UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO" FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CAMPUS JABOTICABAL

USO DE ADITIVOS EM DIETAS PARA BOVINOS CONFINADOS

Richard Vaquero Ribeiro

Zootecnista

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UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO" FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS CAMPUS JABOTICABAL

USO DE ADITIVOS EM DIETAS PARA BOVINOS CONFINADOS

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DADOS CURRICULARES DO AUTOR

Richard Vaquero Ribeiro nasceu em 6 de julho de 1999, no município de Estrela D'Oeste, São Paulo. É filho de Neide Maria Caniato Vaquero Ribeiro e Gésio Carlos Ribeiro. Graduou-se em Zootecnia pela Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), na Faculdade de Medicina Veterinária e Zootecnia do Câmpus de Botucatu, no período de 2018 a 2022. Durante a graduação, foi bolsista em dois projetos de iniciação científica financiados pela Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). Também foi contemplado com a Bolsa Estágio Pesquisa no Exterior (BEPE/FAPESP) na Texas A&M University, em College Station, Texas. Em janeiro de 2023, iniciou o mestrado no programa de pós-graduação em Ciência Animal da Faculdade de Ciências Agrárias e Veterinárias da UNESP, Câmpus de Jaboticabal, sob a orientação do professor Dr. Otávio Rodrigues Machado Neto.

“Não reclame, não se explique. Apenas se apresente e faça o seu melhor”

Tom Brady

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SUMÁRIO

CAPÍTULO 1 - CONSIDERAÇÕES GERAIS 1
1. Introdução 1
2. Revisão de Literatura 3
2.1 Ionóforos 3
2.2 Monensina 5
2.3 Narasina 6
2.4 Virginiamicina e seu uso combinado com monensina 7
2.5 Leveduras 9
3. Referências 10
CHAPTER 2 – EFFECTS OF COMBINING YEAST PRODUCTS WITH ANTIMICROBIALS IN ADAPTATION DIETS FOR FEEDLOT NELLORE BULLS ON PERFORMANCE, FEEDING BEHAVIOUR, CARCASS TRAITS AND SERUM METABOLOME 17
1. Introduction 17
2. Material and Methods 19
2.1 Animals and treatments 19
2.2 Diets and feeding management 20
2.3 Performance and carcass traits 21
2.4 Feeding behavior 22
2.5 Blood sample collection and preparation for metabolomic analyses 22
2.6 Nuclear magnetic resonance spectroscopy 23
2.7 Statistical data analysis and bioinformatics 24
3. Results 25
3.1 Intake, performance and carcass traits 25
3.2 Feeding behavior 27
3.3 Serum Metabolomics Profiling 28
4. Discussion 33
5. Conclusion 37
6. References 37

CHAPTER 3 – A META-ANALYSIS OF THE EFFECTS OF MONENSIN SUPPLEMENTATION ON BEEF CATTLE PRODUCTION IN THREE FEEDING SYSTEMS 44
1. Introduction 44
2. Material and Methods 45
2.1. Data set 45
2.2 Statistical analysis 47
2.2.1 Weighted mean difference and Publication bias 47
2.2.2 Meta-regression and Subgroup analysis 47
3. Results 50
3.1. Pasture 50
3.2 Partial TMR 54
3.3. Total mixed ration/Feedlot 58
4. Discussion 65
5. Conclusion 70
6. References 70

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ATESTADO

Atesto que o Projeto "USO COMBINADO DE ADITIVOS NUTRICIONAIS EM DIETAS DE ADAPTAÇÃO PARA BOVINOS CONFINADOS" **Protocolo CEUA 0585/2023** , a ser conduzido por Richard Vaquero Ribeiro, responsável/orientador Otávio Rodrigues Machado Neto, para fins de pesquisa científica/ensino - encontra-se de acordo com os preceitos da Lei nº 11.794, de 08 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal - CONCEA.

Finalidade	PESQUISA CIENTÍFICA
Vigência do projeto	03/03/2024 a 05/07/2024
Nome Comum / Espécie / Linhagem	BOVINA / BOS INDICUS /
Raça	Nelore
Nº de animais machos	120
Nº de animais fêmeas	0
Nº de animais sexo indefinido	0
Peso médio de animais machos	390 Kg
Peso médio de animais fêmeas	0
Peso médio de animais sexo indefinido	0
Idade	2 ano(s) e 0 mes(es) e 0 dia(s).
Procedência	Faculdade de Medicina Veterinária e Zootecnia

Projeto de Pesquisa aprovado em reunião da CEUA em 09/02/2024

JULIANY GOMES QUITZAN
Presidente da CEUA da FMVZ, UNESP - Campus de Botucatu

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USO DE ADITIVOS EM DIETAS PARA BOVINOS CONFINADOS

RESUMO GERAL: Dois estudos foram conduzidos para avaliar o efeito de aditivos alimentares sobre o desempenho de bovinos durante os períodos de adaptação e confinamento total, bem como em animais em condições de pasto. No primeiro estudo, 120 touros Nelore foram divididos em dois grupos de peso corporal e alocados dentro dos grupos em 24 baias (5 touros/baia). Os seguintes tratamentos dietéticos foram aplicados: MVY: monensina a 18 mg/kg de matéria seca (MS) + virginiamicina a 15 mg/kg de MS + levedura viva a 2 g/animal/dia; NLY: narasina a 13 mg/kg de MS + levedura viva a 2 g/animal/dia; NYC: narasina a 13 mg/kg de MS + cultura de levedura a 7 g/animal/dia; NDY: narasina a 13 mg/kg de MS + levedura seca ativa a 1 g/animal/dia. Os tratamentos foram fornecidos durante o período de adaptação (d1–d21). Amostras de sangue foram coletadas ao final do período de adaptação para avaliar o perfil metabolomico do soro sanguíneo. Durante o período de adaptação de 21 dias, os touros alimentados com NYC tenderam (P = 0,09) a apresentar maior ganho médio diário em comparação aos alimentados com MVY. O consumo de matéria seca (CMS), peso corporal final e a eficiência alimentar foram semelhantes entre os tratamentos. Durante o período de terminação (d22 – d115), os touros alimentados com NLY apresentaram menor (P = 0,04) CMS como porcentagem do peso corporal em relação aos que receberam MVY. Nem a energia líquida observada nem a relação energia líquida observada/esperada diferiram entre os tratamentos (P > 0,05). Não houve efeitos dos tratamentos (P > 0,05) sobre as características da carcaça. Touros alimentados com NDY passaram mais tempo descansando (P = 0,03) e tenderam (P ≤ 0,10) a realizar menos refeições por dia e a consumir mais por refeição em comparação aos alimentados com MVY. O lactato foi o metabólito sérico mais importante para separação de todos os tratamentos, apresentando maior concentração no grupo MVY. Os três metabólitos mais importantes para diferenciar os grupos MVY e NLY foram lactato, creatina e valina, com concentrações mais altas no grupo MVY. Já para diferenciar MVY e NYC, os principais metabólitos foram 3-fenilpropionato, hipurato e betaína, com concentrações mais altas no grupo NYC. Assim, a narasina pode substituir a combinação de monensina e virginiamicina em dietas de adaptação para touros Nelore quando administrada com produtos à base de levedura, e a combinação NYC apresenta resultados promissores. No segundo estudo, uma meta-análise avaliou os efeitos da suplementação de monensina sobre o desempenho de bovinos de corte, parâmetros ruminais e digestibilidade total em diferentes sistemas de alimentação, incluindo ração total (TMR), ração parcial (pTMR) e pasto. O conjunto de dados incluiu 131 publicações revisadas por pares, abrangendo 349 médias de tratamento. Os efeitos da suplementação de monensina foram avaliados usando as diferenças médias ponderadas (WMD) entre os grupos controle (dietas sem monensina) e tratamento (dietas com monensina). A heterogeneidade foi explorada por meio de meta-regressão e análise de subgrupos, considerando fatores como dieta, dose de monensina e características experimentais. A suplementação com monensina reduziu (P < 0,01) o CMS (pasto: -0,92 kg/dia; pTMR: -0,12 kg/dia; TMR: -0,25 kg/dia) e aumentou o ganho médio diário (GMD = 0,06, 0,05 e 0,02 kg/dia, respectivamente) em todos os sistemas de alimentação. No

sistema pTMR, o grupo genético e o delineamento experimental influenciaram (P < 0,01) o efeito da monensina sobre as concentrações ruminais de propionato e butirato. Além disso, o consumo de suplemento e o tipo afetaram o efeito da monensina sobre a conversão alimentar (CA) e a concentração ruminal de propionato. No sistema pTMR, um consumo de monensina entre 200 e 300 mg/dia promoveu a melhor melhora na CA (WMD = -1,07 kg/kg; P = 0,011). No sistema TMR, o grupo genético, classe sexual, período de engorda e os níveis de concentrado e proteína bruta na dieta influenciaram (P < 0,01) o efeito da monensina sobre CMS e GMD. O tipo de forragem afetou o efeito da monensina sobre GMD, acetato, butirato e isovalerato (P < 0,01). A suplementação de monensina até 15 mg/kg MS em dietas TMR melhorou a CA (WMD = -0,54 kg/kg; P = 0,001) e aumentou o GMD (WMD = 0,055 kg/dia; P = 0,001). Os efeitos da monensina sobre GMD, CMS e parâmetros ruminais variaram entre sistemas de alimentação e foram influenciados por fatores como composição da dieta, níveis de consumo de monensina, grupo genético, classe sexual e condições experimentais. Assim, todos esses fatores devem ser considerados ao analisar a resposta à monensina, em vez de focar apenas nos efeitos gerais.

Palavras-chave: Antimicrobianos, bovinos de corte, confinamento, desempenho, leveduras

USE OF FEED ADDITIVES IN DIETS FOR FEEDLOT CATTLE

GENERAL ABSTRACT: Two studies were conducted to evaluate the effect of feed additives on cattle performance during the adaptation and total feedlot periods, as well as in animals under pasture conditions. One hundred twenty Nellore bulls were divided into two body weight groups (lighter half and heavier half) and assigned within groups to 24 pens. The following dietary treatments were applied: MVY: monensin at 18 mg/kg of dry matter (DM) + virginiamycin at 15 mg/kg of DM + live yeast at 2 g/animal/day; NLY: narasin at 13 mg/kg of DM + live yeast at 2 g/animal/day; NYC: narasin at 13 mg/kg of DM + yeast culture at 7 g/animal/day; NDY: narasin at 13 mg/kg of DM + active dry yeast at 1 g/animal/day. Treatments were administered during the adaptation period (d1–d21). Blood samples were collected at the end of the adaptation period for serum metabolome profiling. During the 21-day adaptation period, bulls fed NYC tended ($P = 0.09$) to have a greater average daily gain than bulls fed MVY. Dry matter intake (DMI), final body weight, and feed efficiency were similar ($P > 0.05$) among treatments. During the finishing period, bulls fed NLY had a lower ($P = 0.04$) DMI as a percentage of body weight than bulls receiving MVY. Neither observed net energy nor observed/expected net energy ratio differed among treatments ($P > 0.05$). There were no treatment effects ($P > 0.05$) on the carcass traits. Bulls fed NDY spent more time resting ($P = 0.03$) and tended ($P \leq 0.10$) to have fewer meals per day and greater DMI per meal than animals fed MVY. Lactate was the most important serum metabolite for discriminating all treatment groups, with a higher concentration in the MVY group. The three most important metabolites for discriminating the MVY and NLY groups were lactate, creatine, and valine, whose concentrations were higher in the MVY group. The three most important metabolites for discriminating MVY and NYC were 3-phenylpropionate, hippurate, and betaine, whose concentrations were higher in the NYC group. Thus, narasin can replace the combination of monensin and virginiamycin in adaptation diets for Nellore bulls when administered together with yeast products, with NYC combination showing a great potential. In the second study, a meta-analysis evaluated the effects of monensin supplementation on beef cattle performance, ruminal parameters, and total tract digestibility in different feeding systems, including total mixed ration (TMR), partial TMR, and pasture. The dataset included 131 peer-reviewed publications that comprised 349 treatment means. The effects of monensin supplementation were evaluated using the weighted mean differences (WMD) between the control (diets without monensin) and treatment (diets with monensin) groups. Heterogeneity was explored through meta-regression and subgroup analysis, which considered factors inherent to the animal, such as diet, monensin dose, and experimental traits. Monensin supplementation reduced ($P < 0.01$) dry matter intake (DMI) (pasture: -0.92 kg/day; partial TMR: -0.12 kg/day; TMR: -0.25 kg/day) and increased average daily gain (ADG = 0.06, 0.05 and 0.02 kg/day, respectively) in all feed systems. In the partial TMR system, genetic group and experimental design influenced ($P < 0.01$) the effect of monensin on propionate and butyrate ruminal concentrations. Additionally, supplement intake and type affected the effect of monensin on feed conversion ratio (FCR) and propionate ruminal concentration. In the partial TMR system, a monensin intake of 200–300 mg/day promoted the greatest improvement in FCR (WMD = -1.07 kg/kg; $P = 0.011$). In the TMR system, genetic group, sex class, fattening period, and dietary concentrate and

crude protein levels influenced ($P < 0.01$) the effect of monensin on DMI and ADG. Forage type affected the effect of monensin on ADG, acetate, butyrate, and isovalerate ($P < 0.01$). Monensin inclusion up to 15 mg/kg DM in TMR diets improved FCR (WMD = -0.54 kg/kg; $P = 0.001$) and increased ADG (WMD = 0.055 kg/day; $P = 0.001$). The effects of monensin on ADG, DMI, and ruminal parameters varied across feeding systems and were influenced by factors such as diet composition, monensin intake levels, genetic group, sex class, and experimental conditions. Thus, all these factors should be considered when analyzing the response to monensin rather than focusing solely on the general effects.

Keywords: Antimicrobials, beef cattle, feedlot, performance, yeast

CAPÍTULO 1 - CONSIDERAÇÕES GERAIS

1. Introdução

Considerando as perspectivas de crescimento populacional mundial e concomitante aumento da demanda por alimentos, além da realidade dos impactos ambientais gerados pela cadeia de produção de alimentos Scollan et al. (2011), definiu cinco principais prioridades futuras para produção animal. 1) Aumento da produção para suprir a maior demanda, 2) Minimizar os impactos ambientais, 3) Considerar as opções de mitigação dos impactos ambientais, 4) Melhorar a eficiência de produção e 5) Melhorar a Qualidade dos produtos de origem animal. Referente a produção de ruminantes, diversas são as tecnologias possíveis de utilização para atender essas determinações. A manipulação da dieta com objetivo otimizar os processos fermentativo pode contribuir para uma produção mais eficiente, lucrativa e menos impactante ao ambiente (Tedeschi et al. 2003). Essas manipulações podem ser feitas em diversos fatores dietéticos como qualidade, níveis de inclusão e processamento de alimentos, além da utilização de aditivos para manipulação da população microbiana no trato gastrointestinal (Polizel et al., 2020a).

A quantidade de bovinos provenientes de confinamento abatidos no Brasil tem aumentado significativamente, saindo de 3,05 para 7,62 milhões de cabeças em 2010 e 2022, respectivamente (ABIEC, 2023). Conforme apontado por Silvestre e Millen (2021), a quantidade de nutricionistas brasileiros que utilizam entre 71% e 90% de concentrado em suas formulações para bovinos confinados aumentou 39,1% entre 2009 e 2021. Dietas com maiores teores de concentrado podem proporcionar alguns benefícios para a produção, como a obtenção de energia líquida de ganho (ELg) a um custo menor em comparação com dietas de alta forragem, além de melhorar o desempenho e a eficiência alimentar dos animais (Owens, 2010). No entanto, é importante destacar que a produção de bovinos confinados, alimentados com dietas de alto teor de concentrado, apresenta desafios e requer ajustes em diversos fatores de produção (Fernando et al., 2010).

Dietas ricas em carboidratos não-fibrosos proporcionam o aumento da produção total de ácidos graxos de cadeia curta (AGCC) e uma redução da relação molar acetato/propionato, quando comparadas a dietas de alta forragem, devido ao

maior aporte de substratos fermentáveis no rúmen (Penner et al., 2009). Quando há falta de sincronismo entre a produção e absorção dos AGCC e um tamponamento inadequado do rúmen, o pH do fluido ruminal se torna ácido possibilitando o desenvolvimento e a atuação de microrganismos produtores de ácido lático, como *Streptococcus bovis* (pH < 6) e *Lactobacillus spp*. (pH < 5). Esses processos aceleram a acidificação ruminal, o que pode levar a distúrbios metabólicos como a acidose aguda (pH < 5,2) ou subaguda (pH < 5,6 por pelo menos três horas) (Gozho et al., 2006; Owens et al., 1998). Como consequência, podem ocorrer alterações na microbiota, redução da eficiência de fermentação, lesões no epitélio ruminal, aumento das concentrações de lipopolissacarídeos, abscessos hepáticos, redução na ingestão de matéria seca e piora na eficiência e desempenho dos animais (Gozho et al., 2006; Millen et al., 2016; Owens et al., 1998).

Formulação, fabricação e distribuição correta das dietas, adaptação adequada dos animais, manejo alimentar e utilização de aditivos nutricionais são fatores determinantes no sucesso da produção de bovinos confinados (Coe et al., 1999). A fase de adaptação refere-se ao período no qual, utilizando estratégias nutricionais, os animais são adaptados para receber dietas com alto teor de concentrado. O uso de aditivos nutricionais em dietas de adaptação pode melhorar o desempenho, eficiência alimentar e a saúde animal (Silvestre et al., 2023). No Brasil, a maior parte dos nutricionistas de bovinos confinados utilizam algum tipo de aditivo, sendo que o ionóforo monensina (MON) aparece como principal aditivo primário (86,11%) e virginiamicina (VM) como principal aditivo secundário (65,71%) (Silvestre & Millen, 2021). Embora possuam mecanismos de ação diferentes, esses aditivos atuam sobre a microbiota ruminal, tornando os processos fermentativos mais estáveis e eficientes, devido à maior estabilidade do pH, menores perdas de energia e nitrogênio, bem como à maior produção de propionato. Esses efeitos sobre microbiota ruminal resultam em melhor desempenho e eficiência alimentar dos animais (Owens et al., 1998; Ceconi et al., 2022). O uso associado de MON e VM em dietas de adaptação tem demonstrado um efeito positivo no desempenho e eficiência alimentar de bovinos durante a adaptação e ao longo de todo o período de confinamento (Rigueiro et al., 2020).

Embora ainda pouco estudado, o ionóforo narasina (NAR) vem demonstrando resultados positivo na produção de bovinos confinados. Já é de conhecimento que a

NAR tem potencial para alterar a fermentação ruminal e elevar o pH ruminal de maneira similar a MON em dietas de adaptação (Polizel et al., 2017). Do mesmo modo, as leveduras e seus produtos também promovem alterações na fermentação ruminal devido aos seus efeitos probióticos e prebióticos no rúmen. De acordo Chaucheyras-Durand et al. (2012), as leveduras estimulam o desenvolvimento de bactérias celulolíticas e de utilizadoras de lactato no rúmen, ao mesmo tempo em que limitam a ação das bactérias produtoras de ácido lático. Isso resulta em uma melhor estabilidade do pH ruminal, melhoria na digestibilidade dos alimentos e na conversão alimentar dos animais.

Os efeitos da utilização de MON e VM associadas em dietas para bovinos confinados é bem documentada na literatura (Rigueiro et al., 2020; Ceconi et al., 2022; Estrada-Angulo et al., 2022). No entanto, não há dados referente a associação de MON, VM e Leveduras, bem como a associação de NAR e produtos de leveduras em dieta de adaptação para bovinos Nelore confinados. O objetivo desse estudo será avaliar os efeitos do uso combinado de MON, VM e leveduras vivas, assim como combinações de NAR com leveduras vivas e cultura de levedura em dietas de adaptação, sobre a performance, fisiologia, comportamento alimentar e microbioma fecal de bovinos Nelore confinados.

2. Revisão de Literatura

2.1 Ionóforos

Aditivos alimentares são substâncias intencionalmente adicionadas a alimentos com objetivo de conservar, intensificar ou modificar suas propriedades (Brasil, 2009). Dentre as possibilidades de aditivos os mais utilizados na alimentação de bovinos são os ionóforos (monensina, lasalocida, salinomicina e narasina) (Marques e Cooke, 2021). Os ionóforos são antibióticos carboxílicos, produzidos pelas bactérias *Streptomyces* spp. e recebem esse nome pela capacidade de se ligarem e facilitar o transporte de íons metais através de membranas celulares (Kevin et al., 2009). A capacidade de se ligarem a íons metálicos é determinada tanto pela natureza química quanto pela estrutura dos ionóforos. Os ionóforos são moléculas lipofílicas que possuem uma região externa hidrofóbica e um interior hidrofílico. Essas

características, combinadas com suas estruturas moleculares contendo átomos de oxigênio, possibilitam a ligação com cátions (Pressman, 1976).

As moléculas de ionóforos apresentam mecanismos de ação muito similares (Nagaraja, 2007). Bactérias Gram-negativas possuem uma segunda membrana externa que é impermeável aos ionóforos, ao contrário das bactérias Gram-positivas e dos protozoários, que possuem apenas uma membrana lipídica que permite a aderência e ação dessas moléculas (Marques e Cooke, 2021; Nagaraja, 2007). Uma vez aderido a membrana dos microrganismos sensíveis, os ionóforos viabilizam a solubilização e troca de um cátion externo por outro do meio interno (Russell, 1987). Uma vez que o cátion foi internalizado, o processo inicia-se novamente de maneira rápida, podendo atingir taxas de mais de 1000 trocas por segundo (Pressman, 1976).

As bactérias ruminais utilizam mecanismos fisiológicos para manter um gradiente químico e elétrico com o ambiente ruminal, de forma que o meio interno fique alcalino e negativo (Mitchell, 1961). Essas bactérias apresentam uma concentração mais elevada de potássio e uma concentração mais baixa de sódio em seu interior, além de um pH mais alto em comparação com o ambiente ruminal (Marques & Cooke, 2021). Esses gradientes são fundamentais na sobrevivência desses microrganismos, pois possibilita a realização de trocas e consequente captação de nutrientes do meio externo e síntese de ATP (Russell e Strobel, 1989). Devido a maior concentração de potássio no interior dos microrganismos e alta afinidade geral dos ionóforos pelo cátion sódio, o resultado da interação dos ionoforos com a membrana dos microrganismos será o aumento da concentração de sódio, redução de potássio e PH no meio interno dos microrganismos atingidos (Russell e Strobel, 1989). Para reestabelecer a homeostase, os microrganismos ativam sistemas ativos de transporte, entretanto, mesmo que íons sejam retirados, o ciclo irá ocorrer novamente, exaurindo as reservas energéticas e reduzindo a síntese proteica, prejudicando a viabilidade celular (Russell e Strobel, 1989). Por consequência da maior concentração interna de cátions e o efeito negativo no balanço energético, o equilíbrio osmótico dos microrganismos também será afetado, resultando em maior entrada de água no meio interno e consequente possibilidade de rompimento celular (Rodrigues, 2016).

2.2 Monensina

A MON é um antibiótico ionóforo produzido pela *Streptomyces cinnamonensins* que possui peso molecular de 671 e sequência de seletividade iônica $Na^+ > K^+$, $Li^+ > Rb^+ > Cs^+$ (Nagaraja, 1995). Em função da relação custo/benefício que proporciona para produção, trata-se do aditivo mais utilizado em dietas de bovinos confinados do Brasil. Os benefícios produtivos serão determinados pelos efeitos sobre a fermentação ruminal; vale pontuar que o nível de inclusão irá influenciar a resposta animal (Fox e Black, 1984). Em revisão realizado por Tedeschi et al. (2003), o uso de 28 ppm de MON em dietas de confinamento reduz 4% do consumo de matéria seca, aumenta 1,5 % do ganho de peso diário e melhora 5,3% a eficiência alimentar dos animais. Uma meta-análise demonstrou resultados semelhantes, em qual o uso médio de 28,1 ppm de MON resultou em 3% de redução do consumo de matéria seca, aumento de 2,5% do ganho de peso diário e melhora de 6,4% a eficiência alimentar (Duffield et al., 2012).

A melhor eficiência alimentar se deve a redução do consumo de matéria seca e aumento do ganho de peso. Esse efeito está ligado ao aumento da eficiência do metabolismo energético, de acordo Byers, (1980), a MON aumenta a energia metabolizável (EM) da dieta e a energia liquida para mantença (ELm) sem afetar a energia liquida para o ganho. Segundo o mesmo autor, isso pode ser explicado pelo aumento da digestibilidade da dieta e o aumento da produção de propionato, corroborando com Ryle e Ørskov, (1990) Holter et al., (1970), que demonstraram uma melhor eficiência relativa da conversão de glicose em propionato e menor produção de calor do propionato no metabolismo ruminal, respectivamente. Apesar de não ser completamente elucidado, atribui-se a redução do consumo de alimentos a maior produção de propionato, que por sua vez estimula a oxidação hepática de Acetil-coa aumentando a síntese de ATPs que atuam no controle da saciedade (Allen et al., 2009).

É importante destacar que, embora seja comum entre a maioria dos ionóforos, a MON apresenta alguns efeitos benéficos para a saúde ruminal e para o ambiente. De acordo com Russell e Strobel (1989), a MON é capaz de reduzir a produção de lactato pela ação contra bactérias como *Streptococcus bovis*. Isso resulta em uma fermentação mais estável no rúmen, evitando grandes oscilações no pH ruminal e

distúrbios metabólicos. A MON tem a capacidade de inibir o desenvolvimento de microrganismos que liberam H2, CO2 e formato, enquanto favorece aqueles que utilizam H2, como os produtores de propionato; essa ação indireta pode levar a uma redução significativa na produção de metano, que pode chegar a 25% Tedeschi et al. (2003). Ademais, a MON reduz a degradação de proteína no rúmen e consequentemente a concentração de NH3, o que permite um maior escape de proteína verdadeira para o intestino, aumentando a digestibilidade de N e retenção do mesmo (Chalupa et al., 1980; Tedeschi et al., 2003).

2.3 Narasina

A NAR é um antibiótico ionóforo produzido pela *Streptomyces aureofaciens* que possui peso molecular de 765 e sequência de seletividade iônica $Na^+ > K^+$, Rb, Cs^+,Li^+ (Nagaraja, 1995). Foi observado em um estudo in vitro que a NAR apresenta um maior potencial em aumentar a produção de propionato e diminuir as concentrações de ácido lático em comparação com a MON; adicionalmente, para alcançar o mesmo efeito na redução da concentração de ácido lático foi necessário a metade da dose de NAR em comparação com a MON (Nagaraja et al., 1987). Em um trabalho realizado por Limede et al. (2021), foi constatado que a adição de 13mg/Kg de matéria seca (MS) em dietas compostas por forragem resulta em aumento na produção de propionato no rúmen e no ganho de peso diário de bovinos, em comparação com o grupo controle (sem aditivo), bem como com aqueles que receberam salinomicina e flavomicina. Similarmente, Polizel, et al. (2020a) observaram um aumento na produção de propionato, redução na relação molar acetato/propionato e amônia ruminal quando a NAR foi incluída em dieta de alta forragem. Vale ressaltar que em ambos os trabalhos, não houve efeito da NAR sobre a ingestão de matéria seca.

Avaliando o efeito de doses crescentes de NAR (5,10 e 15 mg/ Kg MS) em comparação a MON (25 mg/ Kg MS) em dietas com alto concentrado para cordeiros, Polizel et al., (2020b), verificaram que os níveis crescentes de NAR aumentaram linearmente o ganho de peso diário e a eficiência alimentar, além disso, a NAR proveu melhores resultados a nível de desempenho quando comparada com MON. De acordo com Polizel et al. (2017), a adição de 20 mg de NAR/Kg de MS na dieta de bovinos em fase de adaptação resultou em um aumento significativo do pH ruminal, em

comparação aos animais do grupo controle (sem aditivo) e àqueles que receberam 10 mg de NAR/Kg de MS. Em relação aos parâmetros de performance e ingestão de alimentos, a adição de 13 mg de NAR/Kg de MS em dietas de adaptação para bovinos resulta em aumento no consumo de matéria seca e mesmo ganho de peso em comparação a adição de 25 mg de MON/Kg de MS (Baggio et al., 2023).

2.4 Virginiamicina e seu uso combinado com monensina

A VM é um antibiótico não ionóforo da classe das estreptograminas, produzida como um metabolito secundário de uma linhagem de *Streptomyces virginae*; composta por uma mistura de dois componentes químicos distintos denominados fator M (C28 H35 N3O7) de peso molecular de 525 e fator S (C43 H49 N7O10) de peso molecular de 823 (Cocito, 1979; De Araújo et al., 2016). Cada um dos componentes possui potencial bacteriostático, entretanto, quando combinados em proporções adequadas, atuam de forma sinérgica intensificando o potencial de ação contra bactérias, passando a ser bactericida (De Araújo et al., 2016). Assim como os ionóforos, devido a permeabilidade celular, a VM atua contra bactérias Gram-positivas e protozoários no rúmen (Coe et al., 1999). O mecanismo de ação da VM é no metabolismo proteico bacteriano, se diferindo dos ionóforos. Uma vez penetrado a parede celular, a VM se liga as unidades ribossomais no citoplasma, impedindo a formação de peptídeos durante a síntese proteica (De Araújo et al., 2016). De forma mais específica, o fator M irá se ligar a porção 50 S do ribossomo, causando alterações na conformação, impedindo a ligação do RNA transportador ao complexo RNA mensageiro/Ribossomo; uma vez ligado a porção 50 S do ribossomo, o fator M irá intensificar a ação do fator S que irá interferir na formação e liberação dos peptídeos (Cocito e Chinali, 1985).

Em função do efeito direto sobre a viabilidade celular de alguns microrganismos ruminais, a VM irá afetar a fermentação e seus produtos. Assim como os ionóforos, a VM irá promover aumento da produção de propionato e do pH ruminal médio, redução da produção de lactato e da população de *Streptococcus bovis, Lactobacillus spp e Fusobacterium necrophorum* (Nagaraja et al., 1987; Coe et al., 1999). Essa mudanças no ambiente ruminal resultam em benefícios biológicos para a o animal como redução

da incidência de abcesso hepático, redução do risco de acidose ruminal, melhoria no ganho de peso e eficiência alimentar (Rogers et al., 1995; De Araújo et al., 2016).

Segundo Navarrete et al. (2017), que avaliaram o efeito da VM sobre densidade energética da dieta e o desempenho de bovinos confinados, a VM aumenta a energia liquida da dieta em comparação ao estimado e reduz a produção de calor no rúmen. Além da menor produção de calor, outro fator que explica essa melhor eficiência energética é o aumento da produção de propionato. Existem alguns resultados controversos quanto ao efeito da VM sobre o ganho de peso de bovinos, entretanto, Rogers et al. (1995) avaliaram dados de sete experimentos com mais de 3.000 animais e quatro doses diferentes (0; 11; 19,3 e 27,6 ppm) e encontraram um efeito linear da VM sobre o ganho médio diário e eficiência alimentar; os modelos gerados nesse trabalho indicam que as doses de VM indicadas para dietas de confinamento variam de 19.3 a 27.3 mg/Kg de MS. De acordo Tedeschi e Gorocica-Buenfil, (2018), a VM na mesma dosagem e mesmo período de alimentação é 2,3 vezes mais efetiva no ganho de peso em relação a monensina; os autores também observaram que houve uma redução linear de 0,42% na incidência de abscesso hepático por mg de VM por kg de matéria seca, sendo que a redução máxima ocorrerá com a dose acima de 24 ppm.

O uso combinado de VM com outros aditivos como MON irá alterar o padrão da resposta animal, quando comparado ao uso exclusivo (De Araújo et al., 2016). De acordo Rigueiro et al. (2020), durante a fase de adaptação, a associação de VM e MON resulta em maior ganho de peso e mesma eficiência alimentar quando comparado ao uso exclusivo de MON; já na fase de terminação o uso exclusivo de VM proveu melhor performance dos animais confinados. De forma similar, (Rigueiro et al. (2023) avaliaram o efeito da associação do uso combinado e exclusivo de MON e VM durante a adaptação e em dois períodos de terminação. Os autores encontraram que a retirada de MON quando associada com VM no terço final de terminação melhora o desempenho dos animais em comparação ao uso exclusivo de MON ou VM durante todo período de confinamento. No entanto, essa melhora não foi observada quando comparada com os animais que tiveram a MON retirada no final da adaptação, ou seja, uso exclusivo de VM durante a fase de terminação.

2.5 Leveduras

As leveduras são fungos unicelulares eucariontes heterotróficos, esféricos ou ovais, que se reproduzem por bipartição ou gemulação; são microrganismos anaeróbios facultativos, podendo tanto utilizar oxigênio ou outro composto orgânico como aceptor final de elétrons, viabilizando a sobrevivência em vários ambientes (Raven et al., 2014). Embora a maioria dos fungos apresente uma fase unicelular e outra filamentosa, algumas espécies, como a *Saccharomyces cerevisiae* (SC), permanecem predominantemente como leveduras e podem alternar para a forma filamentosa em condições específicas (Raven et al., 2014). Os produtos de leveduras, tais como leveduras vivas, parede celular de levedura e cultura de leveduras, que contêm células viáveis, componentes da parede celular, metabólitos e os meios em que as células de levedura foram cultivadas, têm sido estudados devido o potencial de modular a fermentação ruminal e melhorar a saúde e performance de bovinos (Miles et al., 1991; Chaucheyras-Durand et al., 2012).

Em um estudo *in vitro,* foi observado que em condições anaeróbicas, a SC compete com *Streptococcus bovis* por glicose, limitando o desenvolvimento e a produção de lactato dessas baterias; além disso, a SC estimula o desenvolvimento de bactérias lactato-utilizadoras como *Megasphaera Elsdeni* (Chaucheyras et al., 1996). De acordo Dawson et al. (1990) que avaliaram o efeito de leveduras sobre os parâmetros ruminais, a suplementação de bovinos com SC aumenta a concentração e atividade de microrganismos celulolíticos. Cagle et al. (2020), relataram que as leveduras afetam também outros microrganismos, como os protozoários. De acordo com o estudo, houve um aumento significativo da concentração de protozoários em bovinos suplementados com leveduras em dieta de alto concentrado, em comparação com o grupo de controle (sem aditivos).

Apesar de os mecanismos probióticos das leveduras não serem completamente compreendidos, existem algumas propostas de modelos de ação. Um dos principais fatores que implica em benefícios para os microrganismos ruminais é o fato de as leveduras utilizarem o oxigênio dissolvido no rúmen para metabolizar carboidratos, o que reduz o potencial redox no rúmen e melhora as condições para as bactérias estritamente anaeróbicas (Jouany, 2006); é importante ressaltar que a capacidade de utilização de oxigênio é dependente da variedade de levedura

(Newbold et al., 1996). Ademais, as leveduras vivas liberam micronutrientes que podem ser aproveitados por outros microrganismos no meio ruminal; quando as leveduras sofrem autólise, elas liberam minerais, vitaminas, carboidratos e proteínas, que podem ser utilizados por outros microrganismos presentes (Sauvant et al., 2004; Jouany, 2006).

Os dados dos efeitos da suplementação de produtos de leveduras sobre características ruminais, performance e de saúde são variáveis na literatura (Zinn et al., 1999). Entretanto, em uma meta-análise realizado por Batista et al. (2022), que incluiu 27 trabalhos, os autores verificaram que a suplementação de produtos de leveduras melhora a digestibilidade da matéria seca, fibra e proteína, além de melhorar o desempenho dos animais, aumentando o ganho de peso diário, a eficiência alimentar e o consumo de alimentos. Poucos trabalhos na literatura avaliaram o efeito do uso combinado de produtos de leveduras, especialmente em associação com NAR em dietas de adaptação, sobre as características de interesse de bovinos Nelores confinados, tornando necessários estudos para verificar a aplicabilidade dessas combinações.

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CHAPTER 2 – EFFECTS OF COMBINING YEAST PRODUCTS WITH ANTIMICROBIALS IN ADAPTATION DIETS FOR FEEDLOT NELLORE BULLS ON PERFORMANCE, FEEDING BEHAVIOUR, CARCASS TRAITS AND SERUM METABOLOME.

ABSTRACT - This study evaluated the effects of different combinations of yeast products and antimicrobials administered during the adaptation period on performance, feeding behavior, carcass traits, and serum metabolome of feedlot Nellore bulls. One hundred twenty Nellore bulls were divided into two body weight groups (lighter half and heavier half) and assigned within groups to 24 pens. The following dietary treatments were applied: MVY: monensin at 18 mg/kg of dry matter (DM) + virginiamycin at 15 mg/kg of DM + live yeast at 2 g/animal/day; NLY: narasin at 13 mg/kg of DM + live yeast at 2 g/animal/day; NYC: narasin at 13 mg/kg of DM + yeast culture at 7 g/animal/day; NDY: narasin at 13 mg/kg of DM + active dry yeast at 1 g/animal/day. Treatments were administered during the adaptation period (d1–d21). Blood samples were collected at the end of the adaptation period for serum metabolome profiling. During the 21-day adaptation period, bulls fed NYC tended (P = 0.09) to have a greater average daily gain than bulls fed MVY. Dry matter intake (DMI), final body weight, and feed efficiency were similar (P > 0.05) among treatments. During the finishing period, bulls fed NLY had a lower (P = 0.04) DMI as a percentage of body weight than bulls receiving MVY. Neither observed net energy nor observed/expected net energy ratio differed among treatments (P > 0.05). There were no treatment effects (P > 0.05) on the carcass traits. Bulls fed NDY spent more time resting (P = 0.03) and tended (P ≤ 0.10) to have fewer meals per day and greater DMI per meal than animals fed MVY. Lactate was the most important serum metabolite for discriminating all treatment groups, with a higher concentration in the MVY group. The three most important metabolites for discriminating the MVY and NLY groups were lactate, creatine, and valine, whose concentrations were higher in the MVY group. The three most important metabolites for discriminating MVY and NYC were 3-phenylpropionate, hippurate, and betaine, whose concentrations were higher in the NYC group. Thus, narasin can replace the combination of monensin and virginiamycin in adaptation diets for Nellore bulls when administered together with yeast products, with NYC combination showing a great potential.

Keywords: Antimicrobials, adaptation, beef cattle, feedlot, yeast products

1. Introduction

The adaptation of feedlot cattle to high-concentrate diets is a crucial period; the optimization of dry matter intake (DMI) and weight gain during this period significantly influences overall animal performance (Brown et al., 2006; Silvestre et al., 2023). Hence, implementing dietary strategies that maximize DMI and average daily gain (ADG) during this period is important. As demonstrated by Silvestre et al. (2023), feed additives play a crucial role in modulating ruminal and post-ruminal fermentation,

consequently impacting DMI, ADG, and feed efficiency. This fact highlights the importance of feed additives as key components of adaptation diets.

The ionophore sodium monensin (MON) is the feed additive most widely used in American and Brazilian feedlot operations (Samuelson et al., 2016; Silvestre and Millen, 2021). The impact of MON on gram-positive bacteria significantly alters ruminal fermentation and consequently the host's metabolism (Russell & Strobel, 1989; Nagaraja, 2007). It is well established that MON increases ADG while decreasing DMI, thereby improving feed efficiency (Tedeschi et al., 2003; Duffield et al., 2012). Despite these general findings, the effect of MON on animal performance will vary depending on the dose used of this additive, the stage within the feedlot cycle, and combinations with other feed additives. According to Burrin et al. (1988), DMI decreases linearly with increasing MON inclusion level during the adaptation period. Furthermore, increasing MON intake during adaptation tends to decrease gain throughout the finishing period. Additionally, the combined use of MON and virginiamycin (VM) in adaptation diets has shown a positive effect on overall feedlot performance compared to exclusive MON supplementation (Rigueiro et al., 2020).

Another ionophore that has been studied in cattle is narasin (NAR). Although the mechanism of action of NAR is similar to that of MON, an *in vitro* study demonstrated that NAR is more effective in increasing ruminal propionate production and in controlling lactic acid concentration (Nagaraja et al., 1987). The inclusion of NAR in high-forage diets improves ruminal propionate production without altering DMI (Polizel et al., 2020; Limede et al., 2021). Additionally, feeding NAR at 13 mg/kg of dry matter (DM) during the adaptation of feedlot cattle improves DMI compared to MON at 25 mg/kg of DM (Baggio et al., 2023).

Although the effects of yeast product supplementation on ruminal fermentation and feedlot cattle performance are inconsistent, these feed additives offer promising benefits, especially during adaptation (Crossland et al., 2019). Yeast products such as live yeast, yeast cell wall and yeast culture can be classified as prebiotic, probiotic or postbiotic, respectively (Scarpellini et al., 2021). The potential of these feed additives is mainly related to their capacity to stimulate the growth of rumen cellulolytic and lactate-utilizing bacteria (Chaucheyras et al., 1996; Callaway & Martin, 1997).

Metabolomics is an emerging powerful tool in omics science that, coupled with multivariate analysis, has enabled comprehensive metabolic exploration of animals, plants, and microbes over the past decade (Goldansaz et al., 2017). Metabolites are the end products of complex reactions that are regulated by genomic and environmental factors and are therefore sensitive indicators of nutritional modulation (Goldansaz et al., 2017; Cônsolo et al., 2020).

Given the complementary benefits of antimicrobials and yeast products, the potential synergistic effects of their combinations should be investigated. The effects of the combination of MON and VM in diets for feedlot cattle are well-documented in the literature (Rigueiro et al., 2020; Ceconi et al., 2022; Estrada-Angulo et al., 2022) but few studies have investigated the effect of combining antimicrobials and yeast products in adaptation diets for *Bos indicus* cattle, particularly using metabolomics analysis of bio-fluids to better understand beef cattle metabolism and behavior. Within this context, the current study aimed to evaluate the effects of different combinations of MON, VM and NAR with yeast products in adaptation diets on feedlot performance, carcass traits, feeding behavior, and serum metabolome of Nellore bulls.

2. Material and Methods

All animal procedures were approved by the Ethics and Animal Use Committee (CEUA - protocol 0585/2023) of the São Paulo State University, on February 09, 2024.

2.1 Animals and treatments

A total of 120 Nellore bulls with an average initial body weight (BW) of 386.89 ± 16.73 kg and age of 22 to 24 months were used in this experiment conducted at the School of Veterinary Medicine and Animal Science, São Paulo State University, Botucatu, São Paulo, Brazil. Upon arrival at the feedlot, the animals were treated for endo- and ectoparasites, rabies, clostridiosis, bovine infectious rhinotracheitis, and bovine viral diseases and were ear-tagged. During the first seven days, the animals were fed corn silage *ad libitum* to adapt to the facilities and feeding management. On d0, bulls were weighed to determine the initial BW, which was used to divide the animals into two blocks (light and heavy). Bulls were randomly assigned to 24 pens (5 × 5.35 m; 5 bulls/pen) equipped with automatic waterers and feed bunks, providing 1 m of bunk space per bull. Pens within blocks were randomly allocated to one of the

following four treatments: MVY: MON at 18 mg/kg of DM (Rumensin 100, Elanco Brazil, São Paulo, SP, Brazil) + VM at 15 mg/kg of DM (V-Max, Phibro Animal Health Corporation, Guarulhos, SP, Brazil) + live yeast at 2 g/animal/day (*Saccharomyces cerevisiae* strain NCYC 996 Procreatin-7, Phileo Lesaffre Animal Care, Campinas, SP, Brazil); NLY: narasin at 13 mg/kg of DM (Zimprova 100, Elanco Brazil, São Paulo, SP, Brazil) + live yeast at 2 g/animal/day; NYC: narasin at 13 mg/kg of DM + yeast culture at 7 g/animal/day (Postbiotic, XPC-Ultra®, Diamond V, Cedar Rapids, IA); NDY: narasin at 13 mg/kg of DM + active dry yeast at 1 g/animal/day (*Saccharomyces cerevisiae boulardii* strain CNCM I-1079, ProTernative®, Lallemand Animal Nutrition, Montreal, Quebec, Canada).

Dietary treatments were provided only during the adaptation period (d1 – d21). In the transition (d22 – d33) and finishing (d34 – d115) periods all animals were fed the same diets, with the inclusion of MON at 28 mg/kg of DM. The experiment lasted 115 days.

2.2 Diets and feeding management

The animals were fed *ad libitum*, twice daily at 9:00 and 15:00 hours, providing equal amounts on each occasion. The daily adjustment of dietary supply was based on the DMI of the previous days, targeting refusal levels at 3-5%. The orts were removed daily, weighed, sampled by pen weekly, and dried (100^{o}C for 24 h) for the determination of DMI. Feed samples were collected weekly, stored at -18^{o}C, and composited at the end of the trial for nutrient analysis (Table 1).

Feed samples were dried in a forced-air oven at 60^{o}C for 72 h, grounded in a Willey mill (1-mm screen), and analyzed for moisture (# 934.01), crude protein (# 976.05, N * 6.25), ash (# 942.05), and ether extract (# 920.39) (AOAC, 2005). Neutral detergent fiber (NDF) was analyzed according to Mertens (2002), adding heat-stable α-amylase and sodium sulfite. Additionally, NDF content was adjusted for ash.

Table 1 - Composition (%, DM basis) of experimental diets

	Experimental Diets[1]				
Item	Adaptation	Trans. 1	Trans. 2	Trans. 3	Finishing
Days on feed	21	4	4	4	82
Sugarcane bagasse	15.60	23.54	18.51	13.38	12.00
Corn silage	24.97	-	-	-	-
Ground corn	18.31	28.40	38.70	50.74	51.05
Citrus pulp	12.30	20.01	17.35	13.06	15.91
DDGS[2]	25.43	25.27	22.41	19.43	17.63
Supplement[3]	3.39	2.78	3.03	3.39	3.39
Potassium chloride	-	-	-	-	0.02
Nutrient composition					
Crude Protein %	14.50	14.30	14.00	13.75	12.80
Ether Extract, %	4.01	4.15	4.15	4.14	4.04
Neutral detergent fiber, %	38.45	34.86	32.16	32.00	29.23
Ash, %	6.19	6.29	6.05	5.80	5.54
NEm, Mcal/Kg[4]	1.72	1.79	1.86	1.94	1.96
NEg, Mcal/Kg[4]	1.11	1.16	1.23	1.30	1.31

[1] Trans.: transition diet, formulated according to NASEM (2016).
[2] Dried distillers grains with solubles.
[3] Composition (dry matter basis): 15% Ca; 1.9% S; 1.5% Mg; 4.5% Na; 1.6% P; 1,715 ppm Zn; 1,285 ppm Mn; 428 ppm Cu; 21 ppm I; 5.7 ppm Se; 8.5 ppm Co; 285 ppm Fe; 86,000 IU vitamin A; 115,000 IU vitamin D3; 105 IU vitamin E; 17% urea.
[4] Computed from total digestible nutrients using empirical equations as adopted by NASEM (2016). NEm: net energy for maintenance; NEg: net energy for gain.

2.3 Performance and carcass traits

Bulls were weighed at the beginning of the experiment (d0), at the end of the adaptation period (d22), and at the end of the trial (d116), following a 16-h fasting period. Thus, feedlot performance and DMI were reported for the adaptation, finishing, and total periods. To calculate the observed dietary net energy (NE) of the adaptation period, net energy requirements for gain (NEg) and for maintenance (NEm) were estimated according to the equations proposed for *Bos indicus* bulls by Chizzotti et al. (2008). The observed dietary NE was then calculated as described by Zinn and Shen (1998).

On d105, fat thickness at the 12 rib, *Biceps femoris* (BF) fat thickness, and ribeye area (REA) were measured using an Aloka SSD-1100 Flexus RTU ultrasound unit (Aloka, Tokyo, Japan) equipped with a 3.5-MHz probe (17.2 cm), as described by

Pinheiro et al. (2011). Images were digitized and analyzed by an experienced technician using the Echo Image Viewer 1.0 software (Pie Medical Equipment B.V., Maastricht, The Netherlands). At the end of the experiment, the animals were shipped to a commercial abattoir (~500 km). On the following day, bulls were stunned by brain concussion using a captive dart gun and the hot carcass weight (HCW) was obtained from all animals after hide removal and evisceration. Carcass daily gain (CDG) was calculated using the following equation: CDG = (final HCW – (initial BW × 50%))/experimental period (115 days). The dressing percentage was calculated by dividing HCW by final BW.

2.4 Feeding behavior

Bulls were visually observed on d7 and d19 of the adaptation period to evaluate feeding behavior. The observations were conducted at fixed intervals (instantaneous sampling) of 5 minutes over 24 h (Altmann, 1974). The following behavioral categories were recorded according to Robles et al. (2007): eating, ruminating, and resting. The number of meals was calculated from eating behavior data, using criteria to define them based on the minimum time required to consider two periods of eating activity as separate events. The DMI per meal was calculated by dividing the total DMI by the number of meals. The meal length (minutes per day) was calculated by dividing the total time spent eating by the number of meals. Feed and orts were sampled for NDF analysis as previously described to calculate the eating and rumination rates of DM and NDF according to (Niehues et al., 2022).

2.5 Blood sample collection and preparation for metabolomic analyses

Blood samples were collected once at the end of the adaptation period (d22) from 40 randomly chosen bulls: 20 per block and 10 per treatment group. Approximately 10 mL of blood was sampled by puncture of the coccygeal blood vessels into evacuated tubes without anticoagulant (BD Vacutainer, São Paulo, SP, Brazil). The tubes were placed on ice, transported to the laboratory within 30 min of sampling, and centrifuged at 2000×*g* for 15 min at 4ºC. The supernatant (serum) was transferred to labeled plastic tubes and stored at -80ºC until analysis.

Prior to nuclear magnetic resonance spectroscopy (^{1}H-NMR), samples were processed to remove serum proteins. Ultra-15 centrifugal filter units (3-kDa cutoff, Amicon, Merck Millipore Ltd., Ireland) were rinsed five times each with 0.5 µL of Milli-Q water, centrifuged at 13,000×*g* for 5 min at 4ºC, and once with the filter inverted at 7,500×g for 1 min to eliminate any residue of glycerol bound to the filter membranes and Milli-Q water. Next, 500 µL of each serum sample was transferred to the washed filters and centrifuged at 13,000×*g* for 30 min at 4ºC to remove macromolecules (primarily protein and lipoproteins). The filtered content was then vacuum centrifuged and stored at -80ºC.

2.6 Nuclear magnetic resonance spectroscopy

On the day of ^{1}H-NMR analysis, the dried residues were solubilized in 550 µL of phosphate buffer (D_2O-based PBS; 0.1 M, pH = 7.4) containing 0.5 mM of 3-(trimethylsilyl)-2,2',3,3'-tetradeuteropropionic acid (TMSP-d_4) used as internal NMR standard. The solution of each sample was then transferred to a standard 5-mm NMR tube for NMR measurements.

1D ^{1}H-NMR experiments were performed on a Bruker Avance III 14.1 T spectrometer (Bruker Corporation, Karlsruhe, Baden Württemberg, Germany) fitted with a 5-mm Broadband Observe (BBO) probe with ATMA® (Automatic Tuning Matching), a z-field gradient, a BCU-I variable temperature unit, a field gradient generator unit, and a Sample-Xpress™ automatic sample changer. Standard ^{1}H spectra were acquired at 298.15 K using a NOESY-1D pulse sequence (named noesypr1d in the TopSpin Bruker software), with water signal suppression by irradiation at 2821.88 Hz (O1). The acquisition parameters were as follows: number of scans (ns) of 256, spectral width (sw) of 12019 Hz (20.0276 ppm), 90º pulse (P1) of 14.85 µs, acquisition time (aq) of 4.50 s, relaxation delay (d1) of 4 s, data points (TD) of 108170 (106 K), mixing time (d8) of 5 ms, and dummy scans (ds) of 4.

Spectra were processed with line broadening of 0.3 Hz using the TopSpin™ 3.6.1 software (Bruker BioSpin, Germany). Phase and baseline corrections were performed manually using the Chenomix NMR Suite 8.4 (Chenomix Inc., Edmonton, Canada). The metabolites were manually identified in the 1D ^{1}H-NMR spectra using the in-built compounds library on the Chenomix Profiler tool. For metabolite

quantitation, TMSP (signal at 0.00 ppm) was used as the internal standard of known concentration (0.5 mM).

2.7 Statistical analysis and bioinformatics

All phenotypic traits were analyzed using the SAS software (SAS Institute Inc., Cary, NC). The normality of residuals was verified by the Shapiro-Wilk test and outliers were removed using the UNIVARIATE procedure. Homogeneity of variances was verified using the Box-Cox test. Data that did not show a normal distribution were transformed using PROC RANK (SAS 9.4). The data were analyzed in a completely randomized block design using the PROC MIXED procedure, where the pen was the experimental unit, treatments were the fixed effects, and block and pen were random effects. The model can be written as:

$$Yijt = \mu + Bi + Aj + Pt + eijt,$$

where Y = dependent variable, μ = overall mean, Bi = random effect of block, Aj = random effect of pen, Pt = fixed effect of treatment, and $eijt$ = experimental error.

The results were reported as least square means (LSMEANS statement). Significance was set as $P \leq 0.05$ and trends were considered at $0.05 < P \leq 0.10$.

Metabolomic data were analyzed using the R software v.4.4.0 (R Found., Vienna, Austria) and MetaboAnalyst 6.0. The metabolite concentration data were uploaded to MetaboAnalyst and cubic transformation and Pareto scaling were applied before analysis. Supervised (partial least-squares discriminant analysis, PLS-DA) data analysis was performed. The PLS-DA model was cross-validated using the leave-one-out cross-validation method and the performance measure ‘accuracy’. The variable importance in the projection (VIP) plot was used to rank the metabolites based on their importance in discriminating groups. Metabolites with the highest VIP values are the most powerful group discriminators. Typically, VIP values > 1 are significant and VIP values > 2 are highly significant. Pathway analyses were conducted on the metabolite quantification datasets according to group using the *Bos taurus* library (KEGG). Given the exploratory nature of this study, we classified pathways with a raw $P < 0.1$ as being of high impact and interest.

3. Results

3.1 Intake, performance and carcass traits

During the 21-day adaptation period, bulls fed NYC tended (P = 0.09) to have a greater ADG (2.097 ± 0.46 kg) than bulls fed MVY (1.750 ± 0.52 kg) (Table 2). There was no treatment effect (P > 0.05) on DMI, final BW, or feed efficiency. During the finishing period (d22 to d115), bulls fed NLY during adaptation had a lower (P = 0.04) DMI as a percentage of BW (1.92 ± 0.09%) than bulls submitted to the MVY treatment (2.05 ± 0.11%). Consequently, bulls fed NLY tended (P = 0.09) to have a lower overall DMI as a percentage of BW (2.01 ± 0.09%) throughout the total feedlot period compared to bulls fed MVY (2.11 ± 0.11%), with no negative impact on performance. Feed efficiency and final BW were similar among treatments (P ≥ 0.14) for all periods. The effects of the treatments on observed NE and observed/expected NE ratio are shown in Table 3. Neither observed NE nor observed/expected NE ratio differed among treatments (P > 0.05).

Most carcass traits were not influenced (P > 0.05) by treatment (Table 4). However, bulls fed NYC tended (P = 0.07) to have a greater REA (75.47 ± 9.81 cm^2) than bulls fed NLY (69.45 ± 9.94 cm^2). Additionally, bulls fed MVY tended (P = 0.07) to have a greater 12th rib fat thickness (4.68 ± 1.21 mm) than bulls fed NDY (3.78 ± 1.23 mm). HCW, CDG, dressing percentage, or BF fat thickness did not differ among treatments (P ≥ 0.16).

Table 2 - Feedlot performance, intake, and efficiency of Nellore bulls fed different combinations of yeast products with antimicrobials during the adaptation period

Item[3]	Treatments[2]				SEM[1]	*P - value*
	MVY	NLY	NYC	NDY		
Pens (bulls)	6 (30)	6 (30)	6 (30)	6 (30)	--	--
Initial BW (Kg)	388.09	386.53	388.09	387.49	5.75	0.99
0-21 days (Adaptation)						
Final BW (Kg)	425.85	423.81	432.08	425.88	5.82	0.75
ADG (Kg)	1.750	1.825	2.097	1.837	0.096	0.09
Daily DMI (Kg)	9.74	9.86	9.91	9.80	0.161	0.88
Daily DMI (% BW)	2.40	2.44	2.42	2.41	0.019	0.62
G: F (kg/kg)	0.179	0.180	0.210	0.186	0.010	0.14
22-115 days (Finishing)						
ADG (Kg)	1.565	1.458	1.587	1.558	0.056	0.40
Daily DMI (Kg)	10.18	9.44	10.17	10.01	0.214	0.07
Daily DMI (% BW)	2.05a	1.92b	2.01ab	2.00ab	0.029	0.04
G: F (kg/kg)	0.153	0.154	0.154	0.156	0.005	0.97
0-115 days (Total feeding period)						
Final BW (Kg)	571.90	560.93	581.10	572.42	9.142	0.50
ADG (Kg)	1.598	1.516	1.679	1.609	0.049	0.16
Daily DMI (Kg)	10.10	9.52	10.12	9.97	0.197	0.14
Daily DMI (% BW)	2.11	2.01	2.10	2.08	0.027	0.10
G: F (kg/kg)	0.157	0.159	0.164	0.161	0.003	0.56

[1]Standard Error of Mean.

[2] MVY: monensin at 18 mg/kg of dry matter (DM) + virginiamycin at 15 mg/kg of DM + Live Yeast at 2g/head/d; NLY: narasin at 13 mg/kg of DM + Live Yeast at 2g/head/d; NYC: narasin at 13 mg/kg of DM + Yeast culture at 7g/head/d; NDY: narasin at 13 mg/kg of DM + Active dry yeast at 1g/head/d; treatments were provided during the adaptation period.

[3]BW: body weight; ADG: average daily gain; DMI: dry matter intake; G: F: feed efficiency.

Table 3- Observed dietary net energy (adaptation diet) of feedlot Nellore bulls fed different combinations of yeast products with antimicrobials during the adaptation period

Item[2]	Treatments[3]				SEM[1]	*P - value*
	MVY	NLY	NYC	NDY		
Pens (bulls)	6 (30)	6 (30)	6 (30)	6 (30)	--	--
Dietary NE, Mcal/kg						
Maintenance	1.73	1.72	1.90	1.77	0.053	0.111
Gain	1.11	1.10	1.25	1.14	0.047	0.112
Observed/expected NE ratio						
Maintenance	1.00	1.00	1.10	1.02	0.031	0.112
Gain	1.00	1.00	1.13	1.07	0.042	0.112

[1]Standard Error of Mean.
[2] MVY: monensin at 18 mg/kg of dry matter (DM) + virginiamycin at 15 mg/kg of DM + Live Yeast at 2g/head/d; NLY: narasin at 13 mg/kg of DM + Live Yeast at 2g/head/d; NYC: narasin at 13 mg/kg of DM + Yeast culture at 7g/head/d; NDY: narasin at 13 mg/kg of DM + Active dry yeast at 1g/head/d; treatments were provided during the adaptation period.

Table 4 – Carcass traits of feedlot Nellore bulls fed different combinations of yeast products with antimicrobials during the adaptation period

Item[3]	Treatments[2]				SEM[1]	*P - value*
	MVY	NLY	NYC	NDY		
Pens (bulls)	6 (30)	6 (30)	6 (30)	6 (30)	--	--
HCW (Kg)	316.40	310.52	322.78	316.98	4.172	0.24
Carcass daily gain (kg)	1.063	1.017	1.118	1.072	0.029	0.16
Dressing percentage	55.29	55.33	55.61	55.36	0.275	0.85
REA (cm²)	71.11	69.45	75.47	71.00	1.621	0.08
12th rib fat (mm)	4.68	3.98	4.29	3.78	0.232	0.07
BF fat thickness (mm)	9.81	9.12	9.55	9.58	0.416	0.75

[1]Standard Error of Mean.
[2] MVY: monensin at 18 mg/kg of dry matter (DM) + virginiamycin at 15 mg/kg of DM + Live Yeast at 2g/head/d; NLY: narasin at 13 mg/kg of DM + Live Yeast at 2g/head/d; NYC: narasin at 13 mg/kg of DM + Yeast culture at 7g/head/d; NDY: narasin at 13 mg/kg of DM + Active dry yeast at 1g/head/d; treatments were provided during the adaptation period.
[3] HCW: hot carcass weight; REA: ribeye area; BF: *Biceps femoris* muscle.

3.2 Feeding behavior

Nellore bulls fed NDY spent (P = 0.03) more time resting (949.27 ± 26.24 min) than bulls fed MVY (899.06 ± 32.82 min). On the other hand, animals that received

NDY tended (P = 0.06) to have fewer meals per day (16.55 ± 3.62) compared to those submitted to the MVY (19.69 ± 3.62) and NLY (19.84 ± 5.84) treatments. Accordingly, animals fed NDY tended (P = 0.09) to have a greater DMI per meal (0.64 ± 0.30 kg) than those fed MVY (0.54 ± 0.10 kg). The time spent ruminating and eating, meal length, DMI, NDF intake, and eating and rumination rates of DM and NDF were similar among treatments (P ≥ 0.23).

Table 5 Feeding behavior of feedlot Nellore bulls fed different combinations of yeast products with antimicrobials during the adaptation period

Item[3]	Treatments[2]				SEM[1]	*P - value*
	MVY	NLY	NYC	NDY		
Pens (bulls)	6 (30)	6 (30)	6 (30)	6 (30)	--	--
Time spent resting (min)	899.06b	927.45ab	930.62ab	949.27a	11.19	0.03
Time spent ruminating (min)	311.19	299.33	295.78	284.96	10.37	0.37
Time spent eating (min)	216.76	202.73	203.66	197.07	7.94	0.37
DMI (kg)	9.31	9.53	9.51	9.35	0.20	0.56
Meals per day (n)	19.69	19.84	18.89	16.55	0.90	0.06
Meal length (min)	11.04	10.73	11.26	12.36	0.53	0.26
DMI per meal (kg)	0.54	0.54	0.58	0.64	0.02	0.09
NDF intake (kg)	4.44	4.52	4.54	4.45	0.09	0.80
RRDM (min/kg of DM)	30.14	28.66	27.80	26.85	1.11	0.23
RRNDF (min/kg of NDF)	70.86	69.19	65.65	67.33	2.74	0.41
ERDM (min/kg of DM)	21.21	19.74	19.42	19.29	0.83	0.40
ERNDF (min/kg of NDF)	49.72	45.62	42.85	44.19	2.73	0.47

[1]Standard Error of Mean.
[2] MVY: monensin at 18 mg/kg of dry matter (DM) + virginiamycin at 15 mg/kg of DM + Live Yeast at 2g/head/d; NLY: narasin at 13 mg/kg of DM + Live Yeast at 2g/head/d; NYC: narasin at 13 mg/kg of DM + Yeast culture at 7g/head/d; NDY: narasin at 13 mg/kg of DM + Active dry yeast at 1g/head/d; treatments were provided during the adaptation period.
[3]DMI: dry matter intake; NDF: Neutral detergent fiber; RRDM: rumination rate of DM; RRNDF: rumination rate of NDF; ERDM: eating rate of DM; ERNDF: eating rate of NDF.

3.3 Serum Metabolomics Profiling

Forty-seven metabolites were identified in the NMR spectra of serum samples from Nellore bulls, including essential and nonessential amino acids, small peptides,

amino acid derivatives, organic acids, nucleic acids, sugar, vitamins, and cellular antioxidants. PLS-DA was performed to visualize differences in metabolite profiles among the four treatment groups. The variate1/variate2 score plot (Figure 1) shows no clear distinction among the NAR-fed groups (NLY, NYC, NDY), indicating similarities in their serum metabolome. However, there was a clear distinction between the MVY and NYC groups, indicating that their metabolite profiles differ. Furthermore, the MVY group showed a slight overlap with NYC and NLY, suggesting certain similarities despite the overall difference in their serum metabolomes. Since the metabolite profiles of the NAR-fed groups appeared to differ from those of the MVY group, overall and pairwise comparisons using a PLS-DA model and subsequent VIP scores were performed across the four treatments and between MVY vs. NLY and MVY vs. NYC to elucidate specific metabolite differences. The VIP score plots including the top metabolites that discriminate the four treatment groups, as well as MVY from NLY and MVY from NYC, are shown in Figure 2A-2C.

Lactate was the most important metabolite for discriminating all treatment groups (Figure 2A) and MVY from NLY (Figure 2C), as well as the sixth most important for discriminating MVY from NYC (Figure 2B), with consistently higher concentrations in the MVY group. Glucose, dimethylamine, betaine, 3-hydroxybutyrate, alanine, methionine, 3-phenylpropionate, 2-hydroxyisobutyrate, glycerol, tyrosine, and allantoin exhibited VIP scores > 1.0 and were identified as key metabolites for differentiating the four treatment groups (Figure 2A). Comparing the MVY and NYC groups, the latter showed higher concentrations of 3-phenylpropionate, hippurate, betaine, glycolate, butyrate, glucose, tyrosine, alanine, glycine, choline, and glutamine, whereas higher concentrations of lactate, histidine, 2-hydroxyisobutyrate, and methionine were observed in the former (Figure 2B). Comparing the MVY and NLY groups, the latter showed higher concentrations of citrate, alanine, tyrosine, betaine, glycerol, and creatine phosphate, whereas the former exhibited higher concentrations of lactate, creatine, valine, phenylalanine, and isoleucine (Figure 2C).

The pathway enrichment plots discriminating MVY from NYC and MVY from NLY are shown in Figure 3A-3B. The three most relevant pathways differentiating MVY from NYC were methylhistidine metabolism, gluconeogenesis, and sphingolipid metabolism (Figure 3A). The three most relevant pathways differentiating MVY from

NLY were pyruvate metabolism, catecholamine biosynthesis, and thyroid hormone synthesis (Figure 3B).

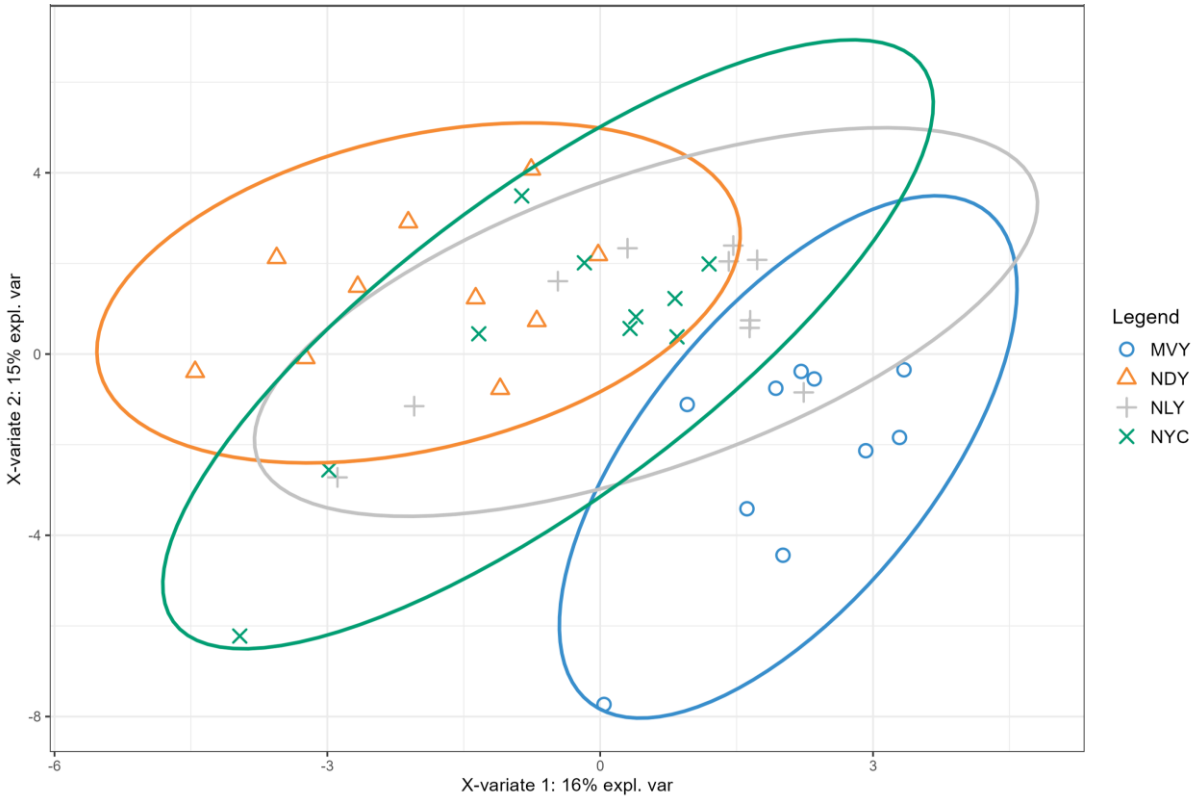


Figure 1: Partial least squares discriminant analysis (PLS-DA) score plot of serum metabolite distribution according to treatment. MVY: monensin + virginiamycin + live yeast; NLY: narasin + live yeast; NYC: narasin + yeast culture; NDY: narasin + active dry yeast.

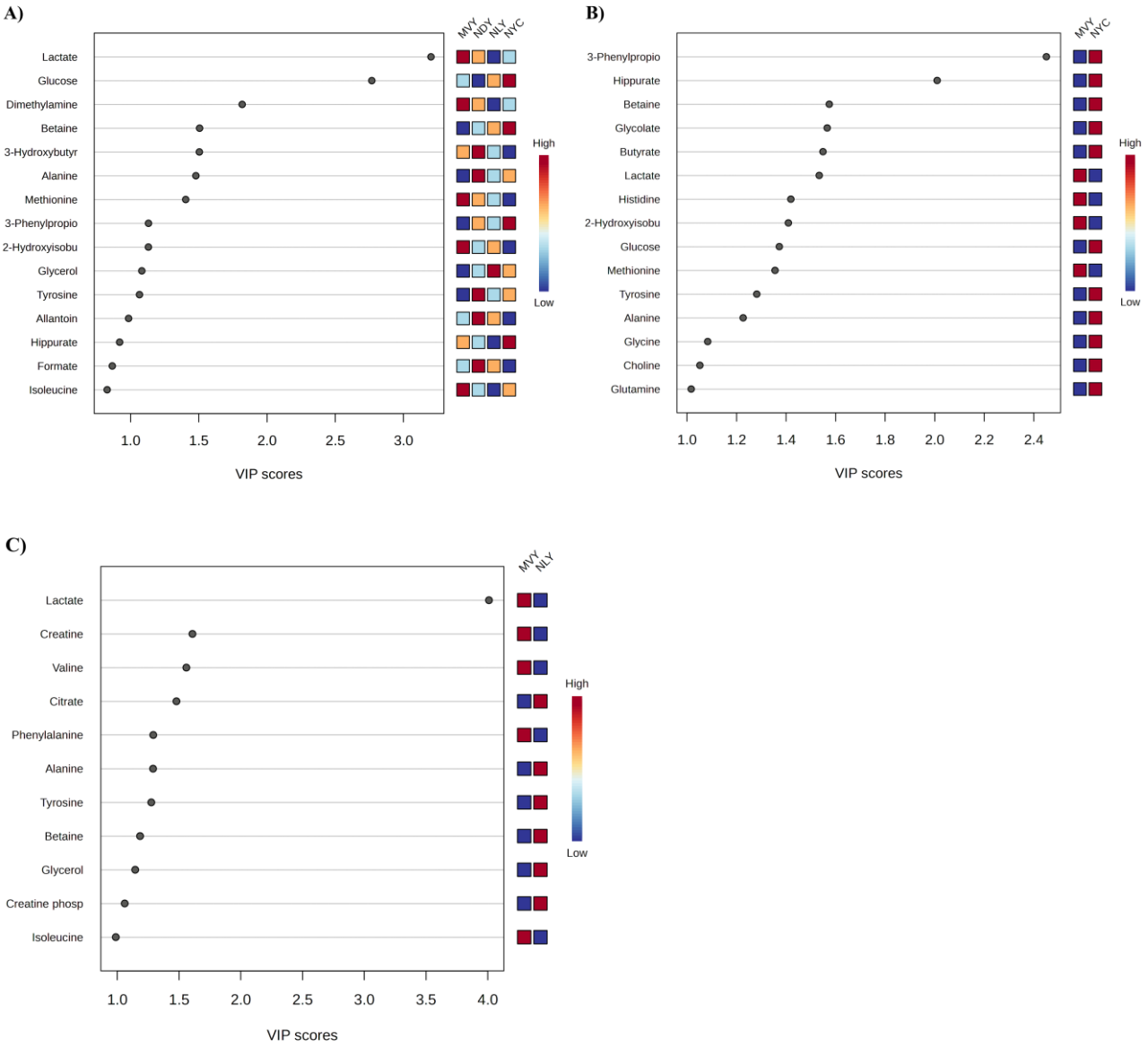


Figure 2: Variable importance in the projection (VIP) analysis ranking the top metabolites whose relative serum levels differ between treatment groups. (**A**) monensin + virginiamycin + live yeast (MVY), narasin + active dry yeast (NDY), narasin + live yeast (NLY), narasin + yeast culture (NYC) (R^2 = 0.99 and Accuracy = 0.28); (**B**) monensin + virginiamycin + live yeast (MVY), narasin + yeast culture (NYC) (R^2 = 0.91 and Accuracy = 0.66); (**C**) monensin + virginiamycin + live yeast (MVY), narasin + live yeast (NLY) (R^2 = 0.94 and Accuracy = 0.55).



Figure 3: Overview of quantitative enrichment analysis showing the top 25 pathways according to *P*-value. (**A**) monensin + virginiamycin + live yeast (MVY) vs. narasin + yeast culture (NYC); (**B**) monensin + virginiamycin + live yeast (MVY) vs. narasin + live yeast (NLY).

4. Discussion

The present study investigated the effects of different combinations of yeast products (live yeast, yeast culture, and active dry yeast) with antimicrobials (MON, VM, and NAR) during adaptation of feedlot Nellore bulls. The results indicated that feeding NYC during the adaptation period tended to improve performance compared to MVY, without any noticeable effects during the post-adaptation period. Animals fed NLY had a lower DMI during the post-adaptation period than those fed MVY. Considering that both treatments contain the same yeast product, the different antimicrobials may have influenced rumen kinetics and, consequently, rumen development differently. Within this context, metabolomics was used to better understand the phenotypic differences between treatments. As previously described by Cônsolo et al. (2022), this study also demonstrated the potential of applying metabolomics to bio-fluids for a better understanding of beef cattle metabolism and behavior, associating the findings with nutritional modulation. Overall, the serum metabolite data suggest differences in energy and protein metabolism between the MVY and NAR-fed groups.

Although some studies have included yeast products in ruminant nutrition, the effect of yeast supplementation on ruminal parameters, digestibility and performance of beef cattle will vary depending on product type, strain, dose, and diet (Sartori et al., 2017; Cagle et al., 2020). Shen et al. (2018) found that, compared to 330 mg MON/day, ruminal yeast culture supplementation increased ruminal acetate concentration and organic matter and NDF digestibility and tended to reduce the time ruminal pH remained below 5.6 in heifers fed high-grain diets. Similarly, Geng et al. (2016) demonstrated that yeast culture supplementation increased the molar percentage of acetate compared to active dry yeast in finishing bulls fed diets containing 31% forage. Both supplements were combined with MON. In the present study, since DMI was similar among treatments, the trend of the NYC treatment to provide greater ADG compared to MVY might be related to a potential increase in fiber digestibility, enhanced by the yeast culture. Although amino acids are not essential for mixed rumen bacteria, aromatic amino acids such as tyrosine and phenylalanine can stimulate the fermentation rate and microbial growth yield (Cotta and Russell, 1982; Guliye et al., 2005). Atasoglu et al. (2001) reported that phenylalanine can be limiting for certain species of cellulolytic ruminal bacteria, including *Fibrobacter succinogenes*. In addition

to minerals, vitamins and NDF, the yeast culture of the NYC treatment contained amino acids such as tyrosine, phenylalanine, glycine, proline, and leucine. Tyrosine can be synthesized from phenylalanine by rumen microorganisms and both metabolites can be converted to phenylpropionic acid, which is similarly beneficial for improving cellulose degradation by stimulating the growth of *Ruminococcus albus* (Hungate and Stack, 1982; Khan et al., 1999). The metabolites 3-phenylpropionate and tyrosine were important for the discrimination of the NYC and MVY groups, with higher serum concentrations in the NYC group. These findings suggest that the yeast culture in the NYC treatment may have enhanced fiber digestibility. Given the high fiber content of adaptation diets, this potential enhancement of fiber digestibility may have contributed to the increase in ADG observed in the NYC group.

Megasphaera elsdenii is the most important ruminal bacteria that utilizes lactic acid and therefore plays a key role in preventing ruminal lactic acid accumulation (Nagaraja and Titgemeyer, 2007). According to Russell (2002), *M. elsdenii* is an ammonia producer but can only use a limited number of amino acids for hydrolysis, such as serine and threonine, highlighting the importance of these amino acids for the growth of this bacteria. Accordingly, Rossi et al. (2004), assessing the effects of peptides fractions from yeast culture on the growth and metabolism of *M. elsdenii*, found that the hydrophilic peptide fraction, which is rich in serine and threonine, stimulated cell growth and the production of butyrate (+100%) and valerate (+76.1%), as well as lactate fermentation (+16.9%). The higher concentration of amino acids in the yeast culture in NYC may have enhanced the growth of *M. elsdenii*, supported by the higher serum levels of butyrate and lower levels of lactate in this group compared to MVY.

The second most important metabolite to differentiate NYC from MVY was hippurate, which is related to the most important metabolite for this differentiation, 3-phenylpropionate. Hippurate is a glycine conjugate of benzoic acid and a component normally found in the urine of animals, which is strongly associated with diet and microbiota (Lees et al., 2013). Pagella et al. (1997) found that most of the 3-phenylpropionic acid infused into the rumen of sheep is absorbed across the mucosal epithelium and transported to the liver, where it undergoes oxidative reactions and is converted to benzoic acid. The latter is then conjugated with glycine to form hippuric

acid, which is subsequently excreted (Chesson et al., 1999). Thus, the increase in 3-phenylpropionate observed in the NYC group may have contributed to the higher serum hippurate levels in bulls of this group, confirming that yeast culture composition positively contributes to increasing the ruminal concentration of these important metabolites for fiber digestibility.

Lactate was important for discriminating the four treatments, with higher levels in the MVY group. Oliveira Júnior et al. (2023) found that, compared to exclusive MON supplementation, the combined use of MON and VM in feedlot diets increased serum D-lactate levels, upregulated the expression of pyruvate carboxylase and lactate dehydrogenase, and tended to increase the expression of propionyl-CoA carboxylase in the liver. These changes indicated higher ruminal metabolic activity in the MON and VM groups, increasing ADG compared to the exclusive MON group (Oliveira Júnior et al., 2023). Increased lactate dehydrogenase expression suggests that, along with D-lactate, serum L-lactate may also be elevated (Ewaschuk et al., 2005). Hence, the higher serum levels of lactate in the MVY group are probably associated with increased ruminal production of D- and L-lactate, as well as with endogenous L-lactate.

One-carbon (methyl) metabolism is a complex network of interrelated biochemical reactions involving the transfer of one-carbon groups, which plays a pivotal role in biological methylation and nucleotide synthesis (Mason, 2003). The two main methyl donors in animal metabolism are betaine and S-adenosylmethionine (SAM), metabolites derived from choline and methionine metabolism, respectively (Pinotti et al., 2002). Since choline is extensively degraded in the rumen, the contribution of dietary choline to the choline body pool is neglectable (Baldi and Pinotti, 2006). For that reason, methionine synthase, the enzyme responsible for the de *novo* synthesis of methionine methyl groups, plays an important role in maintaining the methyl supply of ruminants (Dawson et al., 1981; Pinotti et al., 2002). According to Zeisel & Blusztajn (1994), when methionine and SAM levels are adequate, the latter will increase the level of S-adenosylhomocysteine, which limits the endogenous synthesis of choline through the phosphatidylethanolamine N-methyltransferase pathway. In this context, the higher levels of methionine in the MVY group would explain the lower levels of choline and betaine compared to the NYC group. It is reasonable to believe that there was an increase in microbial protein synthesis due to

a potential increase in rumen metabolic activity in the MVY group as mentioned above, elevating the supply of methionine (Schwab and Broderick, 2017). This suggestion is supported by the elevated levels of other essential amino acids such as histidine, isoleucine, phenylalanine, and valine in the MVY group, which can be synthesized by ruminal bacteria (Schwab, 1995), as well as by enrichment analysis, in which the over-represented pathways were mostly correlated with amino acid metabolism (Figure 3A). Although the MVY treatment appears to enhance the uptake of essential amino acids, it was insufficient to improve animal performance compared to the NYC treatment.

Given the lack of substantial effects of the MVY and NLY treatments on DMI and performance during the adaptation period, the lower DMI of animals fed NLY during the finishing period may be linked to changes in ruminal fermentation patterns and in the serum metabolome. Overall, differences in serum metabolites between these groups were correlated with changes in energy and protein metabolism. Accordingly, the over-represented pathways in serum were correlated with energy metabolism, including pyruvate metabolism and gluconeogenesis (Figure 3B). Cônsolo et al. (2021) found higher serum concentrations of citrate and creatine phosphate in cattle that are more efficient in terms of residual feed intake, suggesting that the higher levels of these metabolites in NLY may represent a metabolic adaptation to substrate uptake. Additionally, in accordance with Dias Batista et al. (2024), who reported a linear increase in branched-chain volatile fatty ruminal concentration with an increase in VM dose in feedlot diets, the higher levels of certain branched-chain amino acids, such as valine and isoleucine, in the MVY group compared to NLY may reflect the impact of this molecule on rumen dynamics, potentially promoting rumen development and thereby explaining the greater DMI observed in MVY compared to NLY post-adaptation. Furthermore, serum lactate may also reflect the occurrence of some alterations in rumen pH and consequently in feeding behavior. According to González et al. (2012), increasing the distribution and reducing the size of meals throughout the day can help control rumen pH since it improves the synchronization between the production and uptake or neutralization of volatile fatty acids. The trend toward increasing meals per day in the MVY group compared to NDY, along with a numerical increase in the time spent ruminating and reduced resting time, may be a response to controlling rumen acidification.

5. Conclusion

The serum metabolome data revealed that Nellore bulls fed NYC during the adaptation period had a higher concentration of metabolites associated with fiber digestibility compared to the other treatments, which likely contributed to the enhanced animal performance. Further research should investigate the effect of combining antimicrobials and yeast culture on rumen fermentation and diet digestibility in feedlot cattle during the adaptation period.

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CHAPTER 3 – A META-ANALYSIS OF THE EFFECTS OF MONENSIN SUPPLEMENTATION ON BEEF CATTLE PRODUCTION IN THREE FEEDING SYSTEMS

ABSTRACT – This meta-analysis evaluated the effects of monensin supplementation on beef cattle performance, ruminal parameters, and total tract digestibility in different feeding systems, including total mixed ration (TMR), partial TMR, and pasture. The dataset included 131 peer-reviewed publications that comprised 349 treatment means. The effects of monensin supplementation were evaluated using the weighted mean differences (WMD) between the control (diets without monensin) and treatment (diets with monensin) groups. Heterogeneity was explored through meta-regression and subgroup analysis, which considered factors inherent to the animal, such as diet, monensin dose, and experimental traits. Monensin supplementation reduced (P < 0.01) dry matter intake (DMI) (pasture: -0.92 kg/day; partial TMR: -0.12 kg/day; TMR: -0.25 kg/day) and increased average daily gain (ADG = 0.06, 0.05 and 0.02 kg/day, respectively) in all feed systems. In the partial TMR system, genetic group and experimental design influenced (P < 0.01) the effect of monensin on propionate and butyrate ruminal concentrations. Additionally, supplement intake and type affected the effect of monensin on feed conversion ratio (FCR) and propionate ruminal concentration. In the partial TMR system, a monensin intake of 200–300 mg/day promoted the greatest improvement in FCR (WMD = -1.07 kg/kg; P = 0.011). In the TMR system, genetic group, sex class, fattening period, and dietary concentrate and crude protein levels influenced (P < 0.01) the effect of monensin on DMI and ADG. Forage type affected the effect of monensin on ADG, acetate, butyrate, and isovalerate (P < 0.01). Monensin inclusion up to 15 mg/kg DM in TMR diets improved FCR (WMD = -0.54 kg/kg; P = 0.001) and increased ADG (WMD = 0.055 kg/day; P = 0.001). The effects of monensin on ADG, DMI, and ruminal parameters varied across feeding systems and were influenced by factors such as diet composition, monensin intake levels, genetic group, sex class, and experimental conditions. Thus, all these factors should be considered when analyzing the response to monensin rather than focusing solely on the general effects.

Keywords: beef cattle, feedlot, monensin, performance, pasture.

1. Introduction

Sodium monensin is a carboxylic polyether produced by *Streptomyces cinnamonensis* that modulates the ruminal environment by altering the metabolism of Gram-positive bacteria (Nagaraja, 1995). Since its registration in 1975, monensin has been one of livestock's most widely studied growth promoters (Goodrich et al., 1984), particularly ruminants. The effect of monensin on Gram-positive bacteria significantly alters ruminal fermentation, consequently impacting the volatile fatty acid (VFA) profile,

ruminal protein metabolism, and host metabolism (Russell & Strobel, 1989; Nagaraja, 2007). It is well documented that monensin increases average daily gain (ADG) while decreasing dry matter intake (DMI), thereby improving the feed efficiency of beef cattle (Tedeschi et al., 2003).

Given the importance of monensin for the beef industry, in addition to experimental studies, meta-analyses and reviews have been performed to establish the overall effect of this feed additive (Goodrich et al., 1984; Tedeschi et al., 2003; Duffield et al., 2012; Ellis et al., 2012; Gadberry et al., 2022). However, it remains unclear how experimental factors such as diet composition and animal characteristics influence the response to monensin in different feeding systems, including pasture, total mixed ration (TMR), and partial TMR systems. So far, no studies in the literature have directly compared these factors' impact.

Meta-analysis is a powerful statistical approach that analyzes large sets of heterogeneous data from multiple independent studies with similar aims to synthesize the results and elucidate data behavior (Sutton and Higgins, 2008). Within this context, studies have identified variations in the effects of monensin on performance and rumen parameters according to diet composition, animal traits, and feeding systems (Clary et al., 1993; Bretschneider et al., 2008; Wood et al., 2016; Martinez et al., 2022). We hypothesized that study-related factors may influence the effect of monensin depending on the feeding system evaluated. Therefore, this meta-analysis aimed to assess monensin's impact on performance, ruminal parameters and digestibility, and to identify covariates that affect these outcomes in different feeding systems.

2. Material and Methods

2.1. Data set

A comprehensive literature search was conducted using the scientific databases PubMed and Science Direct. The publications were retrieved using the search terms "beef cattle and monensin", "monensin and feedlot cattle", and "monensin and pasture cattle". Only articles that met the following predetermined standardized criteria were included in the meta-analysis: 1) control without monensin or other feed additives, 2) treatment with monensin, without additional feed additives, and 3) only data referring

to animals intended for meat production. A flowchart describing the process of study identification and selection for analysis is shown in Figure 1.

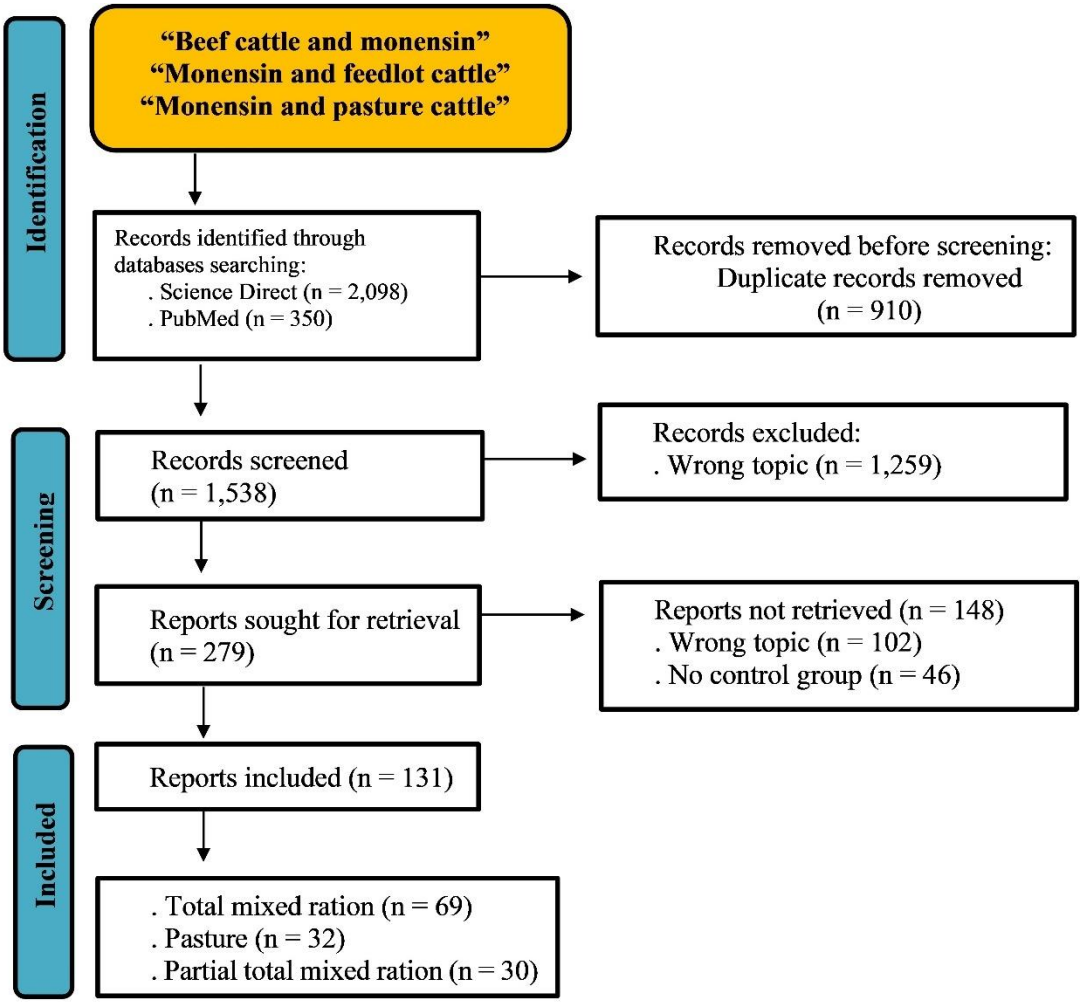


Figure 1: Flowchart showing the inclusion criteria for selecting studies used in the meta-analysis on the effects of Monensin on beef cattle under different feeding systems and diet profiles.

Based on inclusion criteria, 131 peer-reviewed publications were selected. The replicates number, means, and variances (i.e., standard error (SE), standard deviation (SD)) were extracted from the control and monensin treatments for various response variables.

2.2 Statistical analysis

2.2.1 Weighted mean difference and Publication bias

The meta-analysis used the R Statistical Software (Metafor package, version 4.6-0; Viechtbauer, 2010). Forest plots were created using the STATA software (version 16.0; StataCorp LP, College Station, TX). The effects of monensin inclusion in the diet on DMI, digestibility, performance, and carcass and ruminal parameters in beef cattle submitted to different feeding systems were evaluated by examining the weighted mean difference (WMD) between the control group (diets without monensin) and treatment group (diets with monensin). The inverse of the variance-weighted treatment means according to the method DerSimonian & Laird (1986) proposed for random-effects models.

Between-study heterogeneity (i.e., heterogeneity in treatment effects) was evaluated using the chi-square (Q) heterogeneity test and I^2 statistics. The latter measures the percentage of variation due to heterogeneity (Higgins et al., 2003). Zero was assigned to negative I^2 values. I^2 values < 25% indicate low heterogeneity, whereas values between 25 and 50% denote moderate heterogeneity and those > 50% indicate high heterogeneity (Higgins et al., 2003).

Publication bias was evaluated using funnel plots (Light and Pillemer, 1984) and asymmetry between the WMD and SE according to the Egger regression asymmetry test (Egger et al., 1997). Significance was set at $P \leq 0.05$. Outliers were removed when studentized residuals were outside the range of -2.5 to 2.5.

2.2.2 Meta-regression and Subgroup analysis

Meta-regression analysis was conducted to identify the effects of categorical covariates and select those for subgroup analysis. A mixed model was applied to fit the data in the meta-regression analysis using WMD as the dependent variable. The mixed-effect models were given by the equation $\Theta_i = \beta + \beta_i x_{ij} + \ldots \beta_{ip} x_{ip} + \mu_i$, where Θ_i is the true effect of treatment on the i^{th} explanatory variable; β is the overall true treatment effect; x_{ij} is the value of the j^{th} covariate (j = 1, 2, …, p) for the i^{th} explanatory variable; β_i is the change in the true effect size for a unit increase in the j^{th} covariate, and $\mu_i \sim N(0\ t^2)$. The t^2 indicates the heterogeneity the covariate does not explain (Viechtbauer, 2010).

Moment-based estimation of between-study variance (tau-squared, t^2) is used in Der-Simonian and Laird random-effects meta-analysis; however, this approach is less suitable for analysis when covariates are included (Thompson and Sharp, 1999). The restricted maximum likelihood (REML) method was used to estimate t^2 because it is less likely to underestimate or produce biased variance estimates (Thompson and Sharp, 1999; Viechtbauer, 2005).

Tests of the null hypothesis for the covariate coefficients were obtained from the multiparameter Wald test (Harbord and Higgins, 2008). The model's adjusted R^2 represents the proportion of between-study variance (heterogeneity) explained by the covariates (Harbord and Higgins, 2008; Viechtbauer, 2010) and was calculated by comparing the estimated between-study variance when covariates were included in the model (σ^2) with the corresponding values when they were excluded (σo^2): adjusted R^2 (%) = $(\sigma o^2 - \sigma^2)/\sigma o^2 \sigma o$.

Meta-regression criteria were 1) $P \leq 0.05$ for the heterogeneity test; 2) $P \geq 0.05$ for the funnel plot, and 3) absence of studentized residuals outside the range of -2.5 to 2.5 (outliers).

The covariates were divided as following:

Feeding System = Pasture

- Genetic type (*Bos taurus*, *Bos indicus*, and Crossbreed).
- Sex class (Steers, Heifers, Bull and Calf).
- Experiment design (Rotational [Latin square, crossover] and Continuous).
- Fattening period (<30, 60 – 90, 90 – 120, 120 – 150, 150 – 180 and 250 – 350 days).
- Monensin intake (<100, 100 – 150, 150 – 200, 300 – 400 mg/day).
- Experiment Season (Summer, Winter, Winter/Spring and Spring/summer).
- Supplement type (Mineral, Energy and Protein).
- Supplement feed (Self-fed, Hand-fed-daily and Hand-fed-alternate).
- Supplement intake (<0.5, 0.5 – 1.0, 1.0 – 1.5 and 1.5 – 2.5 kg/day).
- Forage type (Grass – C3, Grass – C4, Legume and Grass + Legume).

Feeding system = Partial TMR

- Genetic type (*Bos taurus*, *Bos indicus*, Crossbreed and Holstein).

- Sex class (Steers, Heifers, Bull, Cow and Calve).
- Experiment design (Rotational [Latin square, crossover] and Continuous).
- Fattening period (<60, 60 – 90, 90 – 120, 120 – 150, 150 – 180 and 200 – 300 days).
- Monensin intake (<100, 100 – 200, 200 – 300 and 300 – 400 mg/day).
- Supplement type (Energy and Protein).
- Supplement intake (<0.9, 0.9 – 1.5 and 2.20 – 3.20 kg/day).
- Forage type (Hay, Silage, Straw, Green chop and Fresh).

Feeding system = TMR

- Genetic type (*Bos taurus*, *Bos indicus*, Crossbreed and Holstein).
- Sex class (Steers, Heifers, Bull, Cow and Calf).
- Experiment design (Rotational [Latin square, crossover] and Continuous).
- Fattening period (<30, 30 – 60, 60 – 90, 90 – 120, 120 – 150, 150 – 180 and 180 – 210 days).
- Monensin inclusion (<15, 20 – 25, 25 – 30, 30 – 35, 35 – 40, 40 – 45 and 45 – 50 mg/kg of DM).
- Monensin intake (<100, 100 – 200, 200 – 300, 300 – 400 and 400 – 500 mg/day).
- Concentrate level (<300, 500 – 600, 600 – 700, 700 – 800, 800 – 900 and >900 g/kg).
- Forage type (Hay, Silage, Sugarcane bagasse, Straw, Silage + Hay and Corn stalk).
- Crude protein in diet (<120, 120 – 150 and 150 – 20 g/kg).
- Ether extract in diet (<50 and 50 – 80 g/kg).
- NDF in diet (<200 and 200 – 500 g/kg).

The WMD was evaluated by subgroup analysis when the categorical covariates were significant at $P \leq 0.10$, and when the variables in Table 1 present the P-value ≤ 0.05, subgroup analysis was performed using the covariate the Monensin intake (Pasture and Partial TMR) and Monensin in diet (TMR).

3. Results

The dataset was composed of 131 peer-reviewed publications. The Table 1 are the first approach to determine whether monensin supplementation globally affect each response parameter.

3.1. Pasture

Monensin supplementation in the pasture feeding system reduced ($P \leq 0.05$) DMI, supplement intake, and ruminal butyrate, while it increased ($P \leq 0.05$) final body weight (FBW), ADG, rumen pH, and propionate. On the other hand, monensin had no effect ($P > 0.05$) on total VFA or acetate concentration (Table 1). All individual VFA are expressed in mol/100 mol concentration.

As observed in Table 2 the presence of publication bias from the funnel plot asymmetry test was not evident ($P > 0.05$). In Table 3, the results of the meta-regression analysis are presented. The covariates studied herein explained less than 50% of the heterogeneity (adjusted R^2) for ADG and rumen Ph, suggesting that unidentified factors in this meta-analysis may have influenced the response of monensin on pasture-fed beef cattle (Table 3).

When we evaluated the effect of the covariate monensin intake (Figure 2), an intake < 100 mg/day increased ADG ($P \leq 0.05$). Monensin intake between 100–150 mg/day increased FBW and ADG ($P \leq 0.05$). Monensin intake between 150–200 mg/day increased FBW, ADG, rumen pH, and propionate ($P \leq 0.05$) and reduced butyrate ($P = 0.001$).

Table 1: Effect of including monensin in the diet of cattle in different feeding systems on consumption, digestibility, performance, ruminal and carcass parameters

Item[1]	N[2]	Pasture		N	Partial TMR		N	Total mixed ration		Heterogeneity[4], I² % (P-value)		
		[3]$WMD_{Random\ Effect}$ (95%CI)	*P*-value		$WMD_{Random\ Effect}$ (95%CI)	*P*-value		$WMD_{Random\ Effect}$ (95%CI)	*P*-value	Pasture	Partial TMR	TMR
DMI, kg/d	7	-0.92 (-1.41, -0.42)	0.001	22	-0.12 (-0.18, -0.04)	0.001	132	-0.25 (-0.30, -0.18)	<.0001	8.45 (0.361)	59.26 (0.001)	74.29 (0.001)
DMI Forage, kg/d	--	--	--	5	-0.10 (-0.33, 0.14)	0.410	--	--	--	--	0.00 (0.827)	--
DMI supplement, kg/d	6	-0.04 (-0.07, -0.01)	0.023	--	--	--	--	--	--	85.63 (0.001)	--	--
Digestibility, g/kg of DM												
Dry matter	--	--	--	9	-1.85 (-13.98,10.30)	0.766	25	7.46 (-6.61, 21.53)	0.298	--	0.00 (0.950)	90.21 (0.001)
Crude protein	--	--	--	7	4.30 (-4.18, 12.78)	0.320	8	-18.28 (-44.15, 7.60)	0.166	--	0.00 (0.820)	49.80 (0.052)
NDF	--	--	--	9	8.57 (-6.60, 23.75)	0.268	19	-10.63 (-47.67,26.41)	0.573	--	0.00 (0.841)	74.54 (0.001)
Ether Extract	--	--	--	--	--	--	7	20.98 (12.86, 29.09)	<.0001	--	--	0.00 (0.511)
Starch	--	--	--	--	--	--	16	0.19 (-1.33, 1.69)	0.815	--	--	56.55 (0.002)
Final body weight, kg	37	4.99 (3.21, 6.77)	<.0001	16	4.80 (0.87, 8.72)	0.016	65	2.86 (0.94, 4.78)	0.003	0.00 (0.981)	0.00 (0.980)	0.00 (0.695)
Average daily gain, kg/d	59	0.06 (0.04, 0.07)	<.0001	38	0.05 (0.03, 0.06)	<.0001	126	0.02 (0.01, 0.03)	<.0001	45.86 (0.001)	0.00 (0.759)	42.02 (0.001)
Feed conversion, kg/kg (F: G)	--	--	--	25	-0.79 (-1.01, -0.57)	<.0001	56	-0.44 (-0.58, -0.31)	<.0001	--	76.27 (0.001)	96.31 (0.001)
Feed efficiency, kg /kg (G: F)	--	--	--	-	--	--	58	0.01 (0.01, 0.01)	<.0001	--	--	47.74 (0.001)
Carcass weight, kg	--	--	--	7	4.68 (-0.60, 9.95)	0.082	49	0.74 (-0.71, 2.18)	0.321	--	87.80 (0.001)	2.46 (0.424)
Carcass dressing, %	--	--	--	10	-0.26 (-0.54, 0.01)	0.068	47	-0.03 (-0.29, 0.23)	0.829	--	0.00 (0.491)	89.41 (0.001)
Ribeye area, cm²	--	--	--	--	--	--	21	0.32 (-0.52, 1.16)	0.454	--	--	39.61 (0.032)
SFT, mm	--	--	--	--	--	--	37	-0.12 (-0.37, 0.14)	0.370	--	--	81.78 (0.001)
Ruminal parameters												
Rumen pH	9	0.16 (0.07, 0.23)	0.001	13	0.06 (0.01, 0.10)	0.038	34	-0.01 (-0.04, 0.04)	0.879	60.17 (0.010)	0.00 (0.458)	77.28 (0.001)
N-NH_3, mg/dL	--	--	--	--	--	--	20	-0.55 (-1.09, -0.01)	0.044	--	--	76.44 (0.001)
Total VFA, mmol/L	7	3.39 (-1.48, 8.28)	0.172	15	-3.92 (-6.70, -1.12)	0.005	72	-1.25 (-2.52, 0.03)	0.057	0.00 (0.545)	0.00 (0.866)	77.04 (0.001)
Acetate, mol/100mol	6	-0.46 (-1.13, 0.22)	0.187	21	-2.57 (-3.22, -1.91)	<.0001	72	-1.44 (-1.80, -1.06)	<.0001	0.00 (0.582)	54.56 (0.001)	69.26 (0.001)
Propionate, mol/100mol	7	0.62 (0.01, 1.23)	0.049	25	4.38 (1.91, 6.85)	0.001	70	3.28 (2.83, 3.72)	<.0001	46.08 (0.085)	99.99 (0.001)	73.71 (0.001)
Butyrate, mol/100mol	7	-1.63 (-2.11, -1.15)	<.0001	22	-1.16 (-1.44, -0.88)	<.0001	68	-1.48 (-1.77, -1.20)	<.0001	0.00 (0.691)	98.38 (0.001)	88.44 (0.001)
Valerate, mol/100mol	--	--	--	11	0.01 (-0.07, 0.07)	0.994	49	-0.32 (-0.43, -0.21)	<.0001	--	51.45 (0.024)	97.74 (0.001)
Isovalerate, mol/100mol	--	--	--	11	0.16 (0.05, 0.25)	0.002	48	0.14 (0.04, 0.23)	0.004	--	53.18 (0.018)	94.07 (0.001)
Isobutyrate, mol/100mol	--	--	--	--	--	--	23	0.03 (-0.03, 0.09)	0.345	--	--	82.34 (0.001)
Acetate/Propionate	--	--	--	18	-0.77 (-0.95, -0.59)	<.0001	47	-0.30 (-0.39, -0.21)	<.0001	--	68.34 (0.001)	89.50 (0.001)

[1] DMI = dry matter intake; DM = dry matter; NDF = neutral detergent fiber; VFA = volatile fatty acid; G: F = gain-to-feed ratio; F: G = feed-to-gain ratio; TMR = total mixed ration. [2]N = number of comparisons between control (without monensin) and treatments (with monensin) in the different feeding systems. [3]WMD_{RE} = weighted mean differences between control and treatment in the different feeding systems. [4]I^2 = estimated proportion of the total variation in effect size due to heterogeneity. *P*-value for the χ^2 (Q) heterogeneity test.

Table 2: Egger's regression asymmetry test for intake, digestibility, performance, ruminal, and carcass parameters

Item[1]	Pasture	Partial TMR[2]	Total mixed ration
	Funnel test - *P*-value	Funnel test - *P*-value	Funnel test - *P*-value
DMI, kg/d	0.745	0.331	0.459
DMI Forage, kg/d	--	0.522	--
DMI supplement, kg/d	0.659	--	--
Digestibility, g/kg of DM			
Dry matter	--	0.634	0.567
Crude protein	--	0.598	0.814
NDF	--	0.562	0.473
Ether extract	--	--	0.605
Starch	--	--	0.766
Final body weight, kg	0.135	0.286	0.485
Average daily gain, kg/d	0.468	0.087	0.427
Feed conversation, kg/kg	--	0.184	0.451
Feed Efficiency, kg/kg	--	--	0.576
Carcass weight, kg	--	0.726	0.628
Carcass dressing, %	--	0.376	0.997
Ribeye area, cm^2	--	--	0.180
SFT, mm	--	--	0.164
Ruminal parameters			
Rumen pH	0.806	0.940	0.358
$N\text{-}NH_3$, mg/dL	--	--	0.072
Total VFA, mmol/L	0.501	0.357	0.120
Acetate, mol/100mol	0.583	0.709	0.143
Propionate, mol/100mol	0.225	0.307	0.294
Butyrate, mol/100mol	0.332	0.991	0.343
Valerate, mol/100mol	--	0.335	0.108
Isovalerate, mol/100mol	--	0.519	0.614
Isobutyrate, mol/100mol	--	--	0.288
Acetate/Propionate	--	0.511	0.417

[1]DMI=dry matter intake; NDF= neutral detergent fiber; SFT = subcutaneous fat thickness; VFA = volatile fatty acid.
[2]TMR = Total mixed ration; Funnel test = Egger´s regression asymmetry test.

Table 3: Meta-regression of the Effect of monensin supplementation in different feeding systems on weighted mean differences (WMD), between treatment and control

Dependent variable (Y, WMD) [2]	Meta-regression parameters (*P*-value)[1]																Adjusted R^2 (%)	N[3]
	Genetic Group	Sex class	Exp Design	Fattening period (day)	Monensin in diet (mg/kg of DM)	Monensin intake (mg/day)	CON (g/kg)	Forage type	Supplement type	Supplement intake (kg/d)	Supplement feed	experiment Season	Diet NEg	Diet CP	Diet EE	Diet NDF		
Pasture																		
DMI supplement, kg/d	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	6
Average daily gain. Kg/d	0.458	0.861	--	0.897	0.547	--	--	0.429	0.850	0.209	--	0.881	--	--	--	--	10.83	48
Rumen pH	0.100	0.686	0.292	--	0.666	--	--	--	0.269	--	--	--	--	--	--	--	0.00	9
Partial TMR																		
DMI, kg/d	0.193	0.063	--	0.341	--	0.054	--	--	0.099	0.102	--	--	--	--	--	--	100	22
Feed conversion kg/kg	0.579	0.033	--	0.767	--	0.845	--	0.300	0.070	0.619	--	--	--	--	--	--	77.98	25
Carcass weight, kg	--	--	--	0.078	--	0.001	--	--	0.057	--	--	--	--	--	--	--	100	7
Acetate, mol/100mol	0.829	0.634	0.272	0.809	--	0.612	--	--	--	--	--	--	--	--	--	--	0.00	21
Propionate, mol/100mol	0.092	0.406	0.001	0.062	--	0.014	--	--	0.234	0.073	--	--	--	--	--	--	74.95	25
Butyrate, mol/100mol	0.739	0.026	0.001	0.978	--	0.683	--	--	0.732	--	--	--	--	--	--	--	100	22
Valerate, mol/100mol	0.446	0.359	--	0654	--	0.582	--	--	0.327	--	--	--	--	--	--	--	100	11
Isovalerate, mol/100mol	0.005	0.130	--	0.242	--	0.280	--	--	0.960	--	--	--	--	--	--	--	100	11
Acetate/Propionate	0.080	0.262	--	0.591	--	0.469	--	--	0.757	--	--	--	--	--	--	--	0.00	18
Total mixed ration																		
DMI, kg/d	0.012	0.003	0.054	0.077	0.207	0.192	0.013	0.391	--	--	--	--	0.288	0.018	0.341	0.154	78.34	132
DM digestibility, g/kg	0.793	0.631	0.640	0.748	0.611	0.571	0.981	--	--	--	--	--	--	--	--	--	0.00	25
CP digestibility, g/kg	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.00	8
NDF digestibility, g/kg	0.824	0.134	0.865	0.819	0.001	0.001	--	0.950	--	--	--	--	--	--	--	--	100	19
Starch digestibility, g/kg	0.140	0.804	--	0.437	0.499	0.034	0.001	--	--	--	--	--	--	--	--	--	100	16
Average daily gain. Kg/d	0.002	0.101	--	0.001	0.610	0.086	0.036	0.008	--	--	--	--	0.371	0.040	0.097	0.005	98.94	126
Feed conversion, kg/kg	0.173	0.451	--	0.795	0.770	0.063	0.140	0.875	--	--	--	--	0.613	0.950	0.652	--	72.50	56
Feed efficiency, kg/kg	0.959	0.531	--	0.128	0.591	0.535	0.506	0.872	--	--	--	--	0.557	0.383	0.401	--	7.28	58
Carcass dressing, %	0.953	0.884	--	0.816	0.904	0.719	0.949	0.862	--	--	--	--	0.990	0.891	0.622	--	0.00	47
SFT, mm	0.310	0.116	--	0.361	0.200	0.073	0.905	0.089	--	--	--	--	0.132	0.176	0.763	--	69.44	37
Rumen pH	0.501	0.694	0.320	0.478	0.920	0.199	0.213	0.142	--	--	--	--	--	0.330	--	--	0.00	34
N-NH_3 mg/dL	0.895	0.416	0.606	0.832	0.910	0.486	0.796	--	--	--	--	--	--	0.821	--	--	0.00	20
Acetate, mol/100mol	0.912	0.005	0.870	0.913	0.609	0.640	0.061	0.051	--	--	--	--	0.318	0.117	0.850	0.518	13.66	72
Propionate, mol/100mol	0.419	0.287	0.283	0.133	0.001	0.750	0.721	0.593	--	--	--	--	0.893	0.574	0.362	0.778	76.54	70
Butyrate, mol/100mol	0.553	0.157	0.473	0.586	0.729	0.874	0.460	0.103	--	--	--	--	0.074	0.537	0.474	--	4.25	68
Valerate, mol/100mol	0.426	0.663	0.865	0.360	0.001	0.572	0.967	--	--	--	--	--	0.381	0.921	--	--	71.12	49
Isovalerate, mol/100mol	0.057	0.470	0.093	0.840	0.021	0.073	0.084	0.021	--	--	--	--	0.861	0.869	--	--	56.24	48
Isobutyrate, mol/100mol	0.347	0.635	0.537	0.650	0.070	0.045	0.001	0.600	--	--	--	--	--	--	--	--	100	23
Acetate/Propionate	0.769	0.599	0.950	0.955	0.483	0.667	0.941	0.460	--	--	--	--	0.906	0.551	--	--	20.08	47

[1]CON= concentrate in diet. [2]TMR = total mixed ration; DMI= dry matter intake; CP= crude protein; NDF= neutral fiber detergent; EE= ether extract; NEg = Net energy for gain; SFT = subcutaneous fat thickness; Adjusted R^2 = proportion of the between-study variance (heterogeneity) explained by the covariate.[3]N = number of comparisons between treatment (monensin) and control.

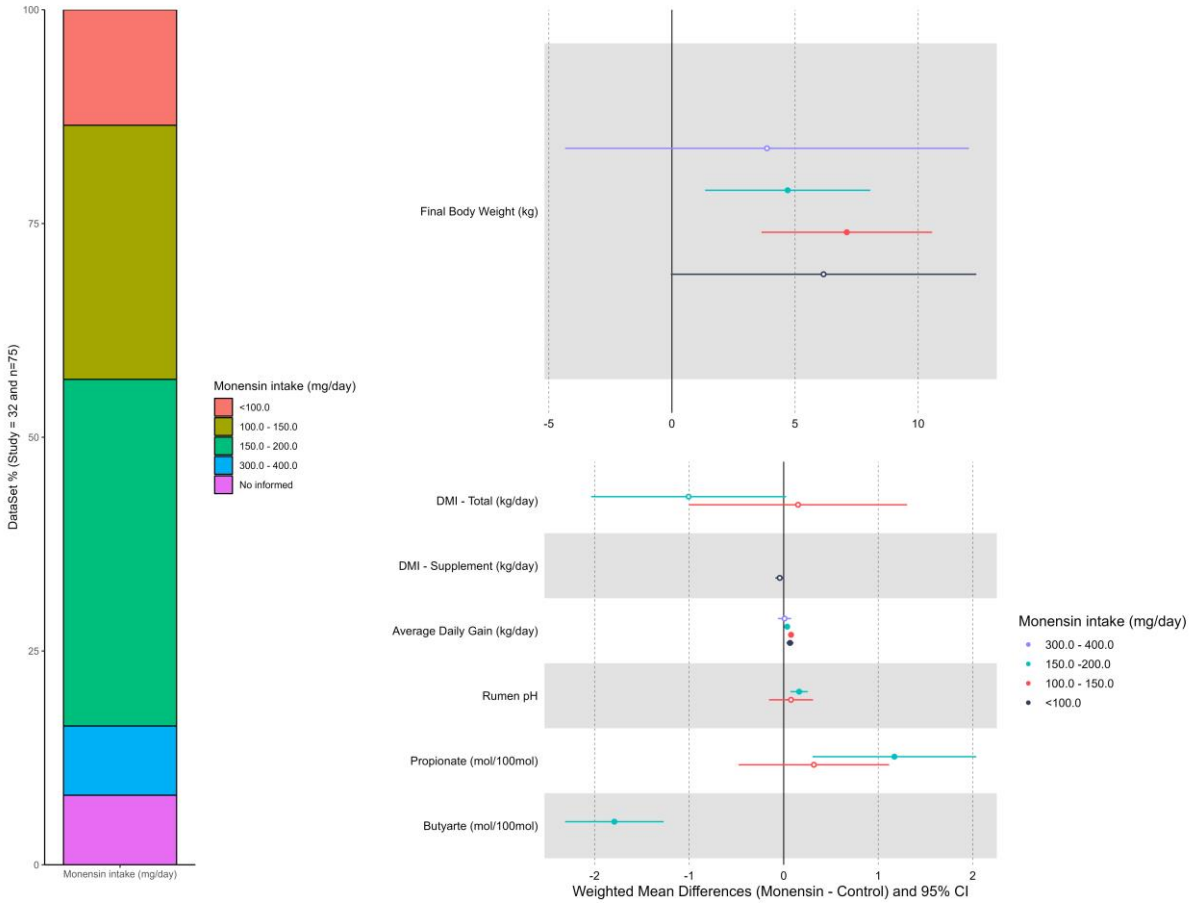


Figure 2: Subgroup analysis (Covariate = Monensin intake (mg/day)) effect of including monensin in the diet of cattle in a feeding system Pasture, WMD = weighted mean differences between treatment and control treatments.

3.2 Partial TMR

Monensin supplementation in the partial TMR feeding system reduced ($P \leq 0.05$) DMI, feed conversion ratio (FCR), total VFA, acetate, butyrate, and the acetate/propionate ratio, while it increased ($P \leq 0.05$) FBW, ADG, rumen pH, propionate, and isovalerate. Monensin supplementation did not affect ($P > 0.05$) forage DMI, DM, CP, NDF digestibility, carcass weight, carcass dressing, or valerate (Table 1).

As observed in Table 2 the presence of publication bias from the funnel plot asymmetry test was not evident ($P > 0.05$). In Table 3, the results of the meta-regression analysis are presented. The covariates studied here explained less than 50% of the heterogeneity (adjusted R^2) for acetate and the acetate/propionate ratio,

suggesting that unidentified factors in this meta-analysis may have influenced the effect of monensin on rumen kinetics in partial TMR animals (Table 3).

Figure 3A shows that monensin supplementation reduced FCR ($P \leq 0.05$) when added to protein or energy supplements. Evaluating the effect of genetic group, monensin supplementation reduced ($P \leq 0.05$) the acetate/propionate ratio (Figure 3B) and butyrate (Figure 4B) and increased ($P = 0.005$) propionate (Figure 4A) in *Bos taurus* animals. In Holstein animals, monensin reduced ($P \leq 0.05$) the acetate/propionate ratio (Figure 3B) and butyrate (Figure 4B) and increased ($P < 0.0001$) propionate. In crossbred animals, monensin increased ($P = 0.005$) propionate and reduced ($P < 0.0001$) butyrate. Additionally, monensin increased ($P \leq 0.05$) ruminal propionate (Figure 4A) and reduced ($P \leq 0.05$) ruminal butyrate (Figure 4B) in both rotational and continuous designs.

When we evaluated the effect of the fattening period, monensin supplementation increased ($P \leq 0.05$) ruminal propionate, regardless of whether the period was < 30, 60–90, or 200–300 days (Figure 4A). Moreover, monensin supplementation increased ($P \leq 0.05$) ruminal propionate, regardless of the level of supplement intake. Evaluation of the effect of sex class on the response to monensin supplementation showed that monensin reduced ($P < 0.0001$) butyrate for steers (Figure 4B).

Monensin intake of < 100 mg/day increased ($P \leq 0.05$) ADG and propionate and reduced ($P \leq 0.05$) DMI and FCR. Monensin intake of 100 – 200 mg/day increased ($P \leq 0.05$) ADG, FBW, propionate, and rumen pH, and reduced ($P \leq 0.05$) FCR, acetate, butyrate, and the acetate/propionate ratio. Monensin intake of 200 – 300 mg/day increased ($P = 0.02$) ADG and reduced ($P \leq 0.05$) FCR, acetate, and the acetate/propionate ratio. Additionally, monensin intake between 300 – 400 mg/day reduced ($P \leq 0.05$) DMI and FCR (Figure 5).

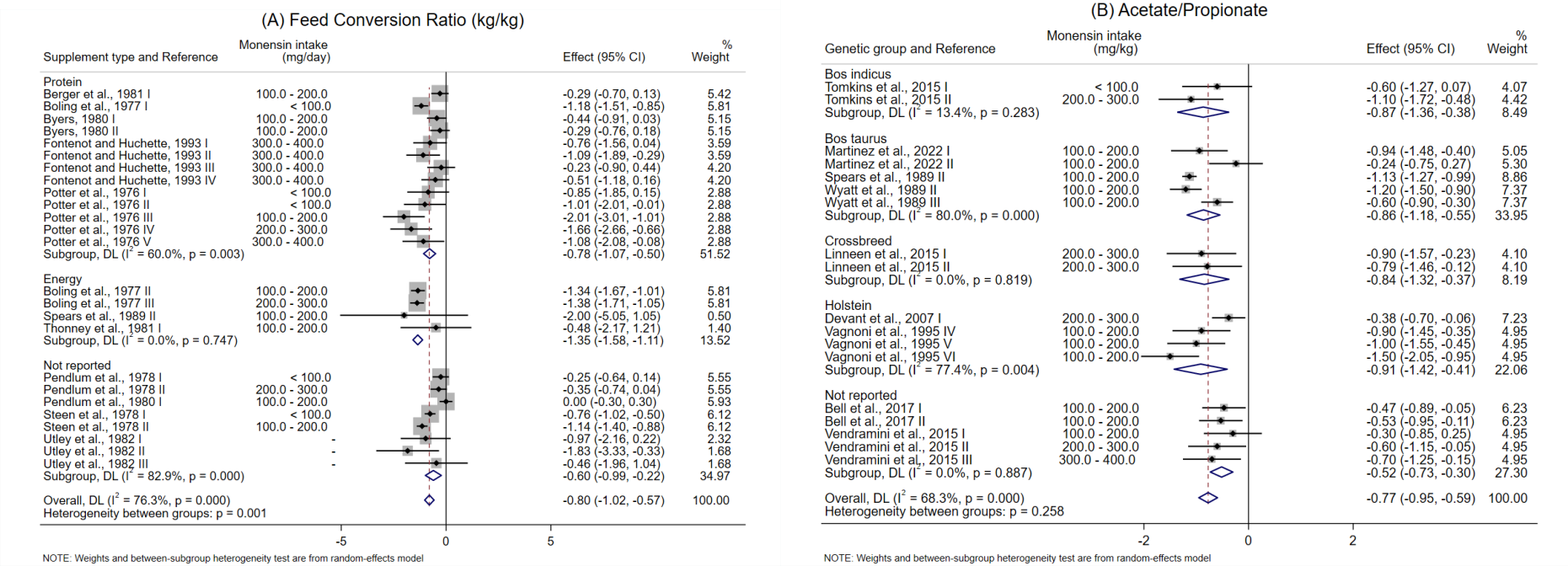


Figure 3: Forest plot, showing the effect of the monensin on the diet the beef cattle in the Feeding system Partial TMR (Total mixed ration) on (A) feed conversion ratio (with subgroup analysis “Covariate = Supplement type) and (B) Acetate/Propionate (with subgroup analysis “Covariate = Genetic group). The x-axis shows the weighted mean difference (Effect); diamonds to the left of the solid line represent a reduction in the measure, whereas diamonds to the right of the line indicate an increase. Each diamond represents the mean size effect for that study, and the size of the diamond reflects the relative weighting of the study to the overall size effect estimate with larger diamonds representing greater weight. The lines connected to the diamond represents the upper and lower 95% confidence interval for the treatment effect. The dotted vertical line represents the overall size effect estimate. The diamond at the bottom represents the mean response across the studies, and the solid vertical line represents a mean difference of zero or no effect.

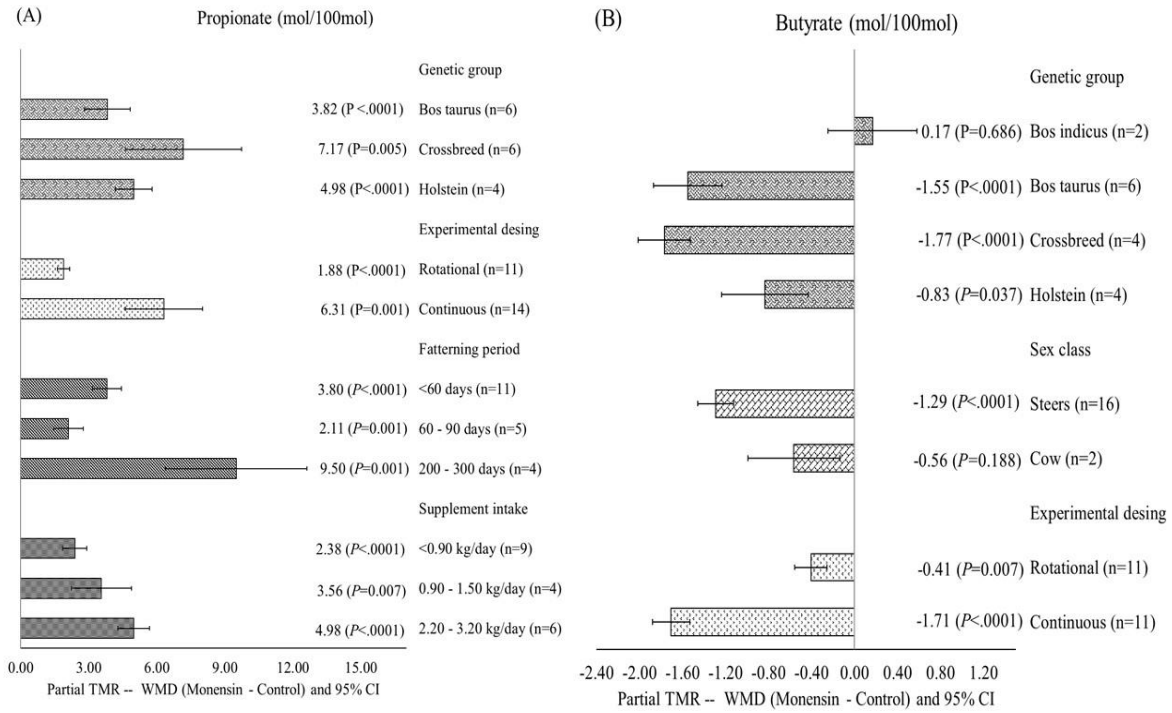


Figure 4: Subgroup analysis effect of including monensin in the diet of cattle in a feeding system Partial TMR, WMD = weighted mean differences between treatment and control treatments; TMR = total mixed ration.

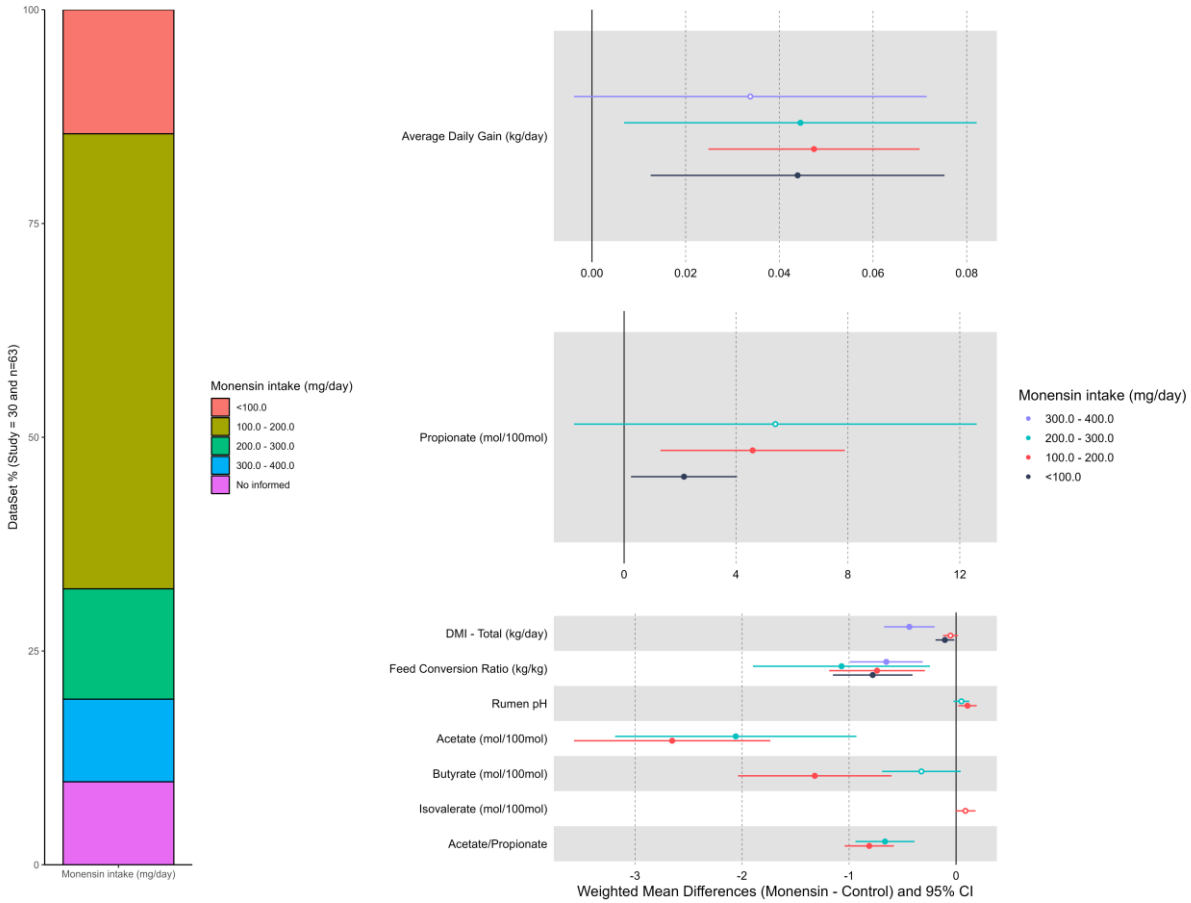


Figure 5: Subgroup analysis (with subgroup analysis “Covariate =Monensin intake (mg/day)) effect of including monensin in the diet of cattle in the feeding system Partial TMR, WMD = weighted mean differences between treatment and control treatments, TMR = Total mixed ration.

3.3. Total mixed ration/Feedlot

Monensin supplementation in the TMR/feedlot feeding system reduced ($P \leq 0.05$) DMI, FCR, ruminal N-NH_3, total VFA, acetate, butyrate, valerate, and the acetate/propionate ratio, while it significantly ($P \leq 0.05$) increased FBW, ADG, feed efficiency, ruminal propionate and isovalerate concentrations, and EE digestibility. Monensin supplementation did not affect ($P > 0.05$) DM, CP, NDF or starch digestibility, carcass weight, carcass dressing, ribeye area, subcutaneous fat thickness, rumen pH, or isobutyrate (Table 1).

As observed in Tables 2 the presence of publication bias from the funnel plot asymmetry test was not evident ($P> 0.05$). In Table 3, the results of the meta-regression analysis are presented. The covariates studied here explained less than

50% of the heterogeneity (adjusted R^2) for DM and CP digestibility, feed efficiency, carcass dressing, Rumen pH, ruminal ammonia, butyrate, and acetate/propionate ratio, suggesting that unidentified factors in this meta-analysis may have influenced the effect of monensin on feedlot cattle (Table 3).

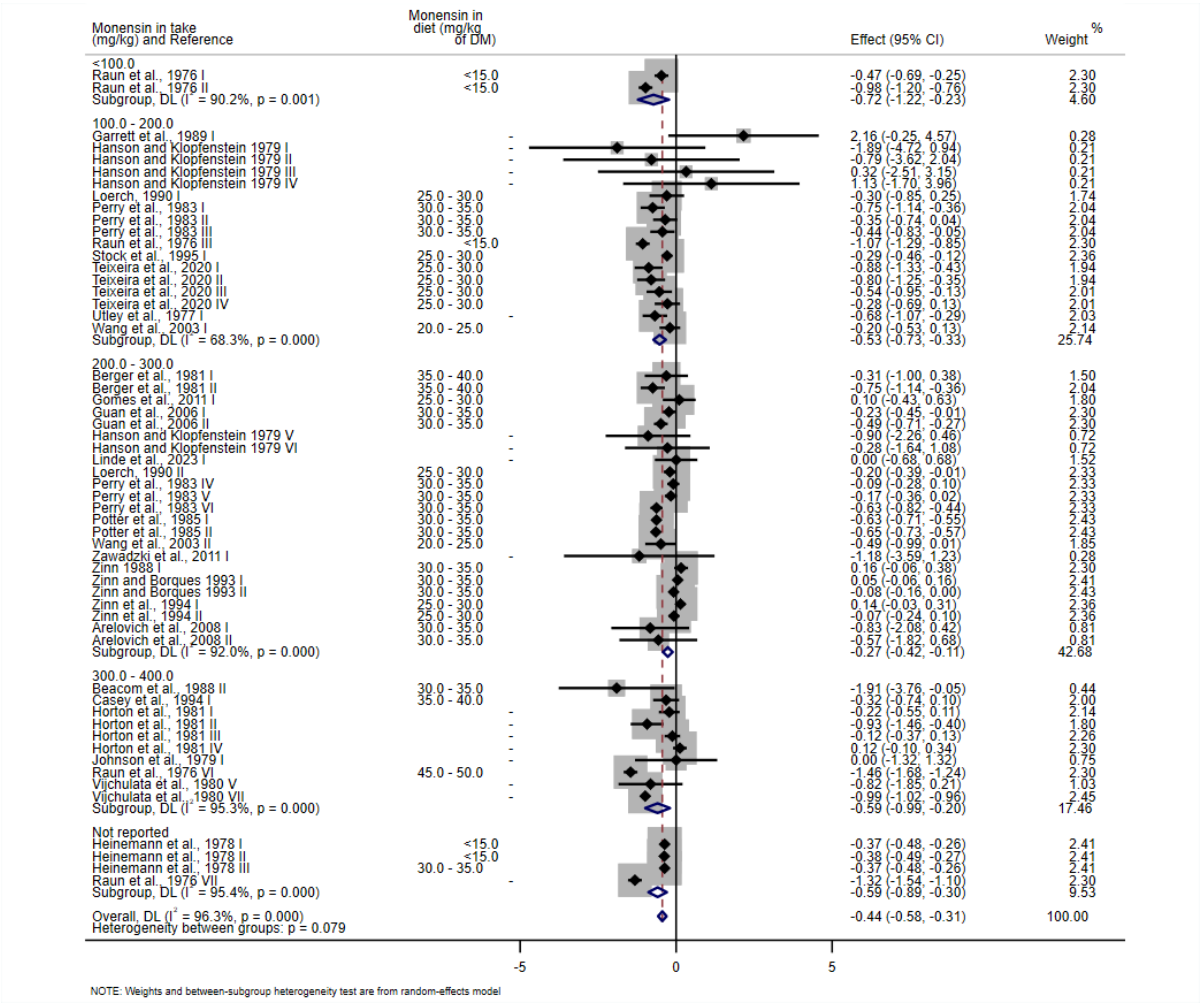


Figure 6: Forest plot, (with subgroup analysis “Covariate = Monensin intake (mg/day)”), showing the effect of the monensin on the diet the beef cattle in the Feeding system TMR (Total mixed ration) on feed conversion ratio. The x-axis shows the weighted mean difference (Effect); diamonds to the left of the solid line represent a reduction in the measure, whereas diamonds to the right of the line indicate an increase. Each diamond represents the mean size effect for that study, and the size of the diamond reflects the relative weighting of the study to the overall size effect estimate with larger diamonds representing greater weight. The lines connected to the diamond represents the upper and lower 95% confidence interval for the treatment effect. The dotted vertical line represents the overall size effect estimate. The diamond at the bottom represents the mean response across the studies, and the solid vertical line represents a mean difference of zero or no effect.

Monensin intake levels of < 100, 100–200, and 300–400 mg/day reduced ($P \leq 0.05$) FCR (Figure 6), while 400 –500 mg/day reduced ($P = 0.007$) ruminal isobutyrate (Figure 7F).

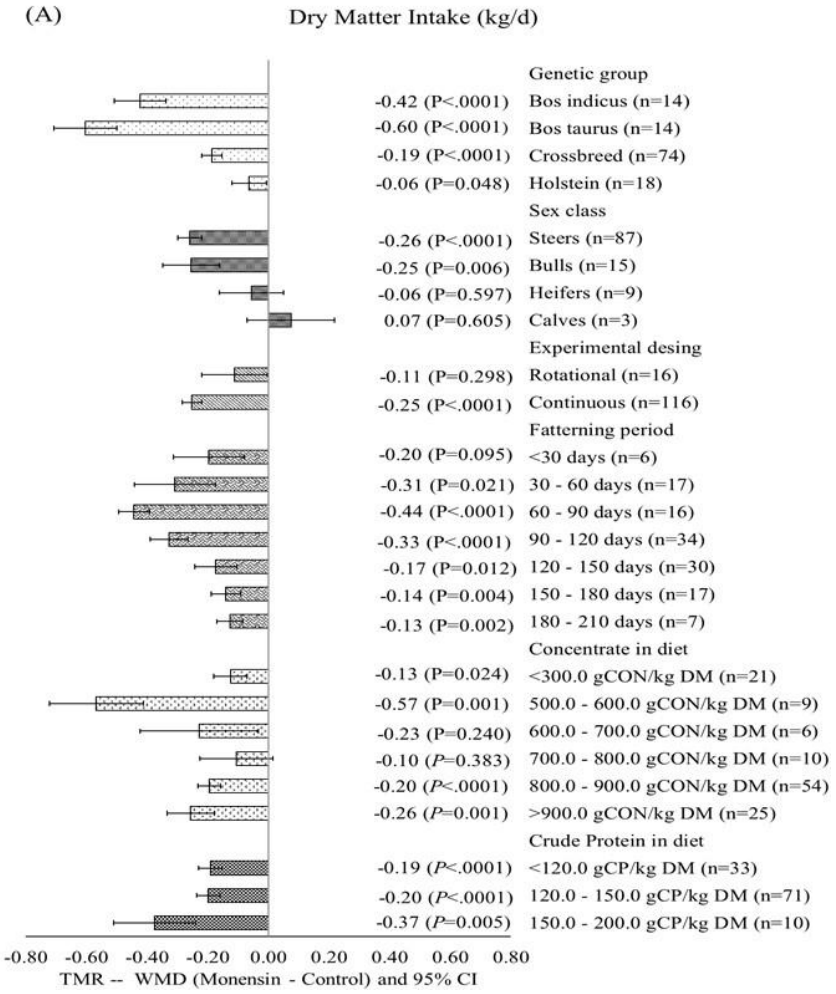
Figure 7A shows that monensin supplementation reduced ($P \leq 0.05$) DMI in *Bos indicus*, *Bos taurus*, crossbred, and Holstein animals. Evaluation of the effect of sex class on the response to supplementation showed that monensin reduced ($P \leq 0.05$) DMI in steers and bulls (Figure 7A) and reduced ($P < 0.0001$) ruminal acetate in steers (Figure 7C). Moreover, monensin increased ($P = 0.001$) ADG in steers (Figure 7B). Evaluation of the effect of the experimental design on the response to supplementation revealed that monensin reduced ($P < 0.0001$) DMI (Figure 7A) and increased ($P = 0.001$) ruminal isovalerate (Figure 7E) in continuous experiments. Furthermore, monensin intake up to 100 and 100 –200 mg/day increased ($P \leq 0.05$) ADG (Figure 7B).

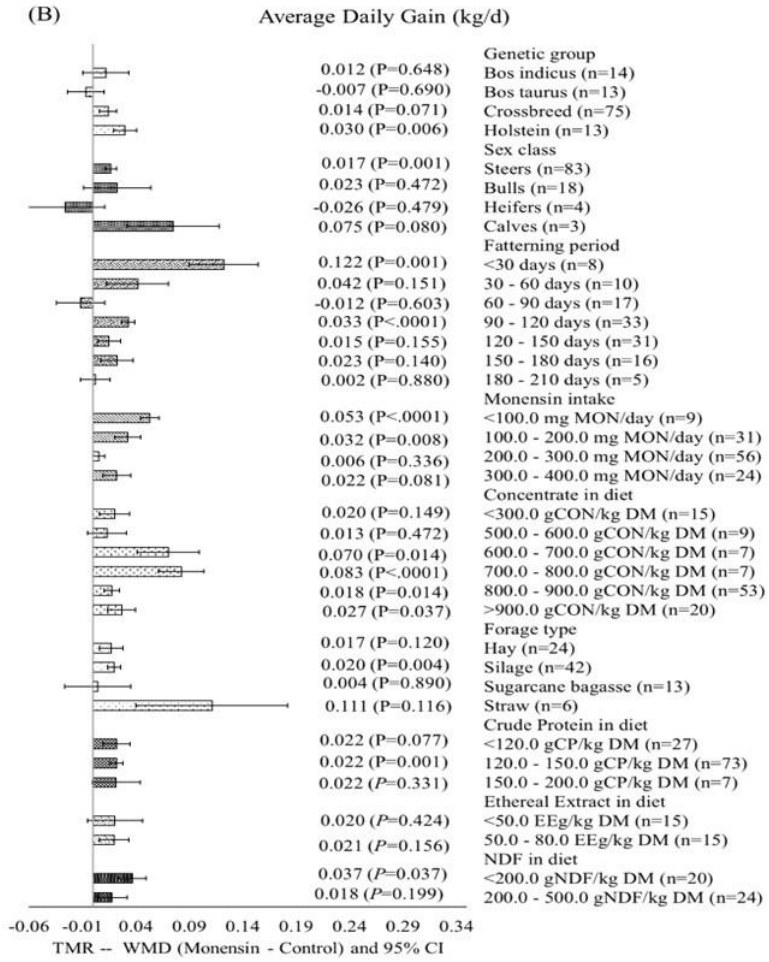
Regarding the effect of the fattening period on the response to monensin supplementation, except for periods < 30 days, monensin reduced ($P \leq 0.05$) DMI for all other fattening periods (Figure 7A). Additionally, monensin increased ($P \leq 0.05$) ADG for the periods < 30 days and 90–120 days (Figure 7B).

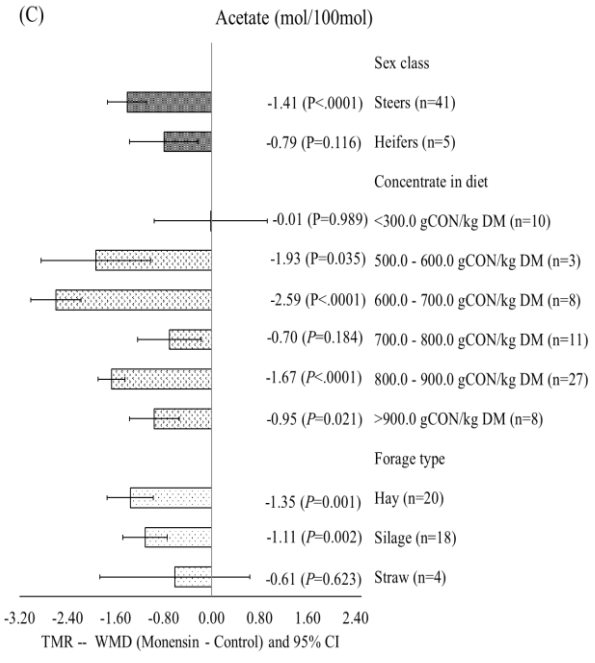
Monensin supplementation in diets with a concentrate level of up to 300 g/kg of DM reduced ($P \leq 0.05$) DMI (Figure 7A) and FCR (Figure 7G) and increased ($P = 0.043$) isovalerate (Figure 7E). At the concentrate intake level of 500–600 g/kg of DM, monensin reduced ($P \leq 0.05$) DMI and acetate (Figure 7C). At 600.0–700.0 g concentrate/kg of DM, monensin increased ($P \leq 0.05$) ADG (Figure 7B) and isovalerate and reduced ($P \leq 0.05$) FCR and acetate. At 700.0–800.0 g concentrate/kg of DM, monensin increased ($P \leq 0.05$) ADG. At the concentrate intake level of 800.0–900.0 g/kg of DM, monensin reduced ($P \leq 0.05$) DMI, FCR, and acetate, and increased ($P = 0.014$) ADG. In diets containing more than 900 g concentrate/kg of DM, monensin reduced ($P \leq 0.05$) DMI, FCR, acetate, and isobutyrate (Figure 7F), and increased ($P = 0.037$) ADG.

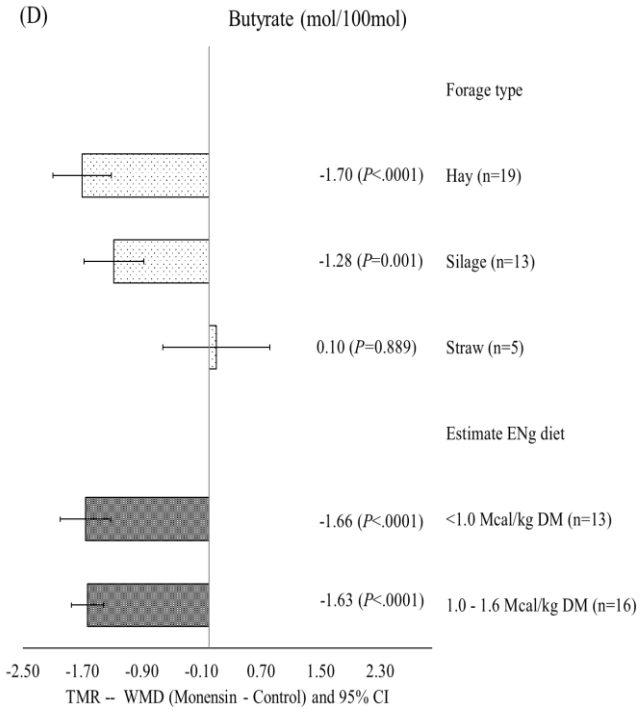
When we assessed the effect of forage type on the response to supplementation, monensin reduced ($P \leq 0.05$) acetate (Figure 7C) and butyrate (Figure 7D) in hay-based diets. However, in silage-based diets, monensin increased ($P \leq 0.05$) ADG but reduced ($P \leq 0.05$) acetate and butyrate. Regarding the influence

of dietary CP on the response to supplementation, monensin reduced ($P \leq 0.05$) DMI in diets containing < 120, 120–150, and 150–200 g CP/kg (Figure 7A). Furthermore, monensin increased ADG in diets with 120–150 g CP/kg. Monensin supplementation in diets with < 200 g NDF/kg increased ($P = 0.037$) ADG (Figure 7B). Moreover, monensin supplementation of diets with estimated net energy for gain (NEg) < 1.0 and between 1.0–1.6 Mcal/kg of DM reduced ($P \leq 0.05$) butyrate (Figure 7D).




(B)
Average Daily Gain (kg/d)
Genetic group
0.012 (P=0.648) Bos indicus (n=14)
-0.007 (P=0.690) Bos taurus (n=13)
0.014 (P=0.071) Crossbreed (n=75)
0.030 (P=0.006) Holstein (n=13)
Sex class
0.017 (P=0.001) Steers (n=83)
0.023 (P=0.472) Bulls (n=18)
-0.026 (P=0.479) Heifers (n=4)
0.075 (P=0.080) Calves (n=3)
Fatterning period
0.122 (P=0.001) <30 days (n=8)
0.042 (P=0.151) 30 - 60 days (n=10)
-0.012 (P=0.603) 60 - 90 days (n=17)
0.033 (P<.0001) 90 - 120 days (n=33)
0.015 (P=0.155) 120 - 150 days (n=31)
0.023 (P=0.140) 150 - 180 days (n=16)
0.002 (P=0.880) 180 - 210 days (n=5)
Monensin intake
0.053 (P<.0001) <100.0 mg MON/day (n=9)
0.032 (P=0.008) 100.0 - 200.0 mg MON/day (n=31)
0.006 (P=0.336) 200.0 - 300.0 mg MON/day (n=56)
0.022 (P=0.081) 300.0 - 400.0 mg MON/day (n=24)
Concentrate in diet
0.020 (P=0.149) <300.0 gCON/kg DM (n=15)
0.013 (P=0.472) 500.0 - 600.0 gCON/kg DM (n=9)
0.070 (P=0.014) 600.0 - 700.0 gCON/kg DM (n=7)
0.083 (P<.0001) 700.0 - 800.0 gCON/kg DM (n=7)
0.018 (P=0.014) 800.0 - 900.0 gCON/kg DM (n=53)
0.027 (P=0.037) >900.0 gCON/kg DM (n=20)
Forage type
0.017 (P=0.120) Hay (n=24)
0.020 (P=0.004) Silage (n=42)
0.004 (P=0.890) Sugarcane bagasse (n=13)
0.111 (P=0.116) Straw (n=6)
Crude Protein in diet
0.022 (P=0.077) <120.0 gCP/kg DM (n=27)
0.022 (P=0.001) 120.0 - 150.0 gCP/kg DM (n=73)
0.022 (P=0.331) 150.0 - 200.0 gCP/kg DM (n=7)
Ethereal Extract in diet
0.020 (P=0.424) <50.0 EEg/kg DM (n=15)
0.021 (P=0.156) 50.0 - 80.0 EEg/kg DM (n=15)
NDF in diet
0.037 (P=0.037) <200.0 gNDF/kg DM (n=20)
0.018 (P=0.199) 200.0 - 500.0 gNDF/kg DM (n=24)
-0.06 -0.01 0.04 0.09 0.14 0.19 0.24 0.29 0.34
TMR -- WMD (Monensin - Control) and 95% CI


(C)
Acetate (mol/100mol)
Sex class
-1.41 (P<.0001) Steers (n=41)
-0.79 (P=0.116) Heifers (n=5)
Concentrate in diet
-0.01 (P=0.989) <300.0 gCON/kg DM (n=10)
-1.93 (P=0.035) 500.0 - 600.0 gCON/kg DM (n=3)
-2.59 (P<.0001) 600.0 - 700.0 gCON/kg DM (n=8)
-0.70 (P=0.184) 700.0 - 800.0 gCON/kg DM (n=11)
-1.67 (P<.0001) 800.0 - 900.0 gCON/kg DM (n=27)
-0.95 (P=0.021) >900.0 gCON/kg DM (n=8)
Forage type
-1.35 (P=0.001) Hay (n=20)
-1.11 (P=0.002) Silage (n=18)
-0.61 (P=0.623) Straw (n=4)
-3.20 -2.40 -1.60 -0.80 0.00 0.80 1.60 2.40
TMR -- WMD (Monensin - Control) and 95% CI


(D)
Butyrate (mol/100mol)
Forage type
-1.70 (P<.0001) Hay (n=19)
-1.28 (P=0.001) Silage (n=13)
0.10 (P=0.889) Straw (n=5)
Estimate ENg diet
-1.66 (P<.0001) <1.0 Mcal/kg DM (n=13)
-1.63 (P<.0001) 1.0 - 1.6 Mcal/kg DM (n=16)
-2.50 -1.70 -0.90 -0.10 0.70 1.50 2.30
TMR -- WMD (Monensin - Control) and 95% CI

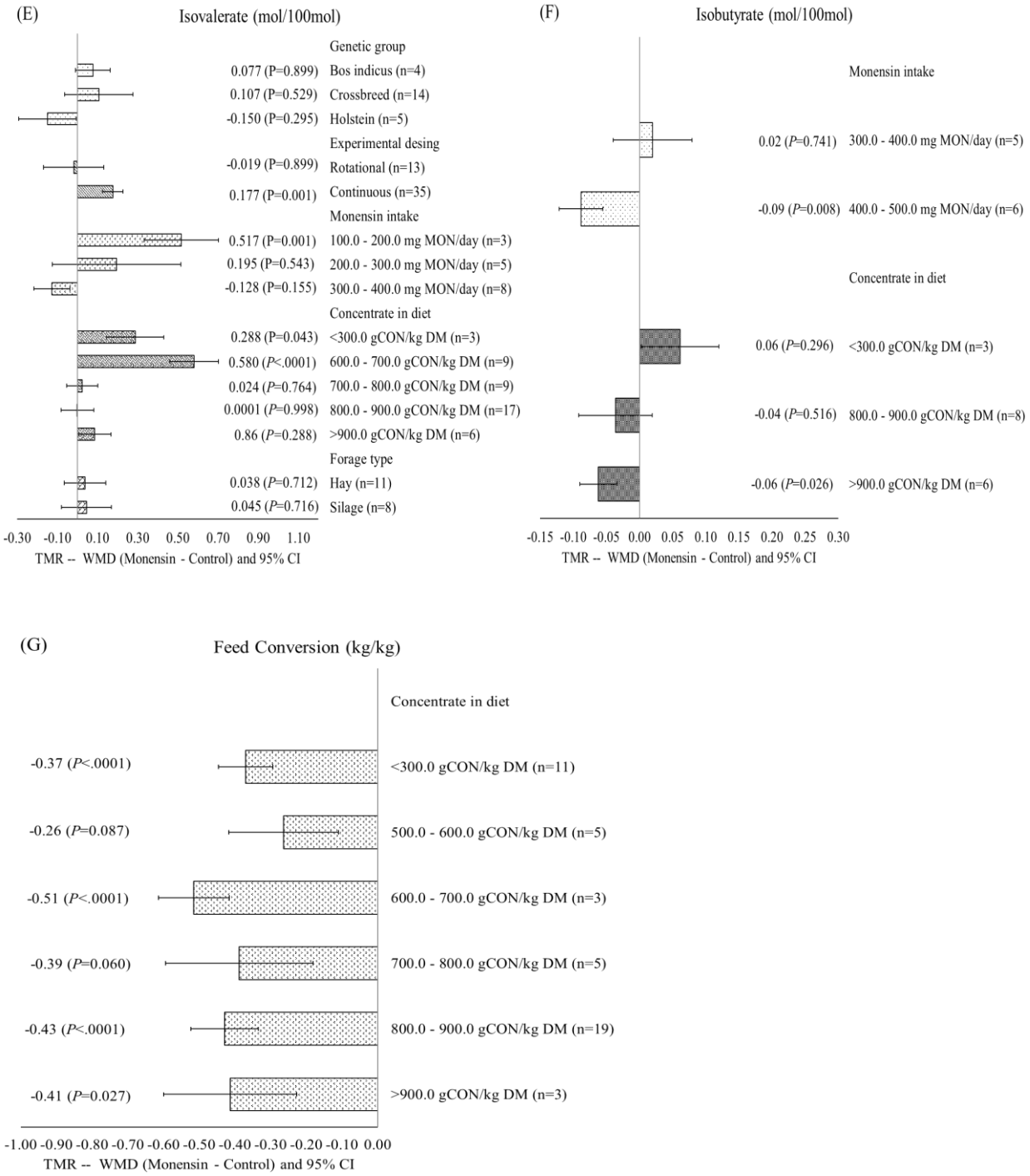


Figure 7: Subgroup analysis effect of including monensin in the diet of cattle in a feeding system TMR, WMD = weighted mean differences between treatment and control treatments; TMR = total mixed ration.

Monensin inclusion up to 15.0 mg/kg of DM increased ($P \leq 0.05$) FBW, ADG, feed efficiency, propionate, and isovalerate, while it reduced ($P \leq 0.05$) FCR, total VFA, acetate, butyrate, and valerate. Inclusion levels between 20.0–25.0 mg/kg of DM reduced ($P \leq 0.05$) DMI, total VFA, and acetate, and increased ($P \leq 0.05$) ADG and feed efficiency. Monensin at 25.0–30.0 mg/kg of DM reduced DMI, FCR, and butyrate, but increased ($P \leq 0.05$) feed efficiency and propionate ruminal concentration. Inclusion levels of 30.0–35.0 mg/kg of DM reduced DMI, FCR, total VFA, ruminal N-NH_3, acetate, butyrate, and acetate/propionate ratio, while these levels increased ($P \leq 0.05$) feed efficiency and propionate. At 35.0–40.0 mg/kg of DM, monensin reduced ($P \leq 0.05$) DMI, FCR, total VFA, acetate, butyrate, and valerate, while an increase ($P \leq 0.05$) in ADG and propionate was observed. Finally, inclusion levels of 40.0–45.0 mg/kg of DM increased ($P \leq 0.05$) propionate and isovalerate and reduced ($P \leq 0.05$) butyrate and valerate (Figure 8).

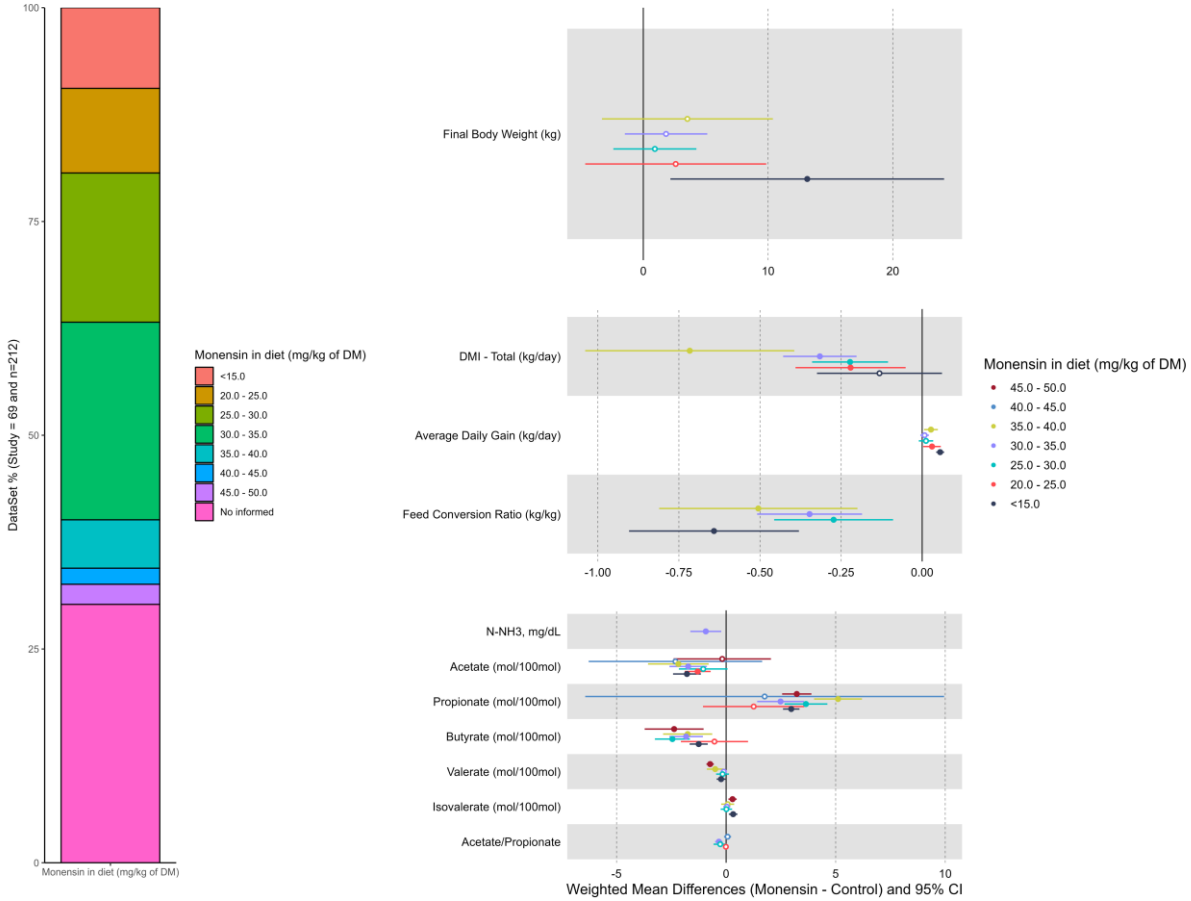


Figure 8. Subgroup analysis (Covariate =Monensin in diet (mg/kg of DM)) effect of including monensin in the diet of cattle in the feeding system TMR, WMD = weighted mean differences between treatment and control treatments, TMR = Total mixed ration.

4. Discussion

Monensin supplementation reduced DMI regardless of the feeding system. However, this reduction was more pronounced in pasture-based systems. In these systems, monensin reduced butyrate and increased propionate concentrations, while acetate and total VFA remained unchanged. Opposite results were found for the partial TMR and TMR systems, in which monensin reduced acetate concentration and total VFA. Since VFA are the main source of energy for ruminants, a decrease in ruminal VFA production is undesirable and can negatively impact animal performance (Benchaar et al., 2008). Additionally, along with propionate, the production of VFA, which are potential metabolic fuels for intermediate energy metabolism in ruminants, can influence DMI (Allen, 2020). Hence, in addition to the increase in propionate, the

lack of changes in total VFA may explain the greater reduction in DMI and the increase in ADG and FBW observed in pasture systems compared to the other systems. Like the pasture system, monensin did not impact ruminal acetate concentration in TMR diets containing less than 300 g of concentrate/kg DM. Although some studies report a greater impact of monensin on the VFA profile of animals receiving high-forage diets, Nagaraja et al. (1997) indicated that this effect is inconsistent for acetate. According to Fernando et al. (2010), the ruminal population of fibrolytic bacteria naturally decreases as the level of dietary concentrate increases. Considering that fibrolytic bacteria are the leading producers of acetate in the rumen (Russell et al., 2009), the potentially greater ruminal population of these bacteria in animals fed pasture, and high-forage TMR diets might have influenced the effect of monensin on acetate.

Monensin supplementation consistently increased propionate concentration across the different feeding systems. An increased propionate supply to the liver was probably responsible for the greater ADG and FBW observed with monensin supplementation in all feeding systems. The relative efficiency of glucose metabolism to propionate is higher (109%) than that observed for butyrate (78%) and acetate (62%) (Ryle and Ørskov, 1990). Furthermore, the heat increment of propionate metabolism is lower than that of acetate (Holter et al., 1970). These processes, combined with reduced methane production and protein degradation, enhance the performance and efficiency of animals fed diets with ionophores (Tedeschi et al., 2003).

Monensin has been shown to reduce ruminal ammonia (NH_3) concentration without affecting nitrogen digestibility in TMR systems (Poos et al., 1979). Moreover, Ogunade et al. (2018) demonstrated that monensin reduces amino acid degradation and ammonia accumulation by suppressing hyper-ammonia-producing bacteria. This finding aligns with Chen & Russell (1991), who found that the effect of monensin on Gram-positive bacteria contributes to ruminal protein sparing. This process can enhance the flux of amino acids to the intestine, increasing the substrate supply for host protein and energy metabolism and consequently improving performance (Tedeschi et al., 2003). However, the lack of effect of monensin on ADG and the more pronounced reduction in DMI in TMR diets containing more than 150 g of CP/kg DM must be highlighted. These results suggest that the increased amino acid flux mediated

by monensin in high-protein TMR diets may negatively affect performance, probably because of the energy cost of removing excess nitrogen (Jennings et al., 2018).

Previous studies have reported a notable effect of the interaction between monensin and dietary fat content on the molar proportions of ruminal acetate, propionate, and estimated methane production (Zinn, 1988; Clary et al., 1993). However, meta-regression analysis revealed no influence of dietary EE on the response of these parameters to monensin. Consistent with Zinn (1988), monensin increaseTMR diets' total tract EE digestibility due to the lower proportion of stearic acid and the greater proportion of oleic acid that reaches the small intestine. These changes could affect EE digestibility since oleic acid is absorbed more efficiently from the small intestine than stearic acid and improves absorption of the latter (Boerman et al., 2015). The effect of monensin on cellulolytic bacteria contributes to inhibiting the biohydrogenation rate, consequently improving the flux of unsaturated fatty acids to the intestine and increasing the net energy value of dietary fat (Fellner et al., 1997; Zinn et al., 2000). Along with propionate, the greater flux of fatty acids through animal metabolism improves feed efficiency and controlled feed intake (Allen, 2000; Zinn et al., 2000).

The effect of monensin on most VFA, particularly propionate, was dose-dependent. In pasture-based systems, a monensin intake of 150–200 mg/day increased propionate concentration and ADG. In TMR systems, diets with 35–40 mg monensin/kg of DM promoted the greatest increase in propionate concentration and represented the range associated with the greatest DMI reduction. The hypophagic effect of propionate is well established by the hepatic oxidation theory (Allen et al., 2009). However, the elevated propionate concentration in TMR diets with less than 15 mg monensin/kg of DM and the lack of an effect of these doses on DMI indicate that the impact of monensin on the latter cannot be attributed solely to the increase in propionate levels. Pereira et al. (2014) showed thaincreasing monensin doses in TMR diets affected the time spent eating and ruminating quadratically. These results suggest that monensin directly or indirectly influences animal behavior, which may affect DMI depending on the dose. Additionally, as observed in the subgroup analysis, the effect of monensin on DMI in TMR systems can vary depending on the genetic group, sex class, experimental design, fattening period, and dietary levels of

concentrate and CP. Therefore, caution is needed when interpreting the dose-response to monensin.

Monensin inclusion in beef cattle diets is primarily associated with improved efficiency, influenced by weight gain and DMI. In TMR systems, monensin at 15 mg/kg DM significantly increased ADG and was the only dose that improved FBW. This response may be due to an increase in ruminal propionate in the absence of an effect on DMI (Byers, 1980). In contrast to Duffield et al. (2012), lower monensin levels (15 mg/kg DM or 100 mg/day) resulted in a better FCR due to increased ADG. Studies found no effect of higher monensin doses on ruminal pH, suggesting that low doses are effective and safe (Wood et al., 2016; Santos et al., 2019). Monensin doses higher than 20–25 mg/kg DM affected DMI and, consequently, FCR, demonstrating the effectiveness of monensin in reducing this ratio by increasing ADG or, reducing DMI, or both. While all monensin levels lowered FCR in partial TMR systems, the greatest FCR reduction did not occur at the lowest intake. Bretschneider et al. (2008) found a quadratic increase in ADG with increasing monensin dose in high-forage diets, with no impact on DMI, similar to what was observed in the partial TMR system. Additionally, monensin was more effective in energy supplements than in protein supplements (> 20% CP) in partial TMR systems, where it increased propionate production and ADG (McLennan et al., 2012; Martinez et al., 2022).

According to Duffield et al. (2012), including corn silage in feedlot diets influences the effect of monensin, resulting in a greater reduction in FCR compared to diets without corn silage. However, our findings suggest that, among all forage types, silage does not affect the effect of monensin on FCR. On the other hand, the effect of monensin on ADG increased with the inclusion of silage in TMR diets. Tarnonsky et al. (2023) found that corn silage contributes more energy to the diet at the same inclusion level as hay. Given the slight difference in the effect of monensin on the VFA profile between silage- and hay-based diets in the TMR system, it is reasonable to suggest that the greater effect of monensin on ADG observed for silage-based diets may be associated with their higher energy content.

Another covariate that affects the effect of monensin is the experimental design. A large part of the studies evaluating ruminal metabolism are carried out in a rotative experimental design, in which all animals are subjected to all treatments. In the partial

TMR system, the effect of monensin on propionate and butyrate was intensified in continuous experimental designs. The same was observed for isovalerate in the TMR system. This suggests potential carryover effects due to an insufficient washout period in rotational studies or even a time effect caused by the shorter experimental period commonly used compared to continuous designs. Dawson & Boling (1983) found that in animals fed monensin, the VFA profile returned to the same level as in non-treated animals after 10 days of monensin withdrawal. However, the relative proportion of monensin-resistant bacteria remained greater 18 days after monensin withdrawal. Similarly, Bell et al. (2017) demonstrated that upon monensin withdrawal, the acetate/propionate ratio of cattle previously fed monensin remained reduced for at least 7 days compared to the negative control group. Although the general washout period aligns with the time required for the VFA profile to return to baseline levels, it may be insufficient for fully reestablishing the rumen bacterial community.

Genetic groups and sex classes also influence the response to monensin. Although *Bos indicus* and *Bos taurus* have distinct rumen microbiomes (Latham et al., 2018), no significant differences in the VFA profile response were observed. This finding suggests that the more pronounced reduction in DMI in both *Bos indicus* and *Bos taurus* contributed to the lack of effect on ADG in these genetic groups in TMR systems. The lack of effect of monensin on acetate in the heifer group in the TMR system agrees with previous results (Utley et al., 1977; Wood et al., 2016), suggesting that, although propionate is increased in the heifer group, the acetate/propionate ratio should be higher than in the steer group. Furthermore, monensin did not affect DMI or ADG in heifers. The NEg requirement of heifers is greater than that of steers (Chizzotti et al., 2008), suggesting a possible difference in response to the increased energy value provided by monensin between these sex classes.

The effect of monensin on FCR was more pronounced at intermediate concentrate levels (600–700 g/kg DM) in TMR diets, consistent with Goodrich et al. (1984), who found the greatest improvement in FCR at intermediate dietary energy levels Additionally, the increase in ADG due to monensin became more pronounced with higher metabolizable energy intake (Goodrich et al., 1984). This is consistent with monensin improving energy metabolism by increasing net energy for maintenance (NEm), reducing the DMI required (Byers, 1980). Alvarez et al. (2004) reported a

quadratic interaction effect between the level of dietary forage and energy intake in feedlot cattle, suggesting that the greater increase in ADG due to monensin observed in TMR diets containing 700–800 g of concentrate/kg DM may be associated with energy intake. Furthermore, although NASEM (2016) reported a stronger effect of monensin on DMI in high-forage diets, our study found the greatest reduction in DMI at intermediate forage levels (400–500 g/kg DM), in the absence of corresponding improvements in ADG or FCR.

After 120 days on feed, monensin no longer affected ADG but continued to reduce DMI, enhancing feed efficiency. As cattle mature and accumulate fat, rising leptin levels may reduce DMI and ADG (Foote et al., 2015). Thus, as the feeding period progresses and animals accumulate fat, the additional effect of monensin on DMI, combined with the naturally declining DMI and increasing NEm requirements (Chizzotti et al., 2008), potentially reduces the effect of the additive on ADG over time.

5. Conclusion

This meta-analysis confirms the well-established effects of monensin supplementation and highlights several experimental factors that can influence these outcomes. Monensin consistently increased propionate concentration, ADG, and FBW, while reducing DMI and FCR in all feeding systems. In pasture systems, a monensin intake of 150–200 mg/day improved ADG and FBW but reduced DMI. In partial TMR systems, an intake of 200–300 mg/day effectively lowered FCR. In TMR systems, monensin inclusion up to 15 mg/kg DM increased ruminal propionate, ADG, and FBW, and reduced FCR. Additionally, in both partial TMR and TMR systems, various dietary, animal, and experimental covariates influenced the effect of monensin on ruminal parameters, DMI, ADG, and FCR, shedding light on the sources of variability in the effects of this additive.

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