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**UNIVERSIDADE ESTADUAL PAULISTA - UNESP CÂMPUS
DE JABOTICABAL**

**COMBINING 3-NITROOXYPROPANOL WITH DIFFERENT
FEED ADDITIVES ON GROWTH PERFORMANCE, CARCASS
TRAITS, MEAT QUALITY, ENTERIC METHANE EMISSIONS,
AND MUSCLE PROTEOMIC PROFILE OF BEEF CATTLE IN A
HIGH-CONCENTRATE FINISHING DIET IN FEEDLOT**

William Luiz de Souza

Zootecnista

**UNIVERSIDADE ESTADUAL PAULISTA - UNESP CÂMPUS
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HIGH-CONCENTRATE FINISHING DIET IN FEEDLOT**

William Luiz de Souza

Orientador: Prof. Dr. Ricardo Andrade Reis

Tese apresentada à Faculdade de Ciências
Agrárias e Veterinárias – Universidade Estadual
Paulista, Câmpus de Jaboticabal, como parte
das exigências para a obtenção do título de
Doutor em Ciência Animal

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Impacto potencial desta pesquisa

Esta pesquisa contribui significativamente para o aumento da produtividade e da eficiência alimentar de bovinos de corte em sistema de confinamento, promovendo práticas sustentáveis por meio da mitigação das emissões de metano entérico. A associação entre o manejo, animais de elevado potencial de resposta nutricional e a combinação de aditivos alimentares com diferentes funções nutricionais não apenas reduz as emissões de gases de efeito estufa, mas também melhora o desempenho de crescimento e as características da carcaça. Isso oferece à indústria de carne uma estratégia eficaz e inovadora para atender à crescente demanda global por proteína animal de maneira ambientalmente responsável.

Potential Impact of This Research

This research significantly contributes to enhancing productivity and feed efficiency in feedlot beef cattle while promoting sustainable practices through the mitigation of enteric methane emissions. The integration of effective management strategies, high-performance animals, and the combination of feed additives with diverse nutritional functions not only reduces greenhouse gas emissions but also improves growth performance and carcass characteristics. This provides the meat industry with an effective and innovative strategy to meet the growing global demand for animal protein in an environmentally responsible manner.

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: COMBINING 3-NITROOXYPROPANOL WITH DIFFERENT FEED ADDITIVES ON GROWTH PERFORMANCE, CARCASS TRAITS, MEAT QUALITY, ENTERIC METHANE EMISSIONS, AND MUSCLE PROTEOMIC PROFILE OF BEEF CATTLE IN A HIGH-CONCENTRATE FINISHING DIET IN FEEDLOT

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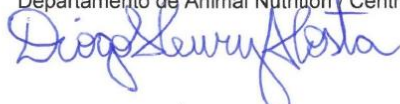
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Jaboticabal, 10 de dezembro de 2024

DADOS CURRICULARES DO AUTOR

William Luiz de Souza was born on February 1, 1994, in Raul Soares, Minas Gerais, Brazil, the son of Nelina Diogo Fortunato de Souza and Marcolino Luiz de Souza. He earned his undergraduate degree in Animal Science (2014–2018) at the Federal Institute of Education, Science, and Technology of Southeast Minas Gerais, Rio Pomba Campus. During his studies, he worked with the SEZOO group under the supervision of Professor Dr. Valdir Botega Tavares (2015–2018) and conducted scientific research under the guidance of Professors Dr. Onofre Barroca de Almeida Neto (2015–2016 and 2017–2018) and Dr. Arnaldo Prata Neiva Júnior (2016–2017 and 2018–2018). In 2018, he completed his internship at the Agência Paulista de Tecnologia dos Agronegócios (APTA) - Polo Regional Alta Mogiana, in Colina, São Paulo, under the guidance of Professor Dr. Flávio Dutra de Resende. In March 2019, he began his master's degree in animal science at the Faculty of Agricultural and Veterinary Sciences of São Paulo State University (UNESP), Jaboticabal, São Paulo, supervised by Dr. Flávio Dutra de Resende. During this period, he was a member of the Ruminant Production Study Group (GEPROR), responsible for conducting scientific projects in beef cattle at the APTA Regional Center in Colina, SP. In 2021, he commenced his PhD. studies in the same program at UNESP, Jaboticabal, supervised by Professor Dr. Ricardo Andrade Reis. During this period, he was a member of the Beef Cattle and Forage Research and Study Group (UNESPFor). Between July 2023 and August 2024, he participated in a research exchange program at Central Queensland University with the Precision Livestock Management (PLM - Nutrition) group, assisting with scientific projects under the supervision of Professor Dr. Diogo Fleury Azevedo Costa at the Institute for Future Farming Systems, Rockhampton, Queensland, Australia.

"The future belongs to those who believe in the beauty of their dreams "

Eleanor Roosevelt

I dedicate this...

To God, to my family, to my friends, and to my
advisors, for their teachings and constant
inspiration!!!

To all of you, I offer this...

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To God, to my mother, Nelina Diogo Fortunato de Souza, and my father, Marcolino Luiz de Souza, for the values instilled in me and their tireless efforts that enabled me to reach this point. To my brother, Leonardo Luiz de Souza, who was always willing to help and offer support.

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DSM PRODUTOS NUTRICIONAIS BRASIL S.A.
CEUA I&AS

Parecer nº 001/22

CERTIFICADO

Certificamos que o protocolo **BR 211215**, intitulado “**Efeitos da combinação de tecnologias com Bovaer® no desempenho de bovinos de corte confinados**”, registrado com o nº 001.22, sob a responsabilidade do pesquisador **Victor Valério de Carvalho** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS DO *INNOVATION AND APPLIED SCIENCE* da DSM PRODUTOS NUTRICIONAIS BRASIL S.A., em reunião de 22/02/2022.

Finalidade	() Ensino (x) Pesquisa Científica
Vigência da autorização	2 anos a contar da data de aprovação
Espécie/Linhagem/raça	Bovinos Nelore
Nº de Animais	224
Peso/Idade	400 kg, 20 meses
Sexo	Machos
Origem	Fazenda Caçadinha, Rio Brilhante/MS

We certify that the protocol BR 211215, entitled "Effects of combining technologies with Bovaer® on the performance of confined beef cattle", under the responsibility of Victor Valério de Carvalho, agree with the Ethical Commission in Use of Animals of the Innovation and Applied Science DSM and was approved.

São Paulo, 22 de fevereiro de 2022.

DocuSigned by:

Leticia Cardoso Bittencourt - Coordenadora



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CERTIFICADO

Certificamos que o protocolo BR 220121, intitulado “Efeitos da combinação de tecnologias com Bovaer® na redução da emissão de metano entérico e desempenho de bovinos de corte confinados”, registrado com o nº 003.22, sob a responsabilidade do pesquisador **Alexandre Perdigão** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS DO *INNOVATION AND APPLIED SCIENCE* da DSM PRODUTOS NUTRICIONAIS BRASIL S.A., em reunião de 22/02/2022.

Finalidade	() Ensino (x) Pesquisa Científica
Vigência da autorização	2 anos a contar da data de aprovação
Espécie/Linhagem/raça	Bovinos Nelore
Nº de Animais	30
Peso/Idade	400 kg, 20 meses
Sexo	Machos
Origem	Fazenda Caçadinha, Rio Brilhante/MS

We certify that the protocol BR 220121, entitled "Effects of combining technologies with Bovaer® in reduction of enteric methane emission and on performance of confined beef cattle", under the responsibility of Alexandre Perdigão, agree with the Ethical Commission in Use of Animals of the Innovation and Applied Science DSM and was approved.

São Paulo, 22 de fevereiro de 2022.

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Leticia Cardoso Bittencourt - Coordenadora

COMBINING 3-NITROOXYPROPANOL WITH DIFFERENT FEED ADDITIVES ON GROWTH PERFORMANCE, CARCASS TRAITS, MEAT QUALITY, ENTERIC METHANE EMISSIONS, AND MUSCLE PROTEOMIC PROFILE OF BEEF CATTLE IN A HIGH-CONCENTRATE FINISHING DIET IN FEEDLOT

Abstract:

The demand for red meat protein has been continuously increasing due to population growth, which places constant pressure on the agricultural sector to produce more efficiently. However, this need for expanded production is accompanied by the requirement to reduce the environmental impacts associated with livestock production, particularly regarding greenhouse gas emissions, such as enteric methane (CH₄) from ruminants. In this context, this study aimed to combine a potent enteric CH₄ inhibitor, 3-nitrooxypropanol, with various feed additives, including monensin sodium and a blend of essential oil, 25-hydroxyvitamin D₃, and chelated minerals such as chromium and zinc. The research assessed the effects of these combinations on performance, carcass traits, enteric CH₄ emissions, physiological responses, and muscle proteomic profiles of feedlot-finished Nellore bulls fed a high-concentrate diet. The study was conducted in two simultaneous experiments. In Experiment 1, one hundred and sixty-eight non-castrated Nellore bulls, with an initial body weight of 410 ± 8 kg and 16 months of age, were used. These animals were allocated to 24 pens, with 7 bulls per pen, totaling 8 pens per treatment in a randomized block design, where performance and meat quality were evaluated. In Experiment 2, thirty non-castrated Nellore bulls, with an initial body weight of 410 ± 3 kg and 16 months of age, were used. These animals were housed in a collective pen equipped with 6 electronic feeders and a Greenfeed unit, with 10 bulls per treatment in a completely randomized design. In this experiment, performance, carcass traits, CH₄ and hydrogen (H₂) emissions, blood parameters, nutrient intake and digestibility, and shotgun proteomic analysis of muscle tissue were evaluated. Both Experiments 1 and 2 received the same treatments and remained under experimentation for 102 days, using a diet with a roughage-to-concentrate ratio of 10:90, supplemented with three different treatments: control (monensin sodium at 26 mg/kg of dry matter - DM), M3NOP (monensin sodium at 26 mg/kg DM and 3-nitrooxypropanol at 100 mg/kg DM), and Combo (3-nitrooxypropanol at 100 mg/kg DM, essential oil at 100 mg/kg DM, 25-hydroxyvitamin D₃ at 0.10 mg/kg DM, chelated chromium at 4 mg/kg DM, and chelated zinc at 60 mg/kg DM). All data were analyzed using SAS 9.4 software with Tukey's test, except for proteomic data, which were analyzed using R software, with the support of websites such as UniProt (<https://www.uniprot.org/>) and STRING (<https://string-db.org/>). A significance level of 5% was adopted. In Experiment 1, Combo bulls showed higher ($P<0.01$) dry matter intake (DMI) on day 28 compared to control and M3NOP. From days 0 to 102, Combo bulls had higher ($P\leq 0.03$) final body weight and average daily gain (ADG) compared to control. Combo and M3NOP bulls showed better ($P<0.01$) feed conversion ratio (FCR) and feed efficiency (FE) compared to control. Hot carcass weight (HCW), carcass ADG, and carcass yield were higher ($P\leq 0.05$) for Combo bulls compared to control and M3NOP. Combo bulls showed higher ($P=0.01$) carcass yield compared to M3NOP. Combo bulls had better ($P=0.02$) biological efficiency compared to control. Combo bulls also had lower ($P<0.01$) carcass pH compared to control and M3NOP. In Exp. 2, Combo bulls had higher ($P=0.04$) DMI on day 28 compared to control and higher ($P<0.01$) DMI on day 102 compared to control and M3NOP. Combo bulls showed higher ($P=0.04$) HCW compared to control and M3NOP, and carcass ADG was higher ($P=0.04$) for Combo bulls compared to M3NOP. Combo and M3NOP bulls showed lower ($P<0.01$) CH₄ production (38.8%, g/d), yield (41.1%, g/kg DMI), intensity (40.8%, g/kg carcass ADG), and higher ($P<0.01$) H₂ emissions (291%, g/d) compared to control. Combo bulls had lower ($P<0.01$) blood glucose and insulin levels, as well as higher nutrient intake and digestibility ($P\leq 0.05$) compared to control and M3NOP. Regarding muscle proteomic profiles, 1,044 proteins were observed across control, M3NOP, and Combo treatments, with 47 proteins showing significant differences in abundance between Combo and control bulls, where 45 proteins were more abundant in the Combo treatment, while 2 were more abundant in the control treatment. When comparing control and M3NOP bulls, 41 proteins exhibited significant differences, with 18 proteins more

abundant in the control treatment and 23 in the M3NOP treatment. Between Combo and M3NOP bulls, 99 proteins showed significant differences, with 77 more abundant in the M3NOP treatment and 22 in the Combo treatment. These proteins contributed to the upregulation of several energy metabolism pathways, such as glycolysis, the citric acid cycle, and supporting pathways, as well as antioxidant metabolism, such as glutathione metabolism, compared to the muscles of control and M3NOP bulls, which showed no differences in any observed pathway in this study. The combination of 3-nitrooxypropanol with different feed additives improved feed conversion and feed efficiency and reduced enteric CH₄ emissions, regardless of the combination. The Combo treatment improved performance, carcass traits, nutrient intake and digestibility, and glucose and insulin responses, reflecting a more efficient energy and antioxidant metabolism in feedlot finishing bulls fed a high-concentrate diet.

Keywords: adoption of technology, animal productivity, carbon footprint, feed additives, feedlot, greenhouse gases, meat quality, muscle energy turnover, proteome, sustainable intensification.

COMBINAÇÃO DE 3-NITROOXIPROPANOL COM DIFERENTES ADITIVOS ALIMENTARES NO DESEMPENHO DE CRESCIMENTO, CARACTERÍSTICAS DE CARCAÇA, QUALIDADE DA CARNE, EMISSÕES DE METANO ENTÉRICO E PERFIL PROTEÔMICO MUSCULAR DE BOVINOS DE CORTE EM DIETA DE TERMINAÇÃO DE ALTA CONCENTRADO EM CONFINAMENTO

Resumo:

A demanda por proteína vermelha tem crescido continuamente devido ao aumento populacional, gerando uma pressão constante sobre o setor agropecuário para produzir de forma mais eficiente. No entanto, essa necessidade de expansão na produção é acompanhada pela exigência de reduzir os impactos ambientais associados à produção animal, especialmente em relação às emissões de gases de efeito estufa, como o metano (CH_4) entérico proveniente dos ruminantes. Diante desse cenário, esta pesquisa teve como objetivo combinar um potente inibidor de CH_4 entérico, o 3-nitrooxipropanol, com diferentes aditivos alimentares, incluindo monensina sódica e um pacote composto por óleo essencial, 25-hidroxivitamina D3 e minerais quelatados, como cromo e zinco. O estudo avaliou os efeitos dessas combinações sobre o desempenho, as características de carcaça, as emissões de CH_4 entérico, a resposta fisiológica e o perfil proteômico muscular de bovinos de corte da raça Nelore em confinamento, alimentados com uma dieta de terminação de alto teor de concentrado. A pesquisa foi realizada em dois experimentos simultâneos. No Experimento 1, utilizaram-se cento e sessenta e oito bois da raça Nelore, não castrados, com peso corporal inicial em jejum de 410 ± 8 kg e 16 meses de idade. Esses animais foram alocados em 24 baias, com 7 bois por baia, totalizando 8 baias por tratamento, em delineamento de blocos casualizados, onde foram avaliados o desempenho e a qualidade da carne. No Experimento 2, foram utilizados trinta bois da raça Nelore, também não castrados, com peso corporal inicial em jejum de 410 ± 3 kg e 16 meses de idade. Esses animais foram alocados em uma baia coletiva equipada com 6 cochos eletrônicos e uma unidade de Greenfeed, sendo 10 bois por tratamento em delineamento inteiramente casualizado. Neste experimento, foram avaliados desempenho, características de carcaça, emissão de CH_4 e hidrogênio entérico, parâmetros sanguíneos, consumo e digestibilidade de nutrientes, além de análise proteômica *shotgun* do músculo. Os Experimentos 1 e 2 receberam os mesmos tratamentos e permaneceram sob experimentação por 102 dias, em uma dieta com uma relação de volumoso e concentrado de 10:90, acrescida de três diferentes tratamentos: Controle (CTL) (monensina sódica em 26 mg/kg de matéria seca - MS), M3NOP (monensina sódica em 26 mg/kg de MS e 3-nitrooxipropanol em 100 mg/kg de MS) e Combo (3-nitrooxipropanol em 100 mg/kg de MS, óleo essencial em 100 mg/kg de MS, 25-hidroxivitamina D3 em 0,10 mg/kg de MS, cromo quelatado em 4 mg/kg de MS e zinco quelatado em 60 mg/kg de MS). Todos os dados foram analisados com o auxílio do software SAS 9.4, utilizando o teste de Tukey, exceto os dados proteômicos, que foram analisados com o software R, com apoio de websites como UniProt (<https://www.uniprot.org/>) e STRING (<https://string-db.org/>). Foi considerado um nível de significância de 5%. No experimento 1, os bois do tratamento Combo apresentaram maior ($P<0,01$) consumo de matéria seca (CMS) no dia 28 em comparação ao CTL e M3NOP. Durante os dias 0 a 102, os bois do tratamento Combo tiveram maior ($P\leq 0,03$) peso corporal final e ganho médio diário (GMD) em comparação ao CTL. Os bois dos tratamentos Combo e M3NOP apresentaram melhor ($P<0,01$) conversão alimentar (CA) e eficiência alimentar (EA) em comparação ao CTL. O peso de carcaça quente (PCQ), o GMD de carcaça e o rendimento de carcaça foram maiores ($P\leq 0,05$) para os bois do tratamento Combo em comparação ao CTL e M3NOP. Os bois do tratamento Combo apresentaram maior ($P=0,01$) rendimento de carcaça em comparação ao M3NOP. Os bois do Combo tiveram melhor ($P=0,02$) eficiência biológica em comparação ao CTL. Os bois do tratamento Combo apresentaram menor ($P<0,01$) pH da carcaça em comparação ao CTL e M3NOP. No Exp. 2, os bois no tratamento Combo tiveram maior ($P=0,04$) CMS no dia 28 em comparação ao CTL e maior ($P<0,01$) CMS no dia 102 em comparação ao CTL e M3NOP. Os bois do tratamento Combo apresentaram maior ($P=0,04$) PCQ em comparação ao CTL e M3NOP, e o GMD de carcaça foi maior ($P=0,04$) para os bois do Combo em comparação ao M3NOP. Os bois dos tratamentos Combo e M3NOP apresentaram menor ($P<0,01$) produção de CH_4 (38,8%, g/d),

rendimento (41,1%, g/kg CMS), intensidade (40,8%, g/kg GMD de carcaça) e maior ($P<0,01$) emissão de H_2 (291%, g/d) em comparação ao CTL. Os bois do tratamento Combo apresentaram menores níveis ($P<0,01$) de glicose e insulina no sangue, além de maior consumo e digestibilidade de nutrientes ($P\leq 0,05$) em comparação ao CTL e M3NOP. Quanto ao perfil proteômico do músculo, foi observada uma abundância de 1.044 proteínas entre os tratamentos CTL, M3NOP e Combo, das quais 47 apresentaram diferenças significativas em abundância entre os bois do tratamento Combo e CTL, sendo que 45 proteínas foram mais abundantes no tratamento Combo, enquanto 2 foram mais abundantes no tratamento CTL. Ao comparar os bois dos tratamentos CTL e M3NOP, 41 proteínas exibiram diferenças significativas em abundância, com 18 proteínas mais abundantes no tratamento CTL e 23 no tratamento M3NOP. Nos bois dos tratamentos Combo e M3NOP, foram observadas 99 proteínas com diferenças significativas em abundância, sendo 77 mais abundantes no tratamento M3NOP e 22 no tratamento Combo. Essas proteínas promoveram a regulação positiva de diversas vias do metabolismo energético, como a glicólise, ciclo do ácido cítrico e vias de suporte, além do metabolismo antioxidante, como o metabolismo da glutatona, em comparação ao músculo dos bois dos tratamentos CTL e M3NOP, que não apresentaram diferenças em nenhuma via observada neste estudo. A combinação de 3-nitrooxipropanol com diferentes aditivos alimentares melhorou a conversão alimentar e a eficiência alimentar e reduziu as emissões de CH_4 entérico, independentemente da combinação. O tratamento Combo melhorou o desempenho, as características de carcaça, o consumo e a digestibilidade de nutrientes, além de melhorar as respostas de glicose e insulina, refletindo em um metabolismo energético e antioxidante mais eficiente em bovinos de corte confinados alimentados com uma dieta de terminação com alta concentração de energia.

Palavras-chave: aditivos alimentares, adoção de tecnologia, *Bos indicus*, confinamento, eficiência energética muscular, gases de efeito estufa, intensificação sustentável, metabolismo energético muscular, pegada de carbono, produtividade animal, proteoma, qualidade da carne

CHAPTER 1 - GENERAL CONSIDERATIONS

1. Introduction

The global demand for beef has grown in recent decades, driven by population growth and rising per capita income in various regions of the world (Henchion et al., 2021). This growth has placed pressure on the livestock industry to maximize meat production in an efficient and sustainable manner (Cardoso et al., 2021; Ponnampalam and Holman, 2023). Key goals in this context include mitigating greenhouse gas emissions and the responsible conversion of animals into nutritious and safe food for human consumption (Ponnampalam and Holman, 2023). Among the most significant environmental challenges, enteric methane (CH₄) emissions stand out, with a global warming potential 28 times higher than carbon dioxide (CO₂) over a 100-year period (Sirigina et al., 2023). The agricultural sector accounts for approximately 14.5% of global greenhouse gas emissions (Gerber et al., 2013), highlighting the urgent need for solutions that reconcile the increased demand for meat with a reduction in environmental impacts.

In this context, sustainable intensification strategies for beef cattle production systems become essential to mitigate these emissions due to their carbon footprint while simultaneously promoting productivity increases. Examples of these strategies include feed supplementation (Cardoso et al., 2021) and the implementation of fertilization systems and appropriate pasture management (Cardoso et al., 2016). Such practices enable better nutritional plans for cattle, allowing them to enter the finishing phase at a young age, resulting in early slaughter and consequently reducing enteric CH₄ emissions per unit of animal product and over the emission period (Cardoso et al., 2016; Skidmore et al., 2022). In addition, selecting beef cattle for the feedlot phase from systems with adequate nutritional plans for continuous growth opens the opportunity to promote nutritional strategies within diets, such as using technological additives for various purposes, including improving feed efficiency, ensuring animal safety, increasing carcass gain, and inhibiting enteric CH₄ production (Duffield et al., 2012; Meschiatti et al., 2019; Baggerman et al., 2020; Honan et al., 2021; Marques and Cooke, 2021; Messersmith et al., 2021).

Given the changes in the phenotype of beef cattle, such as improved feed

efficiency, increased body weight gain, and carcass gains, these are directly associated with changes in different metabolic processes of these animals. Proteomic analyses have been applied to identify how different factors influence health, growth, nutritional conditions, and meat quality, allowing the observation of changes in energy metabolism, such as glycolytic and citric acid cycle pathways, as well as influences on antioxidant metabolism, crucial for cellular defense, through the differential expression of proteins in various biological materials (Gagaoua et al., 2020; Gagaoua et al., 2021; Lopes et al., 2021; Adnane et al., 2024).

This review aims to investigate the impacts of different feed additives on feedlot beef cattle, addressing their origins, mechanisms of action, and effects on nutrition based on scientific evidence. Aspects related to feedlot systems, such as their origin, number of animals, and operational strategies, are also discussed. Furthermore, the challenges associated with mitigating enteric CH₄ in feedlot systems are analyzed, as well as the use of proteomic analyses in studies with cattle, aiming to develop technologies that are sustainable and efficient in meat production, aligned with the growing global demands for environmental sustainability and food security.

5. Conclusion

The proteomic characterization of muscle tissue in beef cattle with varying carcass weights in this study provided valuable insights into the mechanisms governing energy metabolism and antioxidant activity in Nellore muscle cells. The treatment with 3-nitrooxypropanol, in combination with a feed additive package containing essential oils, 25-hydroxyvitamin D₃, and chromium- and zinc-amino-phospho-carbo chelated minerals, led to the upregulation of core energy pathways, including glycolysis and the TCA cycle, thereby enhancing ATP production efficiency. Additionally, the observed increase in muscle glycogen synthesis supports superior animal performance and optimal post-slaughter pH regulation, which are indicative of improved meat quality. Moreover, the significant upregulation of the glutathione metabolism pathway suggests an enhanced cellular antioxidant capacity, which is essential for neutralizing reactive oxygen species and maintaining cellular homeostasis, thereby preserving muscle integrity and function.

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