 0.30 | 0.65 | 0.90 |
| *GSK3B* | 0.99 | 0.90 | 0.91 | 1.03 | 0.07 | 0.91 | 0.76 | 0.19 |
| *EIF4E* | 1.00 | 0.80 | 0.90 | 0.96 | 0.07 | 0.45 | 0.70 | 0.13 |
| *IGFR1* | 1.00 | 0.95 | 1.04 | 1.12 | 0.12 | 0.91 | 0.42 | 0.66 |

888 [a-b] Statistical differences between means are denoted by superscript letters.
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908 **Table 5.** Effects of monensin (MON) and magnesium oxide blend (MG) on feeding
909 intake and behavior and particle sorting of steers fed high-energy diets.

| Item | Treatments[1] | | | | SEM | *P-value* | | |
|---|---|---|---|---|---|---|---|---|
| | CON | MON | MG | MON + MG | | MON | MG | MON × MG |
| | *Feed intake (adaptation)* | | | | | | | |
| DMI, *kg/day* | 10.5 | 10.1 | 11.2 | 10.7 | 0.17 | <0.01 | <0.01 | 0.92 |
| DMI, *g of feed/kg of SBW* | 2.77 | 2.65 | 2.90 | 2.79 | 0.04 | <0.01 | <0.01 | 0.93 |
| Feed efficiency, *g/kg MS* | 0.156 | 0.136 | 0.186 | 0.207 | 1.46 | 0.99 | <0.01 | 0.17 |
| | *Feed intake* | | | | | | | |
| DMI, *kg/day* | 11.4 | 11.2 | 12.1 | 11.7 | 0.22 | 0.18 | 0.01 | 0.65 |
| DMI, *g of feed/kg of SBW* | 2.58 | 2.54 | 2.70 | 2.61 | 0.41 | 0.10 | 0.03 | 0.60 |
| Feed efficiency, *g/kg MS* | 0.127 | 0.139 | 0.134 | 0.140 | 0.36 | 0.02 | 0.20 | 0.43 |
| | *Feeding behavior* | | | | | | | |
| Time spent idleness, *min* | 931.97 | 865.51 | 915.93 | 927.21 | 22.29 | 0.22 | 0.31 | 0.09 |
| Time spent ruminating, *min* | 135.07 | 159.83 | 146.56 | 143.59 | 8.63 | 0.22 | 0.78 | 0.12 |
| Time spent eating, *min* | 168.89 | 195.05 | 171.59 | 184.06 | 9.13 | 0.04 | 0.66 | 0.47 |
| Meal length, *min* | 18.41 | 20.49 | 20.21 | 19.49 | 0.86 | 0.43 | 0.64 | 0.11 |
| Meals per day, *n/day* | 8.02 | 8.62 | 7.48 | 8.45 | 0.34 | 0.09 | 0.49 | 0.68 |
| DMI, *kg of DM* | 12.20 | 11.98 | 12.55 | 12.42 | 0.49 | 0.72 | 0.43 | 0.93 |
| DMI per meal, *kg* | 1.58 | 1.44 | 1.68 | 2.47 | 0.55 | 0.10 | 0.10 | 0.94 |
| Time spent eating/DMI, *min/kg of DM* | 12.94 | 15.65 | 13.26 | 14.74 | 0.76 | 0.01 | 0.69 | 0.42 |
| Time spent ruminating/DMI, *min/kg of DM* | 11.69 | 13.47 | 11.10 | 11.37 | 0.85 | 0.24 | 0.13 | 0.39 |
| | *Particle sorting, arbitrary units* | | | | | | | |
| Long | 1.027 | 1.033 | 1.026 | 1.033 | 0.001 | 0.08 | 0.86 | 0.86 |
| Medium | 1.024 | 1.029 | 1.022 | 1.030 | 0.001 | 0.05 | 0.83 | 0.53 |
| Short | 1.013 | 1.016 | 1.010 | 1.016 | 0.001 | 0.13 | 0.60 | 0.61 |
| Fine | 0.986 | 0.983 | 0.985 | 0.981 | 0.001 | 0.30 | 0.78 | 0.90 |

910 [1] Treatments: CON = diets without additives; MON = basal diet plus 25 mg/ kg of DM of sodium monensin
911 (Rumensin, Elanco Animal Health, Greenfield, IN); MG = basal diet plus a magnesium oxide blend product (pHix-
912 up®, Timab Magnesium, Dinard, France) provided at 0.50% of dry matter; MON + MG = basal diet plus MG +
913 MON, at the same doses of the individual treatments.
914 [a-b] Statistical differences between means are denoted by superscript letters.

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**Table 6.** Effects of monensin (MON) and magnesium oxide blend (MG) on starch concentration in feces and fecal pH of steers fed high-energy diets.

| Item | Treatments[1] | | | | SEM | *P-value* | | |
|---|---|---|---|---|---|---|---|---|
| | CON | MON | MG | MON + MG | | MON | MG | MON × MG |
| | | | | *Starch concentration* | | | | |
| Feces, *%* | 8.93 | 9.86 | 9.05 | 9.44 | 0.369 | 0.08 | 0.69 | 0.47 |
| | | | | *Fecal pH* | | | | |
| Day 35 of feedlot | 5.80 | 6.00 | 6.00 | 5.95 | 0.072 | 0.32 | 0.30 | 0.09 |
| Day 69 of feedlot | 5.83 | 5.98 | 5.96 | 5.94 | 0.068 | 0.34 | 0.51 | 0.24 |

[1] Treatments: CON = diets without additives; MON = basal diet plus 25 mg/ kg of DM of sodium monensin (Rumensin, Elanco Animal Health, Greenfield, IN); MG = basal diet plus a magnesium oxide blend product (pHix-up®, Timab Magnesium, Dinard, France) provided at 0.50% of dry matter; MON + MG = basal diet plus MG + MON, at the same doses of the individual treatments.

[a-b] Statistical differences between means are denoted by superscript letters.

**Table 7.** Effects of monensin (MON) and magnesium oxide blend (MG) on ruminal papilla morphometric measurements of steers fed high-energy diets.

| Item | Treatments[1] | | | | SEM | *P-value* | | |
|---|---|---|---|---|---|---|---|---|
| | CON | MON | MG | MON + MG | | MON | MG | MON × MG |
| Papillae surface area, $cm^2$ | 0.74 | 0.79 | 0.86 | 0.83 | 0.05 | 0.88 | 0.17 | 0.44 |
| Papillae Width, *mm* | 0.35[b] | 0.44[a] | 0.41[ab] | 0.41[ab] | 0.02 | 0.04 | 0.45 | 0.02 |
| Papillae height, *mm* | 1.68 | 1.89 | 1.93 | 1.89 | 0.16 | 0.63 | 0.46 | 0.44 |
| Keratinatized layer thickness, $\mu m$ | 60.50 | 59.34 | 58.17 | 61.23 | 2.44 | 0.71 | 0.93 | 0.41 |

[1] Treatments: CON = diets without additives; MON = basal diet plus 25 mg/ kg of DM of sodium monensin (Rumensin, Elanco Animal Health, Greenfield, IN); MG = basal diet plus a magnesium oxide blend product (pHix-up®, Timab Magnesium, Dinard, France) provided at 0.50% of dry matter; MON + MG = basal diet plus MG + MON, at the same doses of the individual treatments.

[a-b] Statistical differences between means are denoted by superscript letters.

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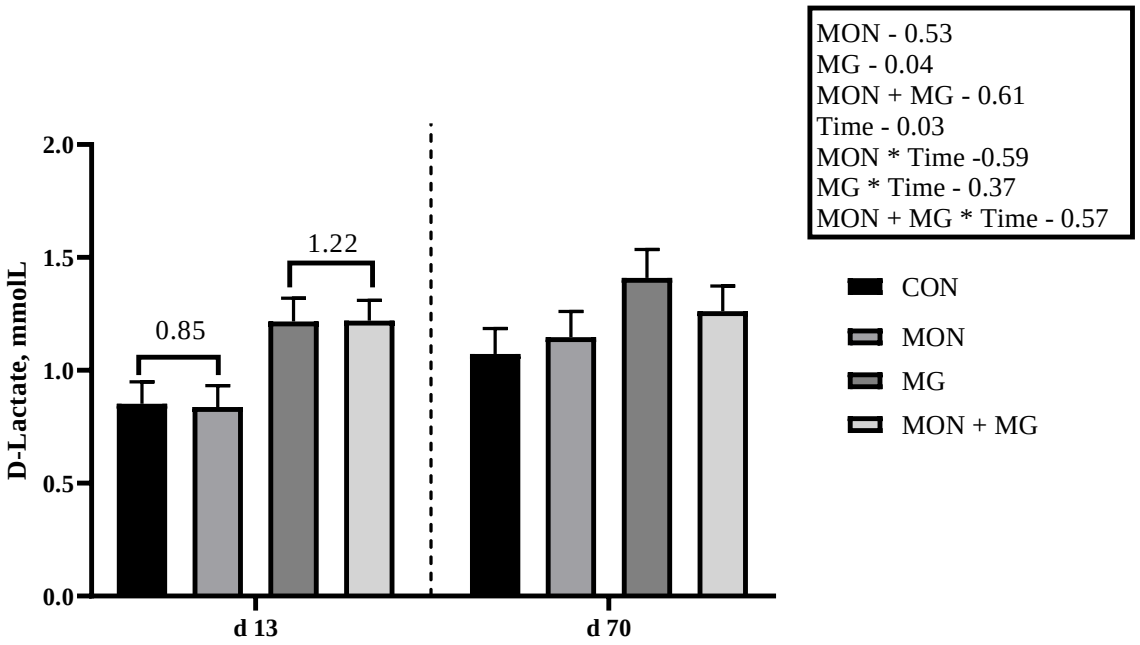


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973 **Figure 1.** Plasma D-lactate concentration in the adaptation (day 13) and finishing (day

974 70) periods of steers fed with high energy density diets using or not different ruminal

975 pH modulators.

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# TERCEIRO CAPÍTULO – ARTIGO

## Beef quality, fatty acid profile, liver gluconeogenesis, and lipid metabolism of Nellore steers fed a magnesium oxide blend combined or not with monensin

Manuscript formatted according to the guidelines of the Meat Science


## Abstract

This study evaluated the effects of a magnesium oxide blend and monensin, both independently and in combination, on beef quality, fatty acid profile, liver gluconeogenesis, and muscle lipid metabolism in feedlot steers. Eighty-four Nellore steers were distributed across 28 shared pens and randomly assigned to the following treatments ($n$ = 7 pens): CON – a basal diet without additives; MgO – basal diet + magnesium oxide blend (0,50% of dry matter); MON – basal diet + sodium monensin (25 mg/kg of dry matter); and MON+MG - basal diet + magnesium oxide blend + sodium monensin. No significant effects on the mRNA expressions of lipogenic markers in the skeletal muscle were observed among the diets ($P$ = 0.213). Steers fed MG increased hepatic expression of Propionyl-CoA carboxylase mRNA (P = 0.018); and the MON × MG interaction tended to increase pyruvate carboxylase mRNA expressions (P = 0.055). Beef chemical composition was similar across dietary additives ($P \geq 0.085$). The 14-day matured beef from the MON group had a higher Warner–Bratzler shear force than the CON and MON+MG groups ($P$ = 0.031). The pH and color of the beef were unaffected by treatments ($P \geq 0.101$). Steers fed MON exhibited lower C18:1 *cis* 9 and monounsaturated fatty acid content than CON ($P \leq 0.019$). The use of the MG blend did not alter the fatty acid profile of beef ($P \geq 0.105$). Overall, the magnesium oxide blend did not induce significant molecular changes in lipogenesis that would directly impact beef quality. Beef chemical composition remained consistent across all dietary additives, while beef from steers not fed monensin exhibited favorable characteristics such as lower levels of certain fatty acids, which may offer health advantages to consumers.

***Keywords*:** acidosis, conjugated linoleic acid, *de novo* synthesis, glucose, ionophore, ruminal biohydrogenation.

## 1. Introduction

Diets with a greater content of rapidly fermenting carbohydrates and low fiber inclusion can lead to digestive and metabolic upsets as a result of changes in the ruminal fermentation patterns (Nagaraja and Lechtenberg, 2007). In addition, rumen pH drop may negatively affect intramuscular fat (Ladeira et al. 2018). Thus, aiming to regulate the ruminal pH and explore safer alternatives for consumers in alternative to antibiotics frequently used in feedlot diets, as monensin, neutralizing agents such, as magnesium oxide, have emerged as an option.

The magnesium concentration in the ruminal fluid facilitates pH neutralization through an electroneutral mechanism (sustained by the $Mg^{2+/}$Cl- transporter), which is stimulated by the volatile fatty acid's availability in the rumen (Schweigel and Martens, 2003). Nevertheless, several factors (such as source, chemical composition, the precise calcining process of magnesium carbonate, and particle size) can affect the response to magnesium oxide (Beed, 2017). Thus, efforts have been made to combine different magnesium sources into a single product to achieve more effective results in ruminant diets.

Recent studies have consistently demonstrated the positive influence of incorporating the magnesium oxide blend on ruminal pH. This effect has been observed in both, dairy cows (Bach et al., 2018; Bach et al., 2023) and beef cattle on feedlot diets (Colombo et al., 2021; Nascimento et al., 2024; Bethancourt-Garcia et al., 2024), indicating improvements in maintaining a stable ruminal pH and animals performance. Nevertheless, despite the potential advantages associated with the utilization of

magnesium oxide blend, there remains a scarcity of information regarding its impact on beef quality and the expression of lipogenic markers in the muscle tissue of animals fed this dietary additive, mainly because its potential to affect rumen biohydrogenation pathways.

A decrease in rumen pH in grain-rich diets might affect the fat deposition and fatty acid profile of the meat, thereby affecting carcass quality (Oliveira et al., 2014; Teixeira et al., 2017; Teixeira et al., 2021). This phenomenon is substantiated by the ability of a drop in ruminal pH to alter polyunsaturated fatty acids biohydrogenation. Ruminal biohydrogenation represents a complex series of biochemical processes involving the transformation of linoleic acid into cis-9, trans-11 CLA (rumenic acid), which is subsequently hydrogenated to form *trans*-11 (vaccenic acid) before eventually becoming stearic acid (Harfoot and Hazlewood 1997; Kim et al., 2000). This intricate process relies on the participation of several bacterial species residing in the rumen, based on the specific products they generate through fermentation (Amin and Mao, 2023).

However, it's worth noting that at a low pH value, changes microbiota and the biohydrogenations pathways (Ladeira et al. 2016)., leading to an increased synthesis of conjugated linoleic acid C18:2 *trans*-10, *cis*-12 (Choi et al., 2005; Maxin et al., 2011). The increased availability of this intermediate influences the expression of genes that encode enzymes involved in lipid metabolism, which in turn, significantly affects the beef quality and its fatty acid profile (Ladeira et al., 2016, Teixeira et al., 2017).

Hence, we hypothesize that incorporating a blend of magnesium oxide sources into finishing diets may upregulate the expression of lipogenic genes and gluconeogenesis, thereby enhancing intramuscular fat and rumenic acid. Therefore, this study had the objective to evaluate the effects of a blend of magnesium oxide and

monensin on beef quality, fatty acid profile, liver gluconeogenesis, and muscle lipid metabolism in feedlot steers. Additionally, considering that many feedlots use a combination of ionophores and non-ionophores in finishing diets (Pinto and Millen, 2018), this study also aimed to investigate the potential effects of the combined use of the magnesium oxide blend with monensin on these parameters.

## 2. Material and methods

### *2.1 Experimental design, treatments, and dietary management*

The procedures detailed in this study were conducted in strict accordance with the guidelines set forth by the Ethics Committee for Animal Experimentation at the Universidade Federal de Lavras (Lavras, Minas Gerais, Brazil), under protocol number 043/2019.

Eighty-four Nellore steers [367.3 ± 37.89 kg of body weight (**BW**)], were accommodated in 28 shared pens in an experimental feedlot, with three steers per pen. The arrangement followed a fully randomized design, structured as a 2 × 2 factorial arrangement, involving four treatment groups (with seven pens per treatment). The animals were evaluated during 100 days of the feedlot phase. The experimental treatments were employed on the pens as follows: (**1**) Control (**CON**) – steers fed a basal diet without any dietary additive; (**2**) Magnesium oxide blend (**MG**) – the basal diet supplemented with a magnesium oxide blend (pHix-up®, Timab Magnesium, Dinard, France) provided at a level of 0.50% of dry matter (**DM**); (**3**) Monensin (**MON**) - the basal diet supplemented with 25 mg/kg of DM of sodium monensin (Rumensin, Elanco Animal Health, Greenfield, IN); and (**4**) MG in combination with MON (**MG + MON**) - the basal diet supplemented with MG + MON at the same doses as in the individual treatments (**Table 1**). The basal diet was formulated according to the Nutrient

Requirements of Zebu and Crossbred Cattle - BR-CORTE 3.0 (Valadares Filho et al., 2016).

All experimental details were previously described in our previous study (Bethancourt-Garcia et al., 2024). Briefly, the steers were gradually adapted to the corn-based finishing diet during a 13-day acclimation period, following a progressive adaptation regimen (days 1-4; days 5-8 and days 9-13 were provided 33.3%, 55.6% and 77.8% of the finishing diet, respectively). Throughout the finishing phase, the diet was offered *ad libitum* and distributed through two daily feedings (0700 am. and 0400 pm.). To prevent excess feed accumulation, the quantity of diet offered was carefully monitored to ensure that leftovers did not exceed 3%.

*2.2 Muscle and liver tissue collections*

Skeletal muscle and liver tissue samples were biopsied from one steer of each pen on day 70 of the experimental phase. Following trichotomy in the lumbar region and the area around the right $10^{th}$ intercostal space, local anesthesia (lidocaine hydrochloride HCl) in a total volume of 6 mL (4 and 2 mL in the muscle and liver sites, respectively) was administered subcutaneously. For skeletal muscle biopsy, a 1 cm incision was made between the $12^{th}$ and $13^{th}$ rib (on the right side), and using sterile Bergström-type needles (Stille, Ekbacken, Thorshälla, Sweden) a 1 g of *longissimus thoracis* muscle tissue was collected. In the liver biopsy, the incision was made at the intersection of the line from the mid humerus to the tuber coxae, and a Tru-Cut needle (ProMedical Equipamentos Médicos Ltd, Juiz de Fora, Minas Gerais, Brazil) was used to obtain approximately 30 mg of liver tissue. Following both procedures, the collected samples were stored in cryovials, labeled, frozen in liquid nitrogen, and subsequently stored at –80º C for subsequent gene expression analysis. Incision sites were cleaned,

sealed with veterinary tissue glue, and treated with an antibiotic spray. Rigorous monitoring of the steers' well-being was performed for 48 hours post-biopsy.

*2.2.1. Beef samples collection*

As the experimental phase concluded, the steers were transported to a commercial slaughterhouse (Campo Belo, Minas Gerais, Brazil). Stringent compliance with the regulatory framework outlined in the Sanitary and Industrial Inspection Regulation for Products of Animal Origin was consistently performed throughout the entire slaughter process. Upon reaching the slaughterhouse, the steers were submitted to the pre-slaughter handling steps. Anesthesia using the captive bolt technique was employed to render the animals insensible, followed by subsequent procedures for exsanguination and dressing. The carcasses were initially identified and then halved. Following this, the half carcasses were individually weighed and subsequently stored in a cold chamber maintained at 2°C for approximately 24 hours. After the 24-hour cooling period, a 15-centimeter segment of the *longissimus thoracis* muscle was excised from the right side of each carcass and transported to the Meat Quality Laboratory, situated within the Animal Science Department at Universidade Federal de Lavras. These samples were frozen for subsequent meat quality analysis.

In the next step, five steaks (each with a thickness of 2.54 centimeters) were taken from each 15-centimeter muscle segment to conduct analyses related to chemical composition, fatty acid profile, color, thawing loss, cooking loss, and Warner-Bratzler shear force (**WBSF**). Each steak was uniquely labeled and vacuum-sealed in nylon-polyethylene packaging. Additionally, meat samples were randomly assigned to two aging times (0- or 14-day post-mortem) at 1 ± 0.5°C for further analysis.

*2.3. Laboratory analyses*

*2.3.1. Gene expression*

In the skeletal muscle tissue, genes namely *Insulin-Like Growth Factor Receptor 1* (*IGFR1*), *Glucose Transporter* 4 (*GLUT4*); *ATP Citrate Lyase* (*ACLY*); *Acetyl-CoA Carboxylase Alpha* (*ACACA*); *Carnitine Palmitoyltransferase I Muscle Isoform* (*CPT1A*); *Peroxisome Proliferator-Activated Receptor Alpha* (*PPARA*); and *Sterol Regulatory Element-Binding Factor 1* (*SREBF1*) were elicited to be evaluated, due to their central role in the energy and lipid metabolism. We also examined genes in liver tissue that encode essential enzymes involved in the gluconeogenesis pathway. Specifically, was assessed the mRNA expression of *propionyl-CoA carboxylase* (*PCCA*), *pyruvate carboxylase* (*PC*), *phosphoenolpyruvate carboxykinase* 1 (*PEPCK1*), and Lactate Dehydrogenase A (*LDHA*).

Total RNA was isolated from frozen muscle and liver tissues utilizing QIAzol reagent (QIAGEN, Valencia, CA, USA) following the manufacturer's instructions, and subsequently treated with DNA-free DNase (Ambion, Austin, TX, USA). The RNA quantity (ng/μL) and quality (measured by 260/280-nm and 260/230-nm optical density ratios) were determined using a nano spectrophotometer (DeNovix DS-11, Wilmington, DE, USA). To assess sample integrity, electrophoresis was performed on 1% agarose gel (*m/v*), stained with GelRed nucleic acid gel stain (Biotium, Hayward, CA, USA), and visualized using a UVItec FireReader XS D-77Ls-20M (UVItec, Cambridge, UK). Following that, cDNA synthesis was conducted using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), in adherence to the manufacturer's instructions. The resultant samples were preserved at a -20°C. For reverse-transcription quantitative PCR (RT-qPCR), an Eppendorf Realplex thermocycler (Eppendorf, Hamburg, Germany) was employed in conjunction with the SYBR Green detection system (Applied Biosystems, Foster City, CA, USA).

The stability of reference genes was assessed using the RefFinder web-based tool, which selected β-actin and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the most stable reference genes for gene expression analyses in bovine muscles and liver, respectively. A validation assay was performed to demonstrate that the amplification efficiencies of the target and reference genes were approximately equivalent. A validation assay was performed to confirm that the amplification efficiencies of target and reference genes were approximately equivalent, and standard curves were constructed for the studied genes at dilutions of 1:5, 1:25, 1:125, 1:625, and 1:3125. The primer sequences were developed based on publicly available sequences from the GenBank database, provided by the U.S. National Center for Biotechnology Information (NCBI) (**Table 2**). This design process was done through the Oligo Perfect™ Design software (Primer Quest Tool). Subsequently, the primer sequences were assessed for their characteristics using Oligo Analyzer 3.1 and Premier Biosoft. Finally, the primers were commercially synthesized by Invitrogen (Invitrogen, Carlsbad, CA, USA). Relative expression levels were calculated following the method described by Pfaffl (2001).

*2.3.2. Chemical composition of beef*

The chemical analysis was performed following the methodology outlined by Ramírez-Zamudio et al. (2022). Briefly, for the chemical analysis of the beef, a steak of approximately 100 grams of non-aged samples of *longissimus thoracis* was used. The steak was submitted to a precise trimming process, eliminating subcutaneous and intermuscular fat, and was then meticulously diced into uniform portions. Subsequently, the samples were blended in a commercial blender until a homogeneous mass was obtained. Subsequently, these homogenized meat samples were subjected to a proximal analysis to assess their chemical composition. This analysis was performed using a

FoodScan™ device (AOAC method: 2007-04; FOSS, Hillerod, Denmark). The near-infrared transmission spectroscopy (NIT) was the analytical technique employed to ensure precision and reliability in the assessment of these meat constituents.

### *2.3.3. Color parameter and pH*

All steaks were submitted to a controlled thawing process within their original packaging, allowing for 24 hours at approximately 4°C. Following this, each sample was meticulously removed from its packaging and exposed to air for 30 minutes to facilitate myoglobin oxygenation (blooming time) (Tapp et al., 2011). To assess the color attributes at two distinct aging periods (0 and 14 days), data were collected by recording the color parameters in the CIELAB color space. These measurements were obtained through the average of five consecutive readings, employing a CM-700 spectrophotometric colorimeter (Konica Minolta Sensing Inc., Osaka, Japan). The colorimeter utilized an 8 mm aperture, illuminant A, and a 10° observer angle. The color attributes, including lightness (L*), redness (a*), and yellowness (b*), were subsequently derived from the readings captured in the SCE mode. In addition to color assessment, the pH of the samples was analyzed using a portable pH meter (Model HI 99163; Hanna, Woonsocket, RI, USA), previously calibrated. Three pH readings were conducted for each beef sample.

### *2.3.3. Thawing and cooking losses*

Thawing losses were determined considering the difference between the frozen and thawed weights of each steak, which were allowed to thaw at 4°C for 16 hours. These results were expressed as a percentage of the original frozen weight. Subsequently, the same steaks were grilled within the temperature range of 160-180 °C and were continuously monitored using a digital thermometer equipped with a K-type thermocouple (TD-880; ICEL, Manaus, AM, Brazil). Once each steak reached an

internal temperature of 71°C, ele foi cuidadosamente retirado da grelha e colocado em temperatura ambiente até que a temperatura se estabilizasse. Following this, the weight of the steaks was meticulously recorded following the AMSA (1995) guidelines. The cooking loss data were computed as the difference between the weights of the thawed and cooked steaks.

### *2.3.4. Warner–Bratzler shear force*

Shear force measurements were conducted post-cooking, following the WBSF method as outlined by Silva et al. (2015). For each steak, five rectangular core samples measuring 1.0 × 1.0 × 2.5 cm and aligned with the longitudinal orientation of the muscle fibers were extracted. These core samples were then subjected to shear force testing, perpendicular to the longitudinal orientation of the muscle fibers, being the force applied at a constant rate of 200 mm/min. This testing was performed using a Warner-Bratzler blade affixed to an XT plus texturometer (Stable Micro Systems Ltd., Godalming, Surrey, UK).

### *2.3.3. Fatty acids profile in the meat*

The initial stage in assessing the fatty acid profile of the beef samples involved the extraction of lipids from the meat. For this purpose, muscle lipids were extracted following the methodology outlined by Hara and Radin (1978). Following the successful extraction of lipids, the subsequent transformation into fatty acid methyl esters or other derivatives suitable for gas chromatography analysis was necessary. This step was performed under the methodology described by Christie (1982). Following transmethylation, the samples were subjected to analysis using a gas chromatograph, specifically the Focus CG-Finnigan model, equipped with a flame ionization detector. A capillary column of CP-Sil 88 (Varian) was utilized, with dimensions of 100 meters in length, 0.25 millimeters in internal diameter, and a film thickness of 0.20 millimeters.

Hydrogen served as the carrier gas, flowing at a rate of 1.8 mL/min. The initial oven temperature program was initiated at 70°C, with a hold time of 4 minutes, followed by a gradual increase to 175°C at a rate of 13°C per minute and a subsequent hold time of 27 minutes. This was succeeded by another temperature increase to 215°C at a rate of 4°C per minute, with a hold time of 9 minutes. Finally, the temperature was elevated by 7°C per minute until it reached 230°C, where it remained for an additional 5 minutes. In total, this temperature program spanned 65 minutes. The vaporizer temperature was set at 250°C, and the detector temperature was maintained at 300°C.

### *2.4. Statistical analysis*

The data analysis considered a 2 × 2 factorial arrangement, taking into account the utilization of monensin, magnesium oxide blend, and their potential interactions as fixed effects. The individual pens were considered as random effects in the analysis. Muscle pH, thawing loss, cooking loss, color parameters, and WBSF data were evaluated using the PROC MIXED procedure available in SAS 9.2 (SAS Inst. Inc., Cary, NC). For the gene expression data, fatty acid profile, and the chemical composition of the beef, the GLM (General Linear Models) procedure, was employed. Statistical significance was determined when $P \leq 0.05$, and trends were discussed when $0.05 < P \leq 0.10$.

## 3. Results

### *3.1. Body weight and dry matter intake*

There were no MON × MG interactions regarding animal performance and voluntary feed intake parameters ($P \geq 0.390$). The shrunk body weight (**SBW**) at the end of the experimental period was similar for steers fed with or without MON in their diet ($P$ = 0.330; **Table 3**). Conversely, animals with MG in their diet exhibited a final SBW ~ 2.7% higher ($P$ = 0.025) than animals fed without this additive in their diet.

298 Furthermore, the inclusion of MG in the diet resulted in an approximately 9.5% higher
299 average daily gain (**ADG**) compared to diets without the MG blend ($P$ = 0.025).
300 Additionally, DMI and DMI expressed in grams of feed per kilogram of SBW were
301 higher (P ≤ 0.035) for steers fed MG (**Table 3**). On the other hand, no effects of MG use
302 in finishing diets on feed efficiency were observed (P = 0.20; **Table 3**). In contrast, the
303 inclusion of MON in the diet promoted approximately higher feed efficiency compared
304 to diets without MON (P = 0.02; **Table 3**).

305 *3.2. Gene expression in skeletal muscle and liver*

306 No interactions between MON and MG were observed regarding the mRNA
307 expression for the analyzed genes in skeletal muscle ($P \geq 0.481$) (**Table 4**). Similarly,
308 there were no effects of MG or MON inclusion in the diet on these molecular markers
309 ($P \geq 0.213$).

310 Steers fed MG in their diet exhibited a higher ($P$ = 0.018) mRNA expression of
311 *PCCA* compared to steers-fed diets without this additive (**Table 4**). In addition, there
312 was a tendency of a MON × MG interaction for *PC* mRNA expressions ($P$ = 0.055),
313 with favorable responses observed for steers fed MON or MG in their diets compared to
314 CON or MON + MG. The mRNA expression of *PCK1* and *LDHA* was similar among
315 the evaluated treatments ($P \geq 0.283$).

316 *3.3. Chemical composition, color, pH, thawing losses, cooking losses, and*
317 *tenderness of the beef*

318 There was a tendency of MON × MG interaction ($P \geq 0.085$) concerning the
319 collagen content in the beef (**Table 5**), with greater content verified for the MON group.
320 The moisture, ash, protein, mineral matter, and ether extract concentrations in the meat
321 were similar ($P \geq 0.106$) between steers-fed diets with or without MG (**Table 5**).

There were no MON × MG interactions ($P \geq 0.111$) observed for thawing and cooking losses, pH or color and brightness parameters in the unaged (time 0) or 14-day matured beef (**Table 6**). In the unaged beef, a MON × MG interaction was observed ($P = 0.060$) for WBSF, where animals fed MON + MG had lower WBSF compared to animals exclusively fed MG (**Table 6**). In addition, there were tendencies towards higher thawing losses ($P = 0.091$) and cooking losses ($P = 0.102$) for animals with MG inclusion in the diet (**Table 6**) in the unaged beef. In 14-day matured beef, steers fed MON exhibited higher WBSF values compared to the CON and MON + MG groups (MON × MG: $P = 0.031$; **Table 6**). There was also a tendency ($P = 0.101$) towards an ~2.6% additional L* value for animals without the inclusion of MON in the diet.

*3.4. Fatty acids profile in the meat*

A MON × MG interaction was observed ($P \leq 0.019$) on the C18:1 *cis* 9 and monounsaturated fatty acid (**MUFA**) concentrations. Steers fed MON had lower C18:1 *cis* 9 and MUFA content than CON (Table 8). Steers-fed diets with MON exhibited a ~24% additional content of C18:1 *trans* 11 ($P = 0.006$) than those fed MON-free diets. There was a tendency towards lower C17:0 ($P = 0.101$) and greater C18:1 *trans* 10 ($P = 0.103$) for steers fed MON compared to those fed diets without MON. The use of the MG blend did not yield any noticeable effects on the beef fatty acid profile. There was only a trend ($P = 0.105$) toward lower C18:1 *trans* 11 for steers fed MG compared to those fed diets without MG inclusion.

## 4. Discussion

*4.1. Feed intake*

The positive impact on voluntary feed intake is likely justified by improvements in ruminal pH associated with using the magnesium oxide blend (Bach et al., 2023). While this study does not directly measure the effects of dietary additives on ruminal pH, available evidence consistently demonstrated the same magnesium oxide blend's effectiveness in controlling ruminal pH in both dairy (Bach et al., 2018; Bach et al., 2023) and beef cattle (Colombo et al., 2021) fed high-energy diets.

In a meta-analysis examining the utilization of monensin in beef cattle diets (Duffield et al., 2012), it was observed that the inclusion of this additive led to enhanced performance and reduced voluntary feed intake. The performance improvement may be attributed to increased propionate supply to the liver due to the monensin use, while the reduction in feed intake could be justified by its potential effects on diet palatability, ingestive behavior, and due to its metabolic feedback effect on the central nervous system (Tseu et al., 2020). However, in the present study, no significant effects associated with the use of monensin on animal performance and intake parameters were detected.

*4.2. Gene expression in skeletal muscle*

In terms of meat quality, the initial hypothesis posited in this study was that incorporating a magnesium oxide blend into the diet, by positively influencing ruminal pH control, could prevent adverse effects on the expression of genes encoding enzymes and transcription factors related to lipid metabolism in skeletal muscle, improving intramuscular fat deposition, as evidenced in prior studies (Oliveira et al., 2014; Teixeira et al., 2017). However, despite the positive effects on ruminal pH documented in scientific literature resulting from the magnesium oxide blend (Bach et al., 2018; Bach et al., 2023; Colombo et al., 2021), it did not demonstrate enhanced expression of genes associated with lipogenesis under the experimental conditions employed. In line with these findings, the results of the proximate composition of beef indicated no effects on ether extract content due to using the magnesium oxide blend, supporting that intramuscular fat deposition was unaffected by its use. These results align with the findings reported by Colombo et al. (2021) who utilized the same magnesium oxide blend. In their study, no significant effects on marbling and yield grade were observed.

Consequently, these combined outcomes indicate that, overall, the magnesium oxide blend does not contribute to enhancements in the carcass merit of feedlot cattle.

Given the potential of monensin to decrease the unsaturated fatty acids biohydrogenation (Van Nevel and Demeyer, 1995), its use might have the potential to influence the expression of genes involved in muscle tissue lipogenesis. Nevertheless, the utilization of monensin also did not exhibit any effects on the expression of genes associated with lipogenesis in muscle tissue. These findings are consistent with those reported by Oliveira et al. (2014), where the inclusion of monensin in finishing diets showed no effect on the expression of lipogenic genes, such as *PPARA*, *SREBF1*, *ACACA*, and *SCD1* in the *longissimus thoracis* muscle of Red North young bulls.

In addition, elevated glucose levels typically stimulate the production of insulin and IGF-1, which, in turn, promote the movement of GLUT-4 from an intracellular compartment to the plasma membrane, enhancing glucose transport (Huang and Czech, 2007; Siddle, 2011; Wang et al., 2012). Although monensin is widely recognized for its role in promoting increased propionate production, available evidence shows that it does not necessarily lead to substantial changes in blood glucose concentrations (Maanen et al., 1978; Johnson et al., 1986). Therefore, this justifies the lack of effects on the mRNA expression of *IFGR1* and *GLUT-4* observed in this study.

### *4.3. Gene expression in liver*

Concerning gene expression in the liver, the glucose demands of ruminants are predominantly satisfied by the *de novo* synthesis of glucose through gluconeogenesis (Aschenbach et al., 2010). Propionate is the primary substrate for gluconeogenesis in ruminants, although glucose can also be derived from lactate, glycerol, and some amino acids (García-Roche et al., 2021). In the present study, steers-fed magnesium oxide blend had a greater *PCCA* mRNA expression compared to those fed diets without this

399 additive. This gene encodes the enzyme propionyl-CoA carboxylase, which catalyzes
400 the carboxylation of propionyl-CoA to methylmalonyl-CoA (Wongkittichote et al.,
401 2017). Subsequently, methylmalonyl-CoA is converted into succinyl-CoA, which is
402 then integrated into the tricarboxylic acid cycle to generate oxaloacetate, which in turn,
403 plays a crucial role in the gluconeogenesis pathway (Wongkittichote et al., 2017). As
404 propionyl-CoA carboxylase requires magnesium as a cofactor (Chourpiliadis and
405 Mohiuddin, 2019), this response can be attributed to the enhanced availability of
406 magnesium through supplementation. These findings suggest that incorporating a
407 magnesium oxide blend into diets with higher rapidly fermentable carbohydrate levels
408 (i.e., with higher glucogenic capacity) stimulates the propionate entry into the
409 gluconeogenesis pathway (Fassah et al., 2018), which could potentially result in a
410 greater glucose pool available for productive purposes, such as fat deposition. However,
411 despite this favorable response in the *PCCA* expression, as mentioned earlier, there were
412 no improvements in intramuscular fat deposition resulting from the use of the
413 magnesium oxide blend.

414 There was a trend towards an increased *PC* gene expression (responsible for
415 encoding the enzyme pyruvate carboxylase) in steers fed MON or MG in their diet, in
416 comparison to those fed CON or MON + MG. This suggests a potential increase in the
417 oxaloacetate pool, leading to the subsequent activation of the gluconeogenesis pathway
418 (Fassah et al., 2018) when MON or MG is included in the finishing diet.

419 On the other hand, the evaluated dietary additives demonstrated no impact on the
420 *PEPCK1* mRNA expressions. Therefore, this response indicates that the use of
421 monensin, magnesium oxide, or their combined association in steers fed high-energy
422 diets does not induce effects at the molecular level in the initial and rate-limiting step of

hepatic gluconeogenesis, where oxaloacetate is converted into phosphoenolpyruvate (Yu et al., 2021).

Lastly, there were no effects of using magnesium oxide, monensin, or their combined application on the *LDHA* expressions. In studies involving beef cattle fed feedlot diets (Oliveira-Junior et al., 2023), higher *LDHA* gene expression was linked to increased availability of D-lactate for use as a gluconeogenic substrate in the liver. The *LDHA* gene encodes lactate dehydrogenase, the enzyme involved in converting lactate to pyruvate (Fassah et al., 2018).

### *4.4. Chemical composition, color, pH, thawing losses, cooking losses, and tenderness of the beef*

In general, the use of the magnesium oxide blend or monensin did not affect the chemical composition of the meat. The absence of effects from the applied treatments on the protein content of the meat was expected. Literature indicates that protein values in meat remain relatively constant (around 19 to 25% protein), with minimal impact from dietary factors (Ladeira et al., 2014). Despite the magnesium oxide blend favoring ruminal pH conditions, the lack of impact on the ether extract content in beef suggests that this additive does not induce significant changes in the biohydrogenation pathway. Consequently, it did not sufficiently influence the expression of lipogenic markers, mainly *PPAR* and *SREBF1* transcription factors that would lead to a substantial effect in intramuscular fat deposition.

The ether extract content in beef also showed no variation with the inclusion of monensin in the diet, despite its well-documented potential to enhance propionate flux for glucose production - a key substrate for intramuscular adipocyte fat synthesis (Gilbert et al., 2003). These results are in line with several studies that reported no effects of monensin on carcass traits (Pendlum et al., 1978; Ericksson et al., 2003;

448 Ladeira et al., 2014; Xu et al., 2014). A tendency towards higher collagen content in
449 beef was verified for steers fed monensin compared to the other treatments. This
450 increase in collagen content, explains the greater WBSF observed for the MON group
451 compared to other treatments after a 14-day maturation period, as connective tissue is
452 recognized as an important determinant of the shear force deformation curve (Girard et
453 al., 2012). Hence, these findings suggest that incorporating monensin into the diet may
454 potentially diminish beef tenderness, as a greater force is required to slice cooked meat.
455 Moura et al. (2017) also reported a greater WBSF in the *Semimembranosus* muscle of
456 lambs due to monensin use. Nevertheless, it is important to highlight that the disparities
457 in shear force values verified in our study could still be deemed negligent, given that the
458 threshold for tender meat is regarded as 53 N (Silva et al., 2015), and all treatments
459 exhibited shear force values below this threshold by the 14th day of aging.

460 There was a trend toward increased loss during thawing and cooking for steers fed
461 magnesium oxide blend at time zero. The supplementation of magnesium enhances
462 water retention capacity. Thus, during the freezing process, the formation of ice crystals
463 occurs, leading to the rupture of cell membranes and resulting in greater cell leakage
464 and subsequent loss during defrosting (Constantino et al., 2014), which explains the
465 observed response to thawing and cooking losses.

466 The utilization of the dietary additives showed no discernible impact on the beef
467 color. Alterations in the final pH of beef can modify the negative charges and structure
468 of the muscle matrix, thereby influencing its color (Ramanathan et al., 2020). Thus, the
469 absence of treatment effects on the beef color may be attributed to the comparable final
470 pH values observed across all treatments. Consistently, Apple et al. (2000), evaluating
471 color measurements for the *triceps brachii*, *longissimus thoracis*, *semimembranosus*,
472 and *semitendinosus* muscles, did not find any effects of the inclusion of magnesium

oxide on CIE L*, a*, and b* values in lambs. For instance, Ladeira et al. (2014) also did not verify the effects of monensin inclusion in the feedlot diets on the color parameters of beef.

### *4.5. Fatty acids profile in meat*

An aspect associated with the quality of beef is its fatty acid profile, which holds significance in ensuring oxidative stability during the cooking process, thereby influencing taste (Wood et al., 2008). Overall, the use of a magnesium oxide blend did not exert an influence on the fatty acid profile of the beef in this study. Hence, these findings suggest that the dosage of magnesium oxide applied in this study was insufficient to significantly modify the biohydrogenation pathway or *de novo* synthesis of fatty acids, which in turn triggers fatty acids composition (Santora et al., 2000; Buttrey et al., 2012).

Ionophores, like monensin, may influence the fatty acid profile in both meat (Ladeira et al., 2014) and milk (Silva-Kazama et al., 2007). In a study integrating metagenomics and metabolomics analysis (Ogunade et al., 2018) it was demonstrated an increased abundance of functional genes involved in lipid metabolism and an upregulation of the linoleic acid metabolism pathway in steers fed monensin. These findings indicated inhibition of the growth of gram-positive bacteria, especially of *Butyrivibrio proteoclasticus*, which in turn impairs the final step of the ruminal biohydrogenation, leading to a lowered C18:0 production and an accumulation of C18:2 *cis* 9-*trans* 11 (Ogunade et al., 2018). Nevertheless, a significant portion of the identified C18:2 *cis* 9-*trans* 11 in meat is a result of the desaturation of vaccenic acid within the muscle facilitated by the enzyme Δ9-desaturase (Xu et al., 2014). Thus, the contribution of C18:2 *cis* 9-*trans* 11 synthesized during ruminal biohydrogenation is relatively minor when considering the overall presence of C18:2 *cis* 9-*trans* 11 in the

beef (Xu et al., 2014). However, in this study it was observed that the concentration of oleic acid increased with trans-10 and trans-11. Therefore, it is clear that monensin reduced the final steps of bioHydrogenation.

A MON × MG interaction was detected for oleic acid (C18:1 *cis* 9) and the sum of monounsaturated fatty acids in the beef, with higher concentrations observed in the beef from the CON group compared to animals in the MON group. Oleic acid, the primary monounsaturated fatty acid in beef, can undergo isomerization, leading to the formation of several C18:1 trans isomers, including vaccenic acid, the precursor of CLA (Dannenberger et al., 2004). Scientific literature highlights the positive impact of oleic acid on human health, acting as an anticarcinogenic agent by suppressing the expression of the Her-2/neu gene associated with breast cancer (Menendez et al., 2005). Additionally, C18:1 cis 9 plays a role as a hypocholesterolemic agent (Schwingshackl and Hoffmann, 2012

## Conclusions

The incorporation of a magnesium oxide mixture into the diet does not positively or negatively influence meat quality when compared to diets without additives or that received monensin. However, the use of magnesium oxide mixture in energy-rich diets has been shown to increase the pool of glucose available for productive functions at the molecular level.

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## Disclosures

The authors declare no conflict of interest.

## Declaration of generative AI in scientific writing

We affirm that artificial intelligence (AI) tools were solely employed to enhance the readability and language of this article.

## Author contributions

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795 **Table 1.** Composition of experimental diets and constituent components.

| Item | Treatments[1] |
|---|---|
| *Ingredients, % of DM* | |
| Corn shredlage | 23.0 |
| Silage of rehydrated corn grain | 10.9 |
| Ground corn | 52.1 |
| Soybean mean | 7.00 |
| Soybean hulls | 4.00 |
| Urea | 1.05 |
| Ammonium Sulfate | 0.11 |
| Salt (NaCl) | 0.35 |
| Calcareous | 0.70 |
| Dicalcium phosphate | 0.30 |
| Mineral nucleus[1] | 0.035 |
| Monensin | 0.01 |
| Magnesium oxide blend | 0,50 |
| Inert material | 0.51 |
| *Chemical composition* | |
| Dry matter, *% of DM* | 71.37 |
| Metabolizable energy, *Mcal/kg DM* | 2.81 |
| Net energy for maintenance, *Mcal/kg* | 1.87 |
| Net energy for gain, *Mcal/kg* | 1.23 |
| Total digestible nutrients, *% of DM* | 77.8 |
| Neutral detergent fiber, *% of DM* | 20.0 |
| Crude protein, *% of DM* | 13.3 |
| Ether extract, *% of DM* | 3.41 |
| Starch, *% of DM* | 52.5 |
| Organic matter, *% of DM* | 95.3 |

796 [1] Mineral nucleus: Inclusion level = 0.1% of the concentrate (1 kg per ton). Assurance levels per kilogram
797 of product: 1.9 g I; 0.56 g Se; 1.50 g Co; 267.2 g Zn; 172 g Mn; 60 g Cu; 350 g Fe.
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804 **Table 2.** List of primer sets used to quantify mRNA expression of bovine genes by quantitative real-time PCR (RT-qPCR)

| Gene name | Gene abbreviation | Access number | Primer | Function | Efficiency |
|---|---|---|---|---|---|
| | | | *Skeletal Muscle Tissue* | | |
| Insulin-Like Growth Factor Receptor 1 | *IGFR1* | HQ703508.1 | **F** TGCGGTTCTGTTGATAGTGG<br>**R** TGGAGTGCTGTATGCCTCTG | Energy metabolism | 100 |
| **Glucose Transporter 4** | *GLUT4* | | | Glucose uptake | |
| Acetyl-CoA Carboxylase Alpha | *ACACA* | NM_174224.2 | **F** TGAAGAAGCAATGGATGAACACA<br>**R** TTCAGACACGGAGCCAATAA | Lipogenesis | 96.6 |
| Peroxisome Proliferator-Activated Receptor Alpha | *PPARA* | NM_001034036.1 | **F** CAATGGAGATGGTGGACACA<br>**R** TTGTAGGAAGTCTGCCGAGAG | Fatty acid metabolism and mitochondrial biogenesis | 99 |
| Sterol Regulatory Element-Binding Protein 1 | *SREBF1* | NM_001113302.1 | **F** GAGCCACACACTTCAACGAA<br>**R** TGTCTTCTATGTCGGTCAGCA | Lipogenesis | 94.6 |
| **ATP Citrate Lyase** | *ACLY* | | | Lipogenesis | |
| | | | *Liver* | | |
| Propionyl-CoA carboxylase | *PCCA* | XM_024999953.1 | **F** TTTGGTTTGCCGTCTGTTGG<br>**R** TTGAATGCCGCTGTCAACTC | Gluconeogenesis | 97.9 |
| Pyruvate carboxylase | *PC* | NM_177946 | **F** GAGGTGGTCCGCAAGATG<br>**R** TCGTGCAGGGAAGTGATG | Gluconeogenesis | 99.5 |
| Phosphoenolpyruvate carboxykinase 1 | *PCK1* | NM_174737.2 | **F** GGGCTGATCGAAACCCTTAAT<br>**R** TTTCCTGGAGCCTGCTATTTC | Gluconeogenesis | 98 |
| Lactate Dehydrogenase A | *LDHA* | NM_174099.2 | **F** TCCAACATGGCAGCCTTTTC<br>**R** ACGCTGGACCAAATTCAGAC | Gluconeogenesis | 99.2 |

**Table 3.** Effects of monensin (MON) and magnesium oxide blend (MG) on performance, and feed intake of steers fed high-energy diets.

| Item | Treatments[1] | | | | SEM | *P-value* | | |
|---|---|---|---|---|---|---|---|---|
| | CON | MON | MG | MON + MG | | MON | MG | MON × MG |
| *Feedlot Performance* | | | | | | | | |
| Initial BW, *kg* | 365 | 367 | 370 | 367 | 14.9 | 0.865 | 0.988 | 0.852 |
| Initial SBW, *kg* | 349 | 347 | 351 | 349 | 13.4 | 0.878 | 0.922 | 0.969 |
| Final SBW, *kg* | 493 | 503 | 511 | 512 | 5.58 | 0.330 | 0.025 | 0.390 |
| ADG, *kg/day* | 1.43 | 1.54 | 1.62 | 1.63 | 0.05 | 0.322 | 0.025 | 0.386 |
| *Feed intake* | | | | | | | | |
| DMI, *kg/day* | 11.4 | 11.2 | 12.1 | 11.7 | 0.22 | 0.187 | 0.012 | 0.659 |
| DMI, *g of feed/kg of SBW* | 2.58 | 2.54 | 2.70 | 2.61 | 0.41 | 0.101 | 0.035 | 0.604 |

[1] Treatment Groups: CON represents diets without additives. MON involves the basal diet supplemented with 25 mg/kg of DM sodium monensin (Rumensin, Elanco Animal Health, Greenfield, IN). MG consists of the basal diet supplemented with a magnesium oxide blend product (pHix-up®, Timab Magnesium, Dinard, France) provided at 0.50% of dry matter. MON + MG combines the basal diet with MG and MON, using the same doses as the individual treatments.

**Table 4.** Effects of monensin (MON) and magnesium oxide blend (MG) on gene relative expression in the skeletal muscle and liver tissues of steers fed high-energy diets.

| Item | Treatment[1] | | | | SEM | *P-value* | | |
|---|---|---|---|---|---|---|---|---|
| | CON | MON | MG | MON + MG | | MON | MG | MON × MG |
| | | | | *Skeletal Muscle Tissue* | | | | |
| *IGFR1* | 1.00 | 0.95 | 1.04 | 1.12 | 0.12 | 0.917 | 0.422 | 0.663 |
| *GLUT4* | 1.00 | 1.02 | 0.80 | 1.00 | 0.21 | 0.617 | 0.619 | 0.702 |
| *ACLY* | 1.00 | 1.11 | 1.27 | 1.15 | 0.16 | 0.997 | 0.352 | 0.482 |
| *ACACA* | 1.00 | 1.15 | 1.25 | 1.50 | 0.22 | 0.396 | 0.213 | 0.828 |
| *CPT1A* | 1.00 | 1.00 | 1.20 | 1.21 | 0.18 | 0.952 | 0.278 | 0.984 |
| *PPARA* | 1.00 | 1.23 | 0.93 | 1.44 | 0.29 | 0.235 | 0.797 | 0.639 |
| *SREBF1* | 1.00 | 0.98 | 0.84 | 1.15 | 0.22 | 0.518 | 0.984 | 0.481 |
| | | | | *Liver* | | | | |
| *PCCA* | 1.00 | 1.29 | 1.66 | 1.40 | 0.13 | 0.882 | 0.018 | 0.069 |
| *PC* | 1.00 | 1.45 | 1.38 | 1.05 | 0.17 | 0.720 | 0.974 | 0.055 |
| *PCK1* | 1.00 | 0.87 | 1.14 | 1.28 | 0.35 | 0.634 | 0.283 | 0.769 |
| *LDHA* | 1.00 | 0.97 | 0.75 | 1.09 | 0.14 | 0.313 | 0.663 | 0.257 |

[1] Treatment Groups: CON represents diets without additives. MON involves the basal diet supplemented with 25 mg/kg of DM sodium monensin (Rumensin, Elanco Animal Health, Greenfield, IN). MG consists of the basal diet supplemented with a magnesium oxide blend product (pHix-up®, Timab Magnesium, Dinard, France) provided at 0.50% of dry matter. MON + MG combines the basal diet with MG and MON, using the same doses as the individual treatments.

**Table 5.** Effects of monensin (MON) and magnesium oxide blend (MG) on the chemical composition of beef (%) of steers fed high-energy diets.

| Item | Treatment[1] | | | | SEM | *P-value* | | |
|---|---|---|---|---|---|---|---|---|
| | CON | MON | MG | MON + MG | | MON | MG | MON × MG |
| Moisture | 72.23 | 72.08 | 72.24 | 72.00 | 0.166 | 0.25 | 0.841 | 0.815 |
| Ash | 3.17 | 3.28 | 3.24 | 3.37 | 0.105 | 0.32 | 0.727 | 0.739 |
| Protein | 22.06 | 21.96 | 22.16 | 21.91 | 0.139 | 0.208 | 0.859 | 0.568 |
| Ether extract | 2.59 | 2.55 | 2.54 | 2.73 | 0.169 | 0.669 | 0.676 | 0.499 |
| Collagen | 1.46 | 1.57 | 1.47 | 1.41 | 0.048 | 0.607 | 0.106 | 0.085 |

[1] Treatments: CON = diets without additives; MON = basal diet plus 25 mg/ kg of DM of sodium monensin (Rumensin, Elanco Animal Health, Greenfield, IN); MG = basal diet plus a magnesium oxide blend product (pHix-up®, Timab Magnesium, Dinard, France) provided at 0.50% of dry matter; MON + MG = basal diet plus MG + MON, at the same doses of the individual treatments.

**Table 6.** Effects of monensin (MON) and magnesium oxide blend (MG) on thawing and cooking losses, pH, color parameters, and tenderness of beef from steers fed high-energy diets.

| Item | Treatment[1] | | | | SEM | *P-value* | | |
|---|---|---|---|---|---|---|---|---|
| | CON | MON | MG | MON + MG | | MON | MG | MON × MG |
| | | | | *Aging time (0 days)* | | | | |
| Thawing losses, *%* | 8,25 | 8,12 | 8,91 | 9,59 | 0.609 | 0.650 | 0.091 | 0.508 |
| Cooking losses, *%* | 21.9 | 22.1 | 24.0 | 23.8 | 1.07 | 0.978 | 0.102 | 0.851 |
| pH | 5.61 | 5.62 | 5.61 | [5.62] | 0.013 | 0.387 | 0.908 | 0.775 |
| Ligthness (L*) | 39.7 | 39.1 | 36.4 | 39.2 | 0.600 | 0.512 | 0.847 | 0.691 |
| Redness (a*) | 12.7 | 12.4 | 12.7 | 12.3 | 0.326 | 0.280 | 0.907 | 0.822 |
| Yellowness (b*) | 12.2 | 11.9 | 12.4 | 11.7 | 0.349 | 0.153 | 0.950 | 0.492 |
| Chroma (C*) | 17.7 | 17.3 | 17.8 | 17.0 | 0.412 | 0.151 | 0.941 | 0.576 |
| Hue angle (h*) | 43.9 | 43.8 | 44.3 | 43.5 | 0.786 | 0.523 | 0.945 | 0.647 |
| WBSF, *N* | 66.39 | 69.63 | 73.26 | 63.55 | 0.336 | 0.339 | 0.900 | 0.060 |
| | | | | *Aging time (14 days)* | | | | |
| Thawing losses, % | 9.11 | 8.43 | 8.89 | 8.83 | 0.632 | 0.558 | 0.895 | 0.628 |
| Cooking losses, *%* | 22.4 | 22.4 | 21.3 | 20.4 | 0.968 | 0.692 | 0.119 | 0.665 |
| pH | 5.52 | 5.54 | 5.54 | 5.55 | 0.023 | 0.593 | 0.468 | 0.882 |
| Ligthness (L*) | 41.5 | 40.4 | 41.8 | 40.8 | 0.612 | 0.101 | 0.583 | 0.964 |
| Redness (a*) | 11.3 | 10.9 | 10.6 | 11.4 | 0.321 | 0.527 | 0.796 | 0.111 |
| Yellowness (b*) | 12.6 | 12.2 | 12.4 | 12.3 | 0.248 | 0.226 | 0.904 | 0.562 |
| Chroma (C*) | 16.9 | 16.4 | 16.4 | 16.8 | 0.291 | 0.804 | 0.771 | 0.132 |
| Hue angle (h*) | 48.4 | 48.2 | 49.6 | 47.2 | 0.990 | 0.224 | 0.938 | 0.283 |
| WBSF, *N* | 35.40[b] | 43.34[a] | 38.34[ab] | 35.01[b] | 0.251 | 0.360 | 0.277 | 0.031 |

[1] Treatments: CON = diets without additives; MON = basal diet plus 25 mg/ kg of DM of sodium monensin (Rumensin. Elanco Animal Health. Greenfield. IN); MG = basal diet plus a magnesium oxide blend product (pHix-up®. Timab Magnesium. Dinard. France) provided at 0.50% of dry matter; MON + MG = basal diet plus MG + MON. at the same doses of the individual treatments.

[a-b] Different sub-scripts represent different means ($P \leq 0.05$).

**Table 7.** Effects of monensin (MON) and magnesium oxide blend (MG) on the fatty acids profile (% of total FA) in the beef of steers fed high-energy diets.

| Item | Treatment[1] | | | | SEM | *P-value* | | |
|---|---|---|---|---|---|---|---|---|
| | CON | MON | MG | MON + MG | | MON | MG | MON × MG |
| C12:0 (Lauric) | 0.06 | 0.06 | 0.07 | 0.06 | 0.004 | 0.971 | 0.137 | 0.542 |
| C14:0 (Myristic) | 2.96 | 2.88 | 2.94 | 2.92 | 0.121 | 0.703 | 0.914 | 0.830 |
| C14:1 c9 (Myristoleic) | 0.93 | 0.78 | 0.98 | 0.89 | 0.070 | 0.125 | 0.293 | 0.713 |
| C16:0 (Palmitic) | 23.57 | 23.65 | 23.78 | 23.81 | 0.362 | 0.871 | 0.609 | 0.933 |
| C16:1 c9 (Palmitoleic) | 4.10 | 3.99 | 4.11 | 4.13 | 0.180 | 0.800 | 0.691 | 0.720 |
| C17:0 (Margaric) | 0.74 | 0.75 | 0.82 | 0.79 | 0.033 | 0.101 | 0.866 | 0.585 |
| C18:0 (Stearic) | 11.60 | 11.96 | 11.90 | 11.98 | 0.352 | 0.543 | 0.663 | 0.691 |
| C18:1 *trans* 10 | 0.42 | 0.49 | 0.28 | 0.42 | 0.064 | 0.103 | 0.127 | 0.480 |
| C18:1 *trans* 11 (Vaccenic) | 0.59 | 0.70 | 0.49 | 0.65 | 0.044 | 0.006 | 0.105 | 0.594 |
| C18:1 c9 (Oleic) | 42.60[a] | 40.31[b] | 40.73[ab] | 41.41[ab] | 0.582 | 0.188 | 0.523 | 0.019 |
| C18:2 c9-c12 (linoleic) | 3.92 | 4.14 | 4.04 | 3.68 | 0.331 | 0.855 | 0.622 | 0.399 |
| C18:3 n-3 (α-linolenic) | 0.42 | 0.41 | 0.42 | 0.40 | 0.019 | 0.415 | 0.814 | 0.817 |
| C18:2 c9-t11 (CLA) | 0.30 | 0.32 | 0.30 | 0.34 | 0.020 | 0.219 | 0.773 | 0.535 |
| C22:0 (Behenic) | 0.28 | 0.28 | 0.27 | 0.27 | 0.028 | 0.602 | 0.889 | 0.887 |
| C20:4 n-6 (Arachidonic) | 1.13 | 1.19 | 1.21 | 1.10 | 0.124 | 0.819 | 0.977 | 0.520 |
| C20:5 n-3 (EPA) | 0.20 | 0.26 | 0.21 | 0.20 | 0.028 | 0.970 | 0.111 | 0.854 |
| C22:6 n-3 (DHA) | 0.09 | 0.09 | 0.08 | 0.07 | 0.011 | 0.739 | 0.282 | 0.970 |
| ∑ Saturated | 39.49 | 41.09 | 41.10 | 41.21 | 0.689 | 0.278 | 0.264 | 0.578 |
| ∑ Unsaturated | 59.76 | 58.03 | 58.27 | 58.08 | 0.640 | 0.173 | 0.463 | 0.295 |
| ∑UFA/ ∑SFA | 1.52 | 1.42 | 1.42 | 1.41 | 0.041 | 0.191 | 0.309 | 0.532 |
| ∑ Monounsaturated | 53.00[a] | 50.03[b] | 50.92[ab] | 51.66 [ab] | 0.719 | 0.141 | 0.765 | 0.018 |
| ∑ Polyunsaturated | 6.75 | 8.00 | 7.33 | 6.41 | 0.729 | 0.828 | 0.503 | 0.158 |
| ∑ n-3 | 0.77 | 0.77 | 0.73 | 0.70 | 0.060 | 0.844 | 0.375 | 0.791 |
| ∑ n-6 | 1.18 | 1.51 | 1.40 | 1.14 | 1.188 | 0.861 | 0.803 | 0.244 |
| ∑ n-6:∑ n-3 | 1.49 | 1.90 | 1.81 | 1.63 | 1.777 | 0.407 | 0.444 | 0.136 |

[1] Treatments: CON = diets without additives; MON = basal diet plus 25 mg/ kg of DM of sodium monensin (Rumensin. Elanco Animal Health. Greenfield. IN); MG = basal diet plus a magnesium oxide blend product (pHix-up®. Timab Magnesium. Dinard. France) provided at 0.50% of dry matter; MON + MG = basal diet plus MG + MON. at the same doses of the individual treatments.

Abbreviations: EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid

[a-b] Different sub-scripts represent different means ($P \leq 0.05$).