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OPTIMIZING NUTRIENT UTILIZATION IN BEEF CATTLE: THE EFFECTS OF NARASIN AND RUMEN-PROTECTED METHIONINE SUPPLEMENTATION

ANA LAURA JANUÁRIO LELIS

Thesis submitted to the Graduate Program in Animal Science as part of the requirements for obtaining the degree of Doctor of Science in Animal Science

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Ana Laura Januário Lelis – born on May 1, 1994, in the city of Tupã, São Paulo, Brazil, is the daughter of Sirlei Januário Marchizetti and Mauro Lelis. She began her undergraduate studies in Animal Science at São Paulo State University "Júlio de Mesquita Filho" – Dracena campus – in 2012, graduating in 2017. During her undergraduate program, she carried out a scientific initiation project with a scholarship funded by the São Paulo Research Foundation (FAPESP), under the supervision of Prof. Dr. Danilo Domingues Millen. She completed her mandatory internship in the United States, under the supervision of Prof. Dr. Antonio Faciola, working in the field of ruminant nutrition for a period of six months. After completing her degree, she returned to the United States, where she remained for another year. In 2019, she began her master's degree in animal science at the University of São Paulo – USP, Pirassununga campus, under the supervision of Prof. Dr. Paulo Henrique Mazza Rodrigues, with a scholarship granted by FAPESP, concluding the program in 2021. The following month, she started her PhD in Animal Science at São Paulo State University – School of Veterinary Medicine and Animal Science – Botucatu campus, under the supervision of Prof. Dr. Danilo Domingues Millen, with a FAPESP-funded scholarship, and is expected to complete the program in August 2025. Her research focuses on ruminant nutrition, with an emphasis on beef cattle.

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GENERAL ABSTRACT

This study addresses nutritional strategies aimed at intensifying beef cattle production through the use of feed additives to improve feed efficiency, ruminal health, and productive performance. Chapters 2 and 3 present two studies with this purpose. Chapter 2 aimed to evaluate the effects of different doses of narasin (13, 20, and 27 ppm) on ruminal fermentation, microbial composition, and nutrient digestibility in rumen-cannulated Angus steers fed a finishing diet containing 87% concentrate. The experiment followed a 3×3 duplicated Latin square design. Narasin supplementation enhanced ruminal pH stability, promoted beneficial changes in microbial diversity, and improved ruminal papillae morphology, especially at doses of 13 and 20 ppm. The intermediate dose (20 ppm) stood out by providing greater microbial diversity and ruminal stability. Conversely, the highest dose (27 ppm) reduced volatile fatty acid production and microbial diversity, suggesting an excessive antimicrobial effect. Acid detergent fiber digestibility improved with 27 ppm, although no significant impact on ruminal degradability was observed. Therefore, doses between 13 and 20 ppm are recommended, with 20 ppm being ideal under less controlled nutritional management. In Chapter 3, the impact of adding rumen-protected methionine (RPM) to supplementation protocols for crossbred heifers (½ Angus × ½ Nellore) finished on pasture was evaluated. The randomized block design included 48 animals distributed across 12 paddocks of *Urochloa brizantha* cv. Marandu and subjected to three supplementation protocols with different levels of energy supplementation, with or without rumen-protected methionine (RPM). Although no significant differences in productive performance were observed among treatments, heifers supplemented with methionine exhibited a larger rib eye area at the end of the study and lower fat deposition in the Biceps femoris muscle, highlighting improvements in carcass quality. RPM supplementation promoted histological changes in the cecum, indicating improvements in intestinal health and nutrient absorption potential. These findings suggest that RPM can optimize supplementation strategies in pasture-based systems, especially when combined with a gradual reduction in initial concentrate supply. Overall, the results demonstrate that the strategic use of additives such as narasin and rumen-protected methionine contributes to more efficient production systems capable of meeting the growing demand for high-quality beef.

Keywords: additives; feed efficiency; narasin; rumen; rumen-protected methionine.

RESUMO GERAL

O presente trabalho aborda estratégias nutricionais voltadas à intensificação da bovinocultura de corte, com ênfase no uso de aditivos alimentares para melhorar a eficiência alimentar, a saúde ruminal e o desempenho produtivo dos animais. Os capítulos 2 e 3 apresentam dois estudos com essa finalidade. No capítulo 2, o objetivo foi avaliar os efeitos de diferentes doses de narasina (13, 20 e 27 ppm) sobre a fermentação ruminal, composição da microbiota e digestibilidade de nutrientes em bovinos Angus canulados, confinados e alimentados com dieta contendo 87% de concentrado. O experimento foi conduzido em delineamento quadrado latino 3×3 duplicado. A suplementação com narasina aumentou a estabilidade do pH ruminal, promoveu alterações benéficas na diversidade microbiana e favoreceu o desenvolvimento morfológico das papilas ruminais, especialmente nas doses de 13 e 20 ppm. A dose intermediária (20 ppm) destacou-se por conferir maior diversidade bacteriana e estabilidade do ambiente ruminal. Em contrapartida, a dose mais elevada (27 ppm) resultou em menor produção de ácidos graxos voláteis e redução da diversidade microbiana, sugerindo efeito antimicrobiano excessivo. A digestibilidade da fibra em detergente ácido foi favorecida com 27 ppm, embora sem impacto significativo sobre a degradabilidade ruminal. Conclui-se que doses entre 13 e 20 ppm são mais recomendadas, com 20 ppm sendo ideal para situações de manejo nutricional menos rigoroso. No capítulo 3, investigou-se o impacto da inclusão de metionina protegida ao rúmen (RPM) em protocolos de suplementação para novilhas mestiças (½ Angus × ½ Nelore) terminadas a pasto em piquetes de Urochloa brizantha cv. Marandu. O delineamento foi em blocos casualizados com 48 animais distribuídos em 12 piquetes e submetidos a três níveis de suplementação com RPM. Embora não tenham sido observadas diferenças significativas no desempenho produtivo entre os tratamentos, as novilhas suplementadas com metionina apresentaram uma maior área do olho de lombo ao final do estudo e menor deposição de gordura no músculo Bíceps femoral, destacando melhorias na qualidade da carcaça. A suplementação com MPR promoveu alterações histológicas no ceco, indicando melhorias na saúde intestinal e no potencial de absorção de nutrientes. Esses achados sugerem que a MPR pode otimizar estratégias de suplementação em sistemas a pasto, especialmente quando combinada com uma redução gradual da oferta inicial de concentrado. De modo geral, os resultados demonstram que o uso estratégico de aditivos como a narasina e a metionina protegida no rúmen contribui para sistemas de produção mais eficientes capazes de atender à crescente demanda por carne bovina de alta qualidade.

Palavras-chave: aditivos; eficiência alimentar; metionina protegida; narasina; rúmen.

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LIST OF ABBREVIATIONS, ACRONYMS, AND SYMBOLS

ADF	Acid Detergent Fiber
ADG	Average Daily Gain
AOAC	Association of Official Analytical Chemists
ASA	Absorptive Surface Area
BW	Body Weight
Ca	Calcium
CP	Crude Protein
CV	Coefficient of Variation
DM	Dry Matter
DMI	Dry Matter Intake
EE	Ether Extract
FCAV	Faculty of Agricultural and Veterinary Sciences
FMVZ	Faculty of Veterinary Medicine and Animal Science
GDP	Daily Weight Gain
HCW	Hot Carcass Weight
IVDMD	In Vitro Dry Matter Digestibility
LM	Longissimus Muscle
LRCpH	Lethbridge Research Centre pH Logger
MAPA	Ministry of Agriculture, Livestock and Food Supply
ME	Metabolizable Energy
MPA	Mean Papillae Area
NDF	Neutral Detergent Fiber
Neg	Net Energy for Gain
NH_3-N	Ammonia Nitrogen
NOP	Number of Papillae per cm^2
OTU	Operational Taxonomic Unit
P	Phosphorus
peNDF	Physically Effective Neutral Detergent Fiber
ppm	Parts Per Million
R Core	R Statistical Computing Environment
REA	Rib Eye Area
RPM	Rumen-Protected Methionine
RPSA	Ruminal Papillae Surface Area
SAS	Statistical Analysis System
SCFA	Short-Chain Fatty Acids
SEM	Standard Error of the Mean
TDN	Total Digestible Nutrients

VFA	Volatile Fatty Acids

%	Percentage
°C	Degrees Celsius
µg	Microgram
µm	Micrometer
kg	Kilogram
g	Gram
mg/dL	Milligram per deciliter
mm	Millimeter
cm	Centimeter
cm^2	Square centimeter
mmol/L	Millimole per liter
mL	Milliliter
h	Hour
min	Minute
$\times 10^3$/mL	Thousands per milliliter (e.g., protozoa counts)
Mcal/kg	Megacalories per kilogram
ng/µL	Nanograms per microliter
$\log_{10}$	Logarithm base 10
P	Probability (statistical significance)
<	Less than
>	Greater than
±	Plus or minus (standard deviation or standard error)
pH	Hydrogen potential (acidity/basicity indicator)

SUMMARY

CHAPTER 1

Initial Considerations

The intensification of beef cattle production has driven the search for nutritional strategies aimed at increasing efficiency in feed, reducing metabolic losses, and improving carcass quality. Among these strategies, the use of feed additives stands out due to their positive effects on ruminal fermentation, nutrient utilization, and gut health in ruminants (Beauchemin et al., 2022). The adoption of such tools has become essential in both intensive systems, such as feedlots, and pasture-based or semi-intensive finishing systems, which are predominant in Brazil.

Among the widely used additives, ionophores represent a class of antimicrobial compounds that modulate the ruminal microbiota by favoring gram-negative bacteria and reducing lactic acid production. This modulation contributes to ruminal pH stabilization and increased energy efficiency of the diet (Russell & Strobel, 1989). Narasin, although less studied than other ionophores like monensin, has shown promising results in terms of ruminal fermentation, increasing propionate concentration, reducing ruminal ammonia levels, and improving animal performance even at lower doses (Cappellozza et al., 2019; Miszura et al., 2023). However, there are still few studies evaluating its use in high-energy diets typical of feedlots, especially regarding its influence on microbial community composition and digestive dynamics.

In addition, essential amino acids such as methionine play a key role in growth and protein synthesis. In forage-based tropical diets, the availability of sulfur-containing amino acids is often limited, which may restrict animal performance. Supplementation with rumen-protected methionine (RPM) has been proposed as an effective solution, allowing the amino acid to bypass ruminal degradation and be absorbed in the small intestine (Zanton et al., 2014; Cooke & Arthington, 2013). Studies have indicated that RPM supplementation can improve ruminal papillae development, nutrient absorption, digestive efficiency, and even carcass traits, particularly in pasture-based systems.

In this context, the present thesis is structured into two experimental chapters. Chapter 2 aimed to evaluate the effects of different narasin doses (13, 20, and 27 ppm) on ruminal fermentation, bacterial community composition, and nutrient digestibility in rumen-cannulated Angus steers fed high-concentrate diets. The central hypothesis was that increasing narasin doses would promote controlled changes in ruminal metabolism and microbiota, resulting in improved fermentation stability and feed efficiency. Chapter 3 evaluated the inclusion of RPM in supplementation protocols for crossbred heifers (½ Angus × ½ Nellore) finished on pasture. The study assessed animal performance, carcass traits, and the morphology of the rumen and cecum. It was hypothesized that RPM would improve nutrient absorption and allow for a gradual reduction in concentrate supplementation without compromising productive outcomes.

This work is relevant due to the need to expand knowledge on the strategic use of additives under different production conditions, offering technical support for the formulation of more efficient and practical nutritional strategies. Although the studies present limitations related to the number of animals and experimental duration, the findings contribute significantly

However, as pasture quality declined over time, particularly after day 84, the positive effects of methionine became less evident. This phase coincided with worsening environmental conditions, including lower mean temperatures (from 24.5 °C in March to 18.4 °C in June), reduced relative humidity (from 80% to 67.9%), and decreased precipitation (from 338 mm to 61.5 mm). These factors promoted pasture senescence, leading to a drop in forage crude protein (down to 7.4%) and an increase in neutral detergent fiber (NDF > 65%) and dead material content (above 50% in T3). Under such conditions, methionine alone was likely insufficient to sustain metabolic benefits, as energy and metabolizable protein limitations constrained the effective utilization of supplemented amino acids (Zanton et al., 2014).

The most striking findings occurred in cecal morphology, especially in the T3 group, which combined methionine supplementation with a gradual increase in energy supply. Heifers in T3 showed significantly lower cecal lesion scores, fewer goblet cells, and reduced numbers of disrupted goblet cells, indicating improved epithelial integrity. Two factors likely contributed to this result: progressive adaptation of the digestive tract to increased concentrate intake and the antioxidant function of methionine, a precursor of glutathione, which helps maintain membrane stability and mitigate oxidative stress in the intestinal mucosa (Wu et al., 2010; Pluske et al., 1996). The shallower crypts and reduced goblet cell counts observed in T3 further indicate a more stable and less inflamed mucosa, supporting improved intestinal function and nutrient absorption.

Regarding meat quality, the most relevant effects of methionine supplementation were observed in marbling scores and final pH. Higher marbling in T2 and T3 treatments suggests selective deposition of intramuscular fat, likely driven by methionine's glucogenic role and its influence on intramuscular lipogenesis (Ma et al., 2021). The observed difference in meat pH between T2 and T3, although all within normal physiological ranges (<5.8), may reflect differences in pre-slaughter muscle glycogen storage. All meat samples were aged for 14 days, allowing for standardized postmortem proteolysis and tenderness development. Despite no significant differences in shear force among treatments, all groups showed low values (<4 kgf), indicating desirable tenderness. Thus, the improved marbling in methionine-supplemented groups may have contributed to enhanced sensory quality, even without measurable changes in mechanical texture.

Overall, the T3 protocol provided cumulative benefits by combining methionine supplementation with a progressive feeding strategy. This approach supported more consistent physiological responses, particularly in digestive tract morphology and carcass traits, even under pasture-based systems transitioning into the dry season. The lack of a response in weight gain reinforces the notion that RPM acts more on metabolic efficiency and qualitative traits than on growth itself, especially in forage-based systems subject to seasonal nutrient limitations.

5. CONCLUSION

Rumen-protected methionine supplementation did not affect weight gain in crossbred heifers finished on pasture but improved carcass traits, ruminal papillae density, cecal integrity, and meat marbling. These benefits were more evident when forage quality was adequate, indicating that methionine effectiveness depends on overall dietary nutritional balance. The T3 protocol, with a gradual increase in supplementation, resulted in the best outcomes, suggesting that adaptation strategies enhance digestive health and nutrient utilization. Methionine supported metabolic efficiency and tissue quality, even without changes in performance. In forage-based systems with seasonal variability, methionine should be combined with sufficient protein and energy supply to optimize its benefits.

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