SAO PAULO STATE UNIVERSITY
SCHOOL OF VETERINARY MEDICINE AND ANIMAL SCIENCE
BOTUCATU CAMPUS

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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AUTHOR'S BIOGRAPHY

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

to the advancement of ruminant nutrition in Brazil, particularly in systems where both intensive and pasture-based methods coexist.

1. Literature Review

1.1 Narasin as a Modulator of Rumen Fermentation

Narasin is an ionophore used as a feed additive to improve rumen fermentation efficiency and, consequently, the productive performance of ruminants. Its selective antimicrobial action primarily affects gram-positive bacteria, promoting a shift in the rumen fermentation profile with higher propionate production and a reduction in methane and ammonia formation (Russell & Strobel, 1989).

Studies have shown that narasin, even at doses lower than those traditionally used for monensin, can promote rumen pH stability, reduce lactic acid production, and favor beneficial bacterial groups, such as *Lachnospiraceae*, associated with butyrate production and the development of rumen papillae (Cappellozza et al., 2019; Limede et al., 2021; Polizel et al., 2022).

The inclusion of narasin in ruminant diets presents a notable advantage due to the absence of negative effects on dry matter intake or the consumption of low-intake supplements, which is commonly observed with other ionophores such as sodium monensin (Soares et al., 2015). In an *in vitro* study, Nagaraja et al. (1987) observed that narasin supplementation increased the molar concentration of propionate at lower doses compared to monensin and lasalocid. Furthermore, narasin proved to be more effective in reducing lactic acid production than the other evaluated additives. The authors highlighted that the effective dose of narasin was three times lower than that required for the other ionophores.

The growing interest in narasin as a feed additive in ruminant nutrition stems, in part, from its higher efficacy in ATPase activity compared to monensin, which grants the compound a superior capacity to modulate the ruminal microbiota, affect fermentation parameters, and, consequently, improve productive performance (Wong et al., 1977).

Initial studies on the use of narasin as a feed additive in ruminant diets were conducted by Strasia et al. (1987), who evaluated various additives (monensin; monensin + tylosin; narasin; narasin + tylosin; and salinomycin) in high-grain diets for feedlot cattle. The authors found no significant statistical differences in carcass performance among treatments. However, the combination of an ionophore (monensin or narasin) with tylosin resulted in a lower incidence of liver abscesses.

In Brazil, the Animal Nutrition and Reproduction Laboratory (LNRA) of ESALQ/USP has been one of the pioneers in evaluating narasin in ruminant diets. In a study by Silva et al. (2015), doses of 0, 650, and 1300 mg of narasin per kg of mineral mix were provided to Nelore heifers fed with Tifton 85 haylage. The inclusion of the additive did not affect the intake of the supplement or the dry matter intake of the forage. However, heifers that received 1300 mg of narasin/kg of mineral mix showed an average intake of 15.62 mg narasin/kg of dry matter and a 19.75% increase in average daily gain (from 0.448 to 0.582 kg/day), along with a 25.26% improvement in feed conversion compared to the control group.

In a study with steers fed a high-forage diet, Miszura et al. (2018) found that supplementation with 13 mg narasin/kg of dry matter increased propionate concentrations and total short-chain fatty acid (SCFA) production, while decreasing the acetate:propionate ratio compared to monensin and lasalocid. These results can be attributed to the similar mechanism of action of ionophores, which modulate the ruminal microbial population by altering ion transfer across cell membranes (Berg & Hamill, 1978). This process leads to increased efficiency in energy and nitrogen metabolism of ruminal bacteria and/or the host animal (Russell & Strobel, 1989; McGuffey et al., 2001; Guan et al., 2006), which translates into improved performance in beef cattle (Duffield et al., 2012; Limede et al., 2021).

Gobato et al. (2017) also evaluated the inclusion of 0 and 1300 mg narasin/kg of mineral mix in high-concentrate diets for heifers. The average narasin intake was 10.6 mg/kg of total dry matter, with no impact on supplement or dry matter intake. Although no significant difference in average daily gain was observed, animals supplemented with narasin showed an 8.5% increase in feed efficiency.

Baggio et al. (2021) investigated the effects of different combinations of monensin (25 mg/kg of dry matter) and narasin (13 mg/kg of dry matter) in finishing cattle. During the adaptation period, narasin-supplemented animals had greater dry matter intake than those receiving monensin. However, no differences were observed in nutrient digestibility, performance, or carcass traits during the finishing phase.

Polizel et al. (2017) evaluated the effects of sodium monensin (25 mg/kg of dry matter) and narasin (10 or 20 mg/kg of dry matter) on rumen fermentation parameters in cattle fed diets containing 90% concentrate during the adaptation period. The authors observed no changes in the molar proportions of acetate, propionate, and butyrate during the first seven days of feeding. However, cattle supplemented with monensin or 20 mg narasin/kg dry matter had higher ruminal pH values compared to the control group and those supplemented with 10 mg narasin/kg dry matter. These findings highlight the ability of such additives to maintain higher

ruminal pH levels during abrupt dietary transitions, underlining the importance of monitoring and testing narasin in high-concentrate diets to support ruminal and intestinal health and improve predictive accuracy for productive performance.

Research also suggests that narasin can positively impact rumen health, with results showing lower ammonia concentrations in the rumen and reduced risk of metabolic disorders, such as acidosis. The narasin supplementation increased carbohydrate utilization efficiency from high-concentrate diets, highlighting the additive's potential in promoting more efficient rumen fermentation.

However, it is important to note that most studies on narasin were conducted in pasture-finished animals using protein-energy supplements at doses ranging from 13 to 20 mg/kg DM (Polizel et al., 2020). These doses, although effective for pasture-based systems, still require validation under confinement conditions with high-concentrate diets. It is this gap that drives research into using these same doses in environments with higher fermentative load and risk of metabolic disturbances. Miszura et al. (2023) reported an increase in fiber digestibility and weight gain in cattle finished on high-energy diets supplemented with narasin.

The literature still lacks standardization in experimental conditions and dietary profiles used, making it difficult to consolidate specific recommendations for confined cattle.

1.2 Protected Methionine in Ruminant Nutrition

Methionine is an essential amino acid that often limits productive performance in beef cattle, especially in diets based on tropical forages. Supplementing with rumen-protected methionine (RPM) seeks to overcome this limitation, ensuring that the amino acid is absorbed in the small intestine, where it can be used for protein synthesis, muscle growth, and lean tissue deposition (Zanton et al., 2014). The "protection" of methionine in the rumen is critical, as it prevents degradation by ruminal microorganisms, ensuring that the amino acid reaches the small intestine intact and available for the animal's metabolism.

Methionine plays a vital role in maintaining ruminal epithelial integrity. Its absorption in the small intestine is directly linked to the synthesis of essential proteins, and its use can improve nitrogen utilization efficiency and microbial protein production. Several studies have shown that RPM has positive effects on rumen development. Cooke & Arthington (2013) observed that RPM increased rumen papilla height and mucosal thickness, indicating an improvement in rumen integrity.

In confinement systems, RPM supplementation has shown improvements in nitrogen retention and muscle deposition in beef cattle, even without altering dry matter intake. Zanton

et al. (2014) also observed that RPM increased nitrogen utilization efficiency and microbial protein production, which may contribute to improved feed efficiency and nutrient utilization. RPM has been associated with better utilization of nitrogen compounds, particularly in diets with high concentrate levels (Pereira et al., 2020).

On the other hand, in pasture-based systems, the effects of RPM on productive performance are more variable.

The "protection" of methionine from ruminal degradation ensures that more methionine is available for the animal, without being lost to the ruminal microbiota. Moreover, methionine is a precursor to other essential compounds, such as cysteine, taurine, and glutathione, whose antioxidant and immune functions are crucial for rumen health and overall animal performance. These aspects underline the importance of RPM, especially under greater nutritional challenges, where nutrient utilization efficiency is essential.

Recent studies by Ortiz et al. (2021) corroborate these findings, demonstrating that RPM supplementation in high-concentrate diets also improves nitrogen utilization and microbial protein synthesis, resulting in better animal performance, particularly under high production conditions.

1.3 Final Considerations

A critical analysis of the literature reveals that both narasin and protected methionine present distinct and complementary mechanisms of action in ruminant nutrition. While narasin primarily modulates fermentation and energy utilization efficiency, protected methionine works in nutrient absorption and maintaining digestive epithelial integrity. Despite the advances, there are still significant gaps regarding the definition of ideal doses, interactions with other nutrients, and responses under different management conditions, which justifies the need for more standardized and comprehensive future investigations.

General Objectives:

- Evaluate the effects of different doses of narasin on rumen fermentation, microbiota, and nutrient digestibility and degradability of high-energy diets in confined Angus cattle.
- Assess the impact of protected methionine supplementation and supplementation levels on performance, rumen morphology, cecal histology, and carcass characteristics of pasture-finished heifers.

Journals for Publication:

- Livestock Science
- Revista Brasileira de Zootecnia

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CHAPTER 2

The effects of increasing dosages of narasin on ruminal fermentation patterns, bacterial community composition, and nutrient digestibility in beef cattle receiving feedlot diets

The effects of increasing dosages of narasin on ruminal fermentation patterns, bacterial community composition, and nutrient digestibility in beef cattle receiving feedlot diets

ABSTRACT

This study evaluated the effects of different narasin doses on ruminal fermentation, nutrient digestibility, and papillae histology in Angus cattle fed feedlot diets. Three Angus steers (average body weight: 680 kg) were used in a 3×3 Latin square design with treatments of 13, 20, and 27 ppm of narasin. Each period included 14 days of adaptation and seven days of sampling. Ruminal degradability was assessed on days 15–17, digestibility from days 15–19, ruminal pH on days 19–20, and samples for SCFA, microbiota, and histology were collected on days 20 and 21. Increasing narasin doses improved ruminal pH stability, with a linear increase in minimum pH ($P = 0.01$) and reduced time below pH 5.6 ($P = 0.10$). At 13 ppm, SCFA production, particularly acetate and propionate, increased ($P < 0.05$), indicating enhanced fermentation efficiency. The 20 ppm dose significantly increased the keratin layer thickness of ruminal papillae ($P = 0.02$), suggesting improved tissue development. At 27 ppm, ammonia ($P < 0.01$), acetate ($P = 0.02$), and butyrate ($P < 0.01$) concentrations decreased, while the acetate-to-propionate ratio increased ($P < 0.01$). A linear decrease in lactate concentration was also observed with increasing narasin doses ($P = 0.03$). Digestibility of acid detergent fiber (ADF) was significantly affected, with optimal results at 27 ppm ($P = 0.01$). These findings suggest that narasin supplementation modulates ruminal fermentation and digestive parameters, with doses between 13 and 20 ppm providing the best balance between fermentation efficiency and ruminal health.

Keywords: additive, behavior, dose, metabolism, rumen.

1. INTRODUCTION

Most livestock production in Brazil occurs in pasture-based systems; however, feedlot systems have become increasingly popular to finish cattle sooner and improve feed efficiency. In these systems, the average inclusion level of concentrate ingredients in the diets is 83.5% (Silvestre and Millen, 2021), with corn being the primary ingredient used. Improving production efficiency in ruminants requires optimizing ruminal fermentation, enhancing the quality of dietary ingredients, or manipulating microbial populations in the rumen (Polizel et al., 2020).

Cattle have evolved to consume forage, and when fed diets high in fermentable carbohydrates, significant ruminal changes may occur. These changes include increased free glucose availability, stimulation of bacterial growth, elevated production of short-chain fatty acids (SCFA) and lactic acid, reduced ruminal pH, and decreased rumen motility. Such changes can trigger a cascade of undesirable physiological processes, ultimately leading to ruminal acidosis (Owens et al., 1998). In a survey of Brazilian feedlot nutritionists, 11.4% of respondents identified acidosis-related issues as the second most significant health problem in feedlot cattle, following respiratory diseases (Silvestre and Millen, 2021).

Feed additives are commonly included in feedlot diets to reduce digestive disorders, such as acidosis, and to optimize ruminal fermentation in a controlled manner. Among these additives, ionophores are widely used (Silvestre and Millen, 2021). Monensin, one of the most studied ionophores, has demonstrated the ability to enhance SCFA production, alter their proportions, and, in certain cases, improve animal performance (Tedeschi et al., 2003). However, narasin, an ionophore originally studied in cattle consuming high-forage diets, has been shown to increase average daily gain (ADG) without affecting dry matter intake (DMI) at lower doses than monensin (Cappellozza et al., 2019; Limede et al., 2021; Miszura et al., 2023). Given its potential, interest is growing in evaluating its effects on feedlot diets.

Despite the limited number of studies on narasin for cattle consuming feedlot diets, some promising findings have emerged. Polizel et al. (2020) examined the effects of narasin in finishing lambs at doses based on previous high-forage research and observed increased nutrient digestibility, a higher proportion of propionate, and reduced ammonia production in the rumen. These results suggest a greater energy supply, leading to improved ADG and feed efficiency. The greatest benefits were observed at a 15-ppm dose, surpassing the effects of a 25 ppm monensin treatment. Additionally, Baggio et al. (2023) evaluated narasin supplementation during the adaptation and finishing phases of cattle at a dose of 13 ppm and reported increased dry matter intake (DMI) during the adaptation phase. However, this effect did not persist into the finishing phase, and no significant impacts on performance or ruminal parameters were noted.

Narasin seems to be a promising ionophore for feedlots that employ diets similar to those fed in Brazil (1.27 vs. 1.50 Mcal of net energy for gain/kg of dry matter in USA [DM; Silvestre and Millen, 2021; Samuelson et al., 2016, respectively]). In feedlots in the U.S. and Brazil, monensin is commonly used to enhance feed efficiency and control and prevent coccidiosis (Duffield et al., 2012; Samuelson et al., 2016), however, due to the lower energy content of finishing diets in Brazil, the use of an ionophore that does not affect DMI, such as narasin, may be desirable. A dose of 13 ppm of narasin is recommended for cattle grazing on or fed high-forage diets (Limede et al., 2021).

Therefore, the dosage of narasin tested in this experiment was based on the most effective responses observed in studies involving high-forage diets for beef cattle. However,

the limited research on narasin in feedlot diets raises questions about its efficacy and optimal dosing for feedlot cattle. We hypothesize that increasing levels of narasin will positively influence ruminal metabolism in Angus cattle, resulting in beneficial changes to ruminal fermentation patterns and bacterial community composition. The objective of this study was to evaluate the effects of narasin inclusion at different doses on ruminal fermentation, bacterial community composition, and nutrient digestibility in Angus cattle cannulated in the rumen.

2. MATERIAL AND METHODS

Animals and Experimental Facility

The study was conducted at the São Paulo State University (UNESP) feedlot, Dracena campus, Brazil. The project was submitted to and approved by the Animal Ethics Committee (CEUA) of Unesp/Dracena under protocol number 03/2022. Three Angus steers, with an average initial body weight (**BW**) of approximately 680 kg, 36 months of age, and cannulated in the rumen, were used.

Experimental Design

The experiment utilized a duplicated 3×3 Latin square design, in which the three animals were rotated among treatments over two experimental periods replicated over time. The treatments included doses of narasin: Treatment 1: 13 ppm, Treatment 2: 20 ppm, and Treatment 3: 27 ppm.

The 13-ppm treatment is commonly used in supplements for grazing systems (Gobato et al., 2020; Soares et al., 2021; Miszura et al., 2023). Since feedlot diets have a higher energy content than forage diets, the tested doses were equal to or greater than the typical dose for grazing animals.

Management, Feeding, and Animal Handling

Adaptation in each period took place over 14 days, followed by 7 days of sampling, leading to a total of 21 days per experimental period and 156 days for the entire experiment. Feeding occurred once daily in the morning at 08:00. The diet amount provided was based on the leftovers from the previous day's feeding. These leftovers were collected for chemical analysis and then discarded. The adaptation protocol utilized a step-up method and included three adaptation diets. The initial diet contained 75% concentrate, with a 4% increase in concentrate during each adaptation phase. Tifton hay was used as the sole source of forage and was gradually removed from the diets throughout the adaptation period. The transition from adaptation diets to the finishing diet was carried out as follows: 5 days on adaptation 1, 4 days on adaptation 2 (79% concentrate), and 5 days on adaptation 3 (83% concentrate). The finishing diet consisted of 87% concentrate ingredients (Table 1). The pens were partially concreted, with good air circulation, and had a stocking density of 1 animal per pen (72 m² and 6 m of linear bunk per animal), always providing water through automatic drinkers (090x10x20 m). At the end of each period, the animals underwent a 5-day washout, during which they were released into pasture to standardize the ruminal microbiota and prevent interference from previous treatments.

Table 1. Feed ingredients and chemical composition of the experimental diets provided to rumen-cannulated Angus cattle during the adaptation and finishing phases.

Diets	Adaptation 1	Adaptation 2	Adaptation 3	Finishing
Concentrate (%)	75.0	79.0	83.0	87.0
Ingredients (% DM)				
Tifton hay	15.0	11.0	7.0	3.0
Peanut hulls	10.0	10.0	10.0	10.0
Finely-ground corn	51.0	56.0	61.0	66.0
Soybean meal	10.0	8.0	6.0	4.0
Cottonseed cake	11.0	12.0	13.0	14.0
Urea	0.5	0.5	0.5	0.5
Mineral supplement	2.5	2.5	2.5	2.5
Nutritional Content (% DM)				
TDN[1]	78.0	80.0	81.0	82.0
Crude protein	15.8	15.1	14.4	13.8
Ether extract	4.0	4.1	4.2	4.3
Neutral detergent fiber	33.9	31.7	29.6	27.5
peNDF[2]	23.0	21.0	18.0	16.0
NE_g, Mcal/kg	1.12	1.15	1.19	1.23
Ca	0.52	0.50	0.48	0.47
P	0.41	0.41	0.41	0.42

[1]Total digestible nutrients, [2]Physically effective neutral detergent fiber.

The experimental diets were formulated according to the LRNS (Large Ruminant Nutrition System (Fox et al., 2004), level 2. Feed ingredients were mixed in a truck-mounted mixer. Narasin was administered using soybean meal as a carrier, applied on top of the diet, and mixed immediately after being placed in the bunk feeder.

Experimental diets and ingredients were sampled weekly, as outlined by Pereira et al. (2020), to determine DM (AOAC, 1990; method 930.15), ether extract (AOAC, 1990; method 920.39), crude protein (AOAC, 1995; method 990.02), neutral detergent fiber (**NDF**; Van Soest et al., 1991), and ash (AOAC, 1990; method 942.05).

Ruminal degradability in situ

The in situ degradability assay was conducted on days 15, 16, and 17 of the experimental periods following the methodology proposed by Mehres and Ørskov (1977). Nylon bags with a porosity of 50 microns, measuring 100 x 190 cm, were used to contain approximately 25 g of the experimental diet samples, which had been dried in an oven at 65°C for 72 hours. The bags were incubated in the animals for 0, 6, 12, 24, 48, and 72 hours on the specified days. For each incubation period, three replicas of nylon bags were utilized, and after being removed from the rumen, they were washed in running water until the wash fluid was colorless, then dried at 65°C for 72 hours for subsequent analysis of starch (POORE et al., 1993), crude protein (**CP**; AOAC, 1995), NDF, ADF (VAN SOEST et al., 1991), and ash (AOAC, 1995). Degradability data were adjusted according to the Ørskov and McDonald (1979) model, while potential and effective degradability utilized models proposed by Ørskov et al. (1980).

Total dry matter and nutrient digestibility

The total nutrient digestibility (DM, CP, NDF, ADF, starch, and ash) was determined using titanium dioxide as an external marker (Pezzato et al., 2002). Between days 10 and 19 of the experimental periods, 1 g/kg DM supplied was administered via cannula, half at 08:00 and the remainder at 16:00. From days 15 to 19, samples of feces, diets, and leftovers were collected. At the end of the collection period, the samples were ground through a 2 mm sieve to form a composite sample representing the five days of collection. The apparent digestibility coefficient of the nutrients was calculated based on the concentration of titanium dioxide in the diet, leftover, and fecal samples.

Continuous ruminal pH measurement

The pH, temperature, and redox potential values in the rumen were recorded every 5 minutes during days 19 and 20 of each period. The measurement system utilized was the Lethbridge Research Centre (LRCpH), developed with a data logger (model T7-1 LRCpH Dascor, Escondido, CA). Two 900g weights were attached to the logger to ensure it remained close to the ruminal epithelium (PENNER et al., 2006). The collected data were used to calculate minimum pH, average pH, maximum pH, the duration pH remained below 5.2, 5.6, and 6.2 in minutes, as well as the area under the pH curves below 5.2, 5.6, and 6.2 according to Bevans et al. (2005). The sensor was calibrated using solutions with pH 7.0 and 4.0, facilitating the adjustment of the measured data.

Evaluation of ruminal fermentation products

Ruminal fluid samples were collected at 0, 4, 8, and 12 hours after the diet was provided on day 20 of each experimental period. At each time point, samples were collected from various locations in the rumen and squeezed through cheesecloth. Approximately 50 mL of ruminal fluid was harvested and transported to the laboratory, where it was prepared for future analyses of SCFA, lactate, and NH3-N. The 0-hour sample was obtained before feeding. During sample preparation, 25 mL of ruminal fluid was centrifuged for 15 minutes at 3500 rpm, and aliquots of the supernatant were used for the analyses mentioned earlier. The determination of SCFA was performed using the methodology proposed by Erwin et al. (1961) via high-performance gas chromatography. A test tube containing 2 mL of supernatant was sealed with a stopper, treated with 4 mL of formic acid, and stored in a freezer (-20 °C) until analysis. The gas chromatograph used for the analysis was a Finnigan model 9001, equipped with a Megabore column from Ohio Valley, model OV-351, which measured 1 micron, 30 mm in length, and 0.53 mm in diameter. The total concentration of lactic acid was determined using the colorimetric technique described by Pryce (1969). To determine ammonia nitrogen concentration, 2 mL of the supernatant was placed in test tubes with 1 mL of 1N sulfuric acid and stored in a freezer (-20°C) until colorimetric analysis according to the method described by Kulasek (1972) and adapted by Foldager (1977).

Ruminal bacterial community sequencing

On day 20 of each experimental period, four hours after feeding, 50 mL of ruminal content (solid and liquid parts) was collected in sterile tubes and frozen at -80°C. The total DNA was extracted using a mechanical disruption and phenol extraction protocol. All samples with extracted DNA were resuspended in DNA elution buffer, then quantified using the Qubit Fluorometer (Invitrogen, San Diego, CA, USA), and stored at -80°C. All samples were diluted

to 10 ng of DNA per µl to ensure at least 50 ng per PCR reaction. The PCR procedure was conducted using universal primers that flank the variable region 4 (V4) of the bacterial 16S rRNA gene. A total of 50 ng of DNA, 0.4 µM of each primer, 12.5 µl of 2X Hot Start Ready Mix (KAPA Biosystems, Wilmington, MA, USA), and water (DNA/RNA free water up to a volume of 25 µl were utilized for the sample amplification reaction. Each cycle proceeded as follows: initial denaturation at 95°C for 3 minutes, followed by 25 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 5 minutes. After amplification, 25 µL of each sample was transferred to a low-melt agarose gel along with an additional 5 µL of 6x Orange loading dye. Samples displaying bright bands around 380 bp were collected from the gel for subsequent extraction and sequencing using MiSeq (Illumina, San Diego, CA, USA) with 5% PhiX for DNA control in MiSeq v4 kits (Illumina, San Diego, CA, USA; Dill-McFarland et al., 2017). The sequences obtained from Illumina MiSeq were processed using Mothur software v.1.40.0 (www.mothur.org/wiki). The bacterial community sequences obtained were aligned with the reference database known as SILVA 16S rDNA, as described by Dill-McFarland et al. (2017). Alpha diversity, which refers to the diversity of bacterial communities within each animal, was calculated using the Chao estimate, a measure of species richness, and the Shannon index, which assesses microbial diversity. Similarly, beta diversity, assessing differences in bacterial community composition among animals, was calculated using the Bray-Curtis and Jaccard tests. Additionally, the relative abundances of noteworthy bacterial communities and those that were most prevalent were determined (WEIMER et al., 2017). Bacterial sequencing was performed at the Department of Technology of FCAV/UNESP in Jaboticabal campus.

Ciliated protozoa

Samples for the differential count of ciliated protozoa in the rumen were collected on day 20 of each experimental period, 4 hours after feeding. In this process, 10 mL of the collected material for bacterial sequencing analysis were stored in tubes containing 20 mL of 50% formaldehyde (v/v). A total of 1 mL of the sample diluted in formalin had brilliant green and glycerol added, resulting in a 30-fold dilution. The count was performed using a "Sedgwick Rafter" counting chamber with internal dimensions of 50 mm x 20 mm x 1 mm (capacity 1 mL), as outlined by Dehority (1993), where 100 optical fields were counted using a reticle.

Ruminal papillae histology

For the histological evaluation of papillae, samples were collected from the ruminal wall on day 21 of the experimental period. The samples were processed through a series of solutions, spending 1 hour each in 70% alcohol, 90% alcohol, 100% alcohol, absolute ethyl alcohol I, absolute ethyl alcohol II, xylene I, xylene II, paraffin I (Histosec ®), and paraffin II (Histosec ®). Following these stages, the samples were embedded in aluminum molds filled with paraffin III (Histosec ®) and the ruminal papillae sample according to Odongo et al. (2006). Sections were cut at 6 µm using a microtome to prepare histological slides. The slides were stained with hematoxylin-eosin and Harris's hematoxylin. The following histological measurements were performed: height, width, and area of papillae, thickness of the keratinized epithelium, and mitotic index. The Leica Qwin Image Analyzer was utilized for histological analysis through a Leica electronic light microscope.

Statistical Analysis

Residual normality and heteroscedasticity tests were conducted before proceeding with the analysis of variance. When necessary, the data were transformed. The treatments' effects were categorized as fixed; however, when repeated measures over time were involved, the effects of these measures and their interaction with the treatments were classified as random. Similarly, the effects of the period and the animal were treated as random in the model. Data were analyzed using the PROC MIXED procedure in SAS (2003), with a significance level of 5%. Trends were discussed at $P < 0.08$. The bacterial sequencing data were analyzed using R software (R CORE TEAM, 2015) after sequence processing with Mothur v.1.40.0 (www.mothur.org/wiki).

3. RESULTS

Ruminal degradability in situ

Including narasin in the diet of rumen-cannulated Angus cattle did not significantly affect the degradability of DM, ash, CP, starch, NDF, or ADF. Different levels of narasin (13, 20, and 27 ppm) showed no significant impact on the ruminal degradation of these nutrients, with all effects being statistically non-significant (Table 2).

Table 2. In situ degradability of the moderate-energy diet with the addition of narasin provided to rumen-cannulated Angus cattle.

	Narasin (ppm)				P Value	
Item	13	20	27	SEM[1]	L[4]	Q[5]
In situ Degradability						
Dry Matter, %	60.54	60.00	61.52	3.01	0.37	0.19
Ash, %	74.68	74.97	74.79	1.28	0.89	0.79
NDF[2], %	38.19	31.43	35.42	3.37	0.52	0.13
ADF[3], %	21.30	21.96	22.03	4.09	0.70	0.85
Crude Protein, %	60.20	60.34	58.59	3.02	0.56	0.67
Starch, %	74.13	75.77	75.60	1.25	0.41	0.49

[1]Standard error of the mean; [2]Neutral detergent fiber; [3]Acid detergent fiber; [4] Linear effect; [5] Quadratic effect.

Total dry matter and nutrient digestibility

Adding narasin to the diet of rumen-cannulated Angus cattle did not significantly affect the total apparent digestibility of DM, ash, CP, or starch. However, a significant quadratic effect was found for the digestibility of ADF ($P = 0.01$), with 34.55%, 30.45%, and 38.05% for the 13, 20, and 27 ppm treatments, respectively (Table 3).

245 **Table 3.** Total apparent digestibility of the moderate-energy diet with the addition of narasin
246 provided to rumen-cannulated Angus cattle.

	Narasin (ppm)				P Value	
Item	13	20	27	SEM[1]	Linear	Quadratic
Total Apparent Digestibility						
Dry Matter, %	83.60	83.55	83.45	0.08	0.20	0.82
Ash, %	61.94	70.22	58.27	6.51	0.69	0.22
NDF[2], %	66.38	70.18	72.55	2.51	0.08	0.80
ADF[3], %	34.55	30.45	38.05	2.01	0.14	**0.01**
Crude Protein, %	86.03	87.09	85.39	1.07	0.68	0.31
Starch, %	95.56	95.57	95.58	0.16	0.89	0.96

247 [1]Standard error of mean; [2]Neutral detergent fiber; [3]Acid detergent fiber.

248

249 **Continuous ruminal pH measurement**

250 The results shown in Table 4 revealed a quadratic effect on DMI, measured in kg (P =
251 0.04) and as a percentage of BW (P = 0.01), and for mean pH (P = 0.04) with the lowest values
252 found in cattle receiving 20 ppm of narasin. Moreover, a linear increase in minimum ruminal
253 pH was identified as narasin supplementation rose (P = 0.05). In contrast, the time spent with
254 ruminal pH below 5.2 and the area under pH 5.6 decreased linearly with higher narasin doses,
255 indicating a beneficial effect on ruminal pH stability. Additionally, the duration of pH levels
256 below 6.2 increased linearly as the doses of narasin rose (P = 0.07), and cattle consuming 20
257 ppm of narasin exhibited the largest pH area under 6.2 (P = 0.02).

258

259 **Table 4.** Dry matter intake, ruminal pH, temperature, and oxidation-reduction potential of
260 rumen-cannulated Angus cattle fed moderate-energy diets supplemented with narasin.

	Narasin (ppm)				P Value	
Item	13	20	27	SEM[1]	Linear	Quadratic
DMI, kg	20.70	20.19	23.47	0.86	0.01	**0.04**
DMI, kg/%BW	2.46	2.37	2.79	0.12	0.01	**0.01**
Maximum pH	6.54	6.33	6.38	0.11	0.23	0.24
Minimum pH	5.39	5.44	5.70	0.14	**0.01**	0.19
Mean pH	5.93	5.75	5.97	0.12	0.73	**0.05**
pH<5.2, min	160.83	73.33	0.00	84.51	**0.08**	0.76
pH<5.6, min	333.30	429.00	10.00	170.29	0.10	0.13
pH<6.2, min	980.00	1344.17	1240.83	82.33	**0.08**	0.76
pH area <6.2	462.19	662.12	358.04	161.26	0.85	**0.03**
pH area <5.6	146.03	101.05	0.65	82.33	**0.07**	0.36
pH area <5.2	52.05	8.30	0.00	16.90	0.12	0.81
Temperature, ºC	39.99	39.91	39.44	0.29	0.24	0.54
ORP[2]	-253.79	-253.79	-253.72	30.47	0.17	0.41

pH at 4h[3]	6.02	5.93	5.99	0.10	0.75	0.39

[1]Standard error of mean; [2]Ox-Redox Potential; [3]post-feeding.

Evaluation of ruminal fermentation products

The results of ruminal fermentation products are shown in Table 5. Supplementing with 27 ppm of narasin significantly reduced NH_3, acetate, and butyrate concentrations, as well as total SCFA ($P = 0.01$). Additionally, cattle receiving 20 ppm of narasin demonstrated a decrease in propionate concentration ($P < 0.01$) and an increase in the acetate-to-propionate ratio ($P < 0.01$). A notable linear decrease ($P = 0.03$) in ruminal lactate concentration was observed across all three treatments.

Table 5. Short-chain fatty acids profile, NH3, and lactate of rumen-cannulated Angus cattle fed moderate-energy diets supplemented with narasin.

	Narasin (ppm)				P Value	
Item	13	20	27	SEM[1]	L[3]	Q[4]
NH_3, mg/dL	11.40	14.12	3.52	1.69	<0.01	**0.01**
Acetate, mmol/L	65.54	67.95	46.85	5.12	0.02	**0.01**
Propionate, mmol/L	49.93	27.04	35.78	4.52	0.10	**<0.01**
Butyrate, mmol/L	13.09	17.93	10.83	2.18	0.30	**<0.01**
Lactate, mmol/L	0.55	0.60	0.46	0.13	**0.03**	0.97
Total SCFA, mmol/L	128.72	111.36	93.15	8.86	**0.01**	0.94
ACET:PROP[2]	1.43	2.76	1.46	0.16	0.93	**<0.01**

[1]Standard error of mean; [2]Acetate to propionate ratio; [3]Linear effect; [4]Quadratic effect.

Ruminal bacterial community sequencing

Figure 1 illustrates the data on ruminal bacterial richness. Regarding alpha diversity, narasin supplementation did not have a significant effect on ruminal bacterial richness (Chao richness), with no differences noted across the various dosages ($P = 0.71$). However, regarding beta diversity (Jaccard), bacterial richness significantly differed between animals supplemented with 20 ppm and 27 ppm of narasin.

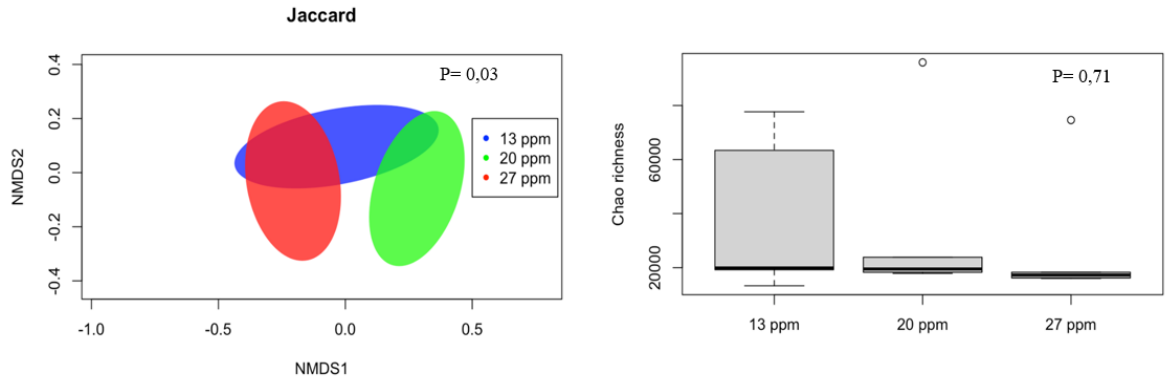


Figure 1. Ruminal bacterial richness (beta and alpha diversity) of rumen-cannulated Angus cattle fed moderate-energy diets supplemented with narasin.

The alpha bacterial diversity (Shannon's diversity) in animals supplemented with varying dosages of narasin exhibited a quadratic response across treatments (P = 0.01), with those supplemented with 20 ppm of narasin showing the highest diversity of ruminal bacterial communities. Regarding beta bacterial diversity (Bray-Curtis), differences in diversity were observed between animals supplemented with 20 ppm and 27 ppm of narasin (Figure 2).

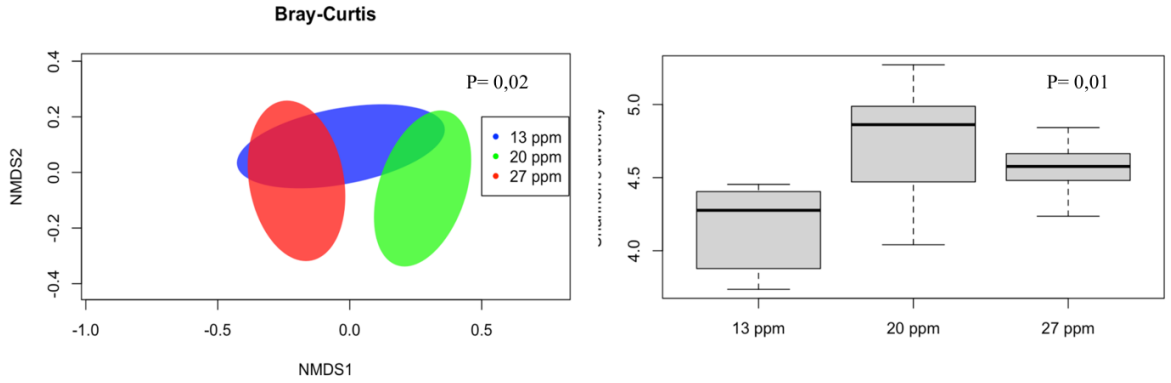


Figure 2. Ruminal bacterial diversity (alpha and beta) in rumen-cannulated Angus cattle fed moderate-energy diets supplemented with narasin.

The results shown in Figures 4 and 5 offer a descriptive overview of the effects of various narasin doses on the five most abundant genera and the seven most abundant phyla of ruminal bacteria identified. The phylum Firmicutes, along with Lactobacillus species from this phylum, emerged as the most prevalent in this study. Notably, increasing the narasin dosage to 20 and 27 ppm decreased the abundance of lactic acid bacteria.

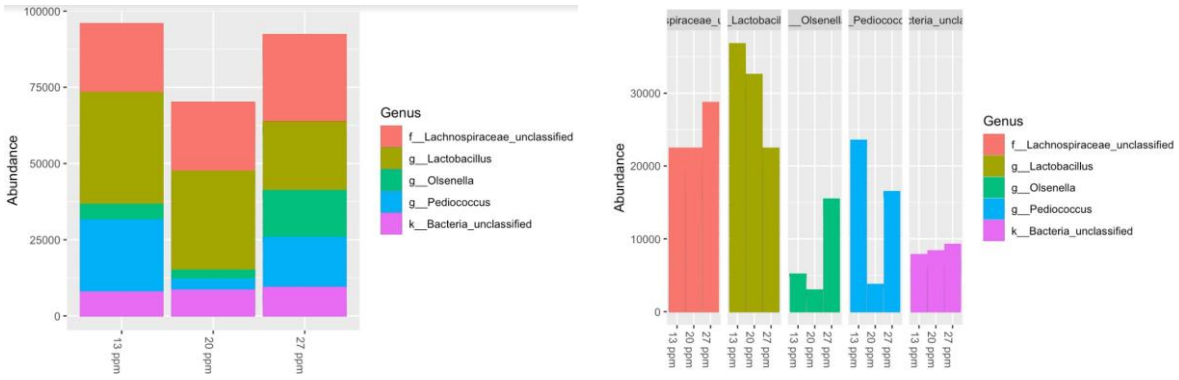
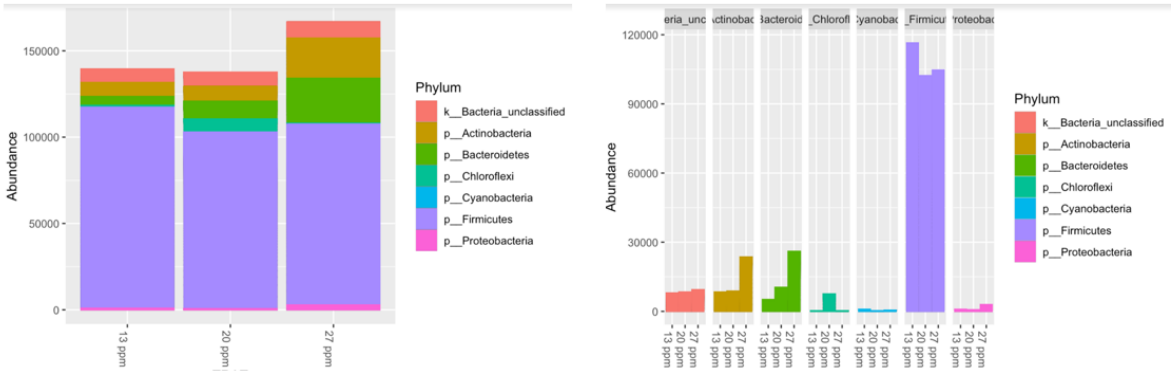


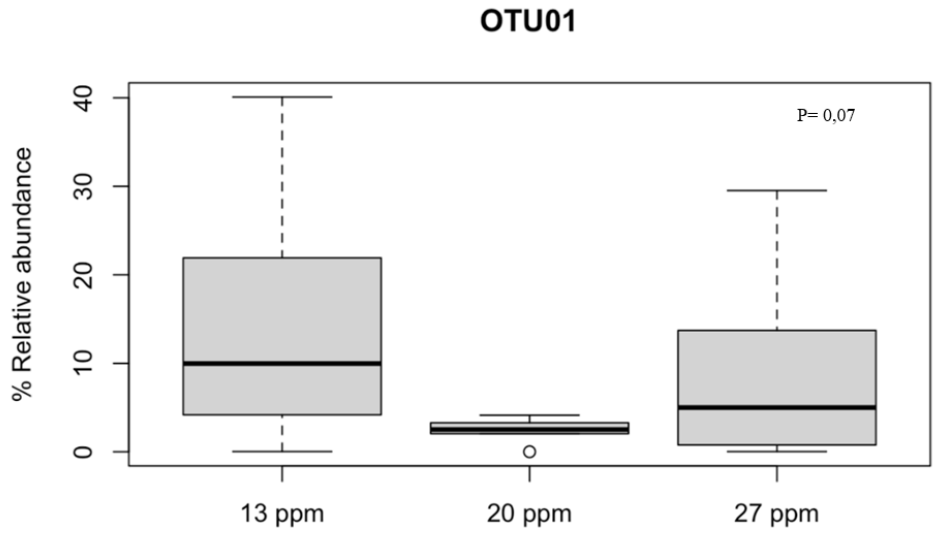
Figure 3. The five most abundant ruminal bacterial genera in rumen-cannulated Angus cattle fed moderate-energy diets supplemented with narasin.



303

304 **Figure 4.** The seven most abundant phyla of ruminal bacteria in rumen-cannulated Angus cattle
305 fed moderate-energy diets supplemented with narasin.

306 A lower abundance of *Pediococcus acidilactici* (lactic acid-producing bacteria) was
307 observed in the rumen of cattle receiving 20 ppm of narasin, indicating a quadratic effect (P =
308 0.07; OTU01; Figure 5). Similarly, a quadratic effect was noted on the abundance of bacteria
309 from the Lachnospiraceae family (which includes the genus Butyrivibrio) across treatments
310 (OTU04; Figure 6), with animals consuming 20 ppm of narasin showing the highest abundance.



311

312 **Figure 5.** Abundance of Pediococcus acidilactici bacteria found in the rumen of rumen-
313 cannulated Angus cattle fed moderate-energy diets supplemented with narasin.

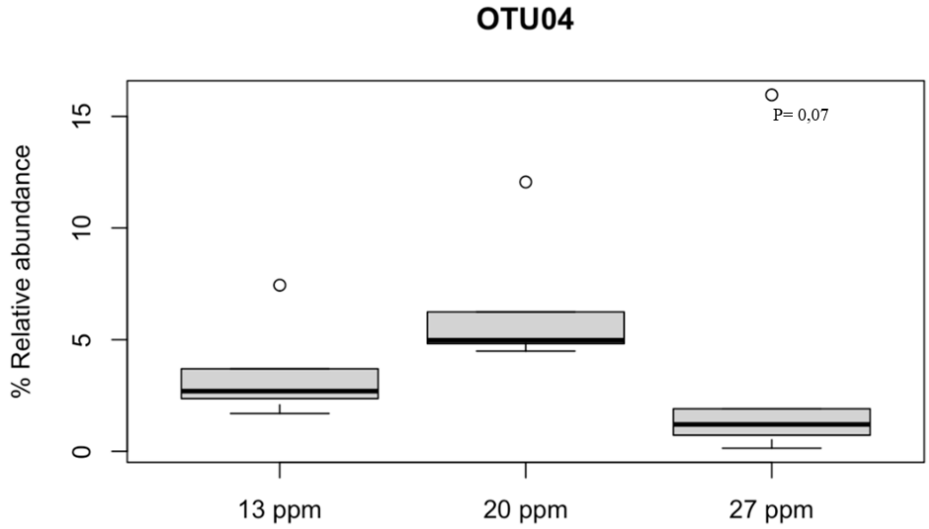


Figure 6. Abundance of bacteria from the Lachnospiraceae family found in rumen-cannulated Angus cattle fed moderate-energy diets supplemented with narasin.

In Figure 7, the abundance of lactic acid-consuming bacterial communities in the rumen is shown. It is important to note that there was no significant effect of narasin dosages on these bacterial populations. However, it is worth mentioning that narasin, in addition to reducing lactic acid bacteria, may also increase lactate-consuming bacteria. This potential effect warrants further investigation in future studies.

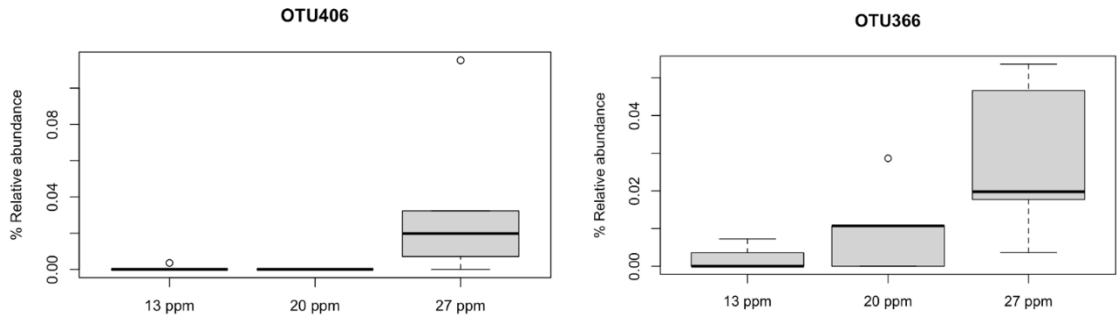


Figure 7. Abundance of Megasphaera (OTU406) genus and Selenomonas ruminantium (OTU366) found in the rumen of Angus cattle, which were rumen-cannulated and fed moderate-energy diets supplemented with narasin.

Ciliated protozoa

In Table 6, a linear increase was observed (P=0.04) in the number of Isotricha as the narasin dose increased. It is worth noting that the Isotricha genus is more prevalent under

higher ruminal pH conditions. Additionally, cattle fed 20 ppm of narasin had the highest number of protozoa in the rumen environment.

Table 6. Differential protozoan count of rumen-cannulated Angus cattle fed moderate-energy diets supplemented with narasin.

	Narasin (ppm)				P Value	
Item	13	20	27	SEM[1]	Linear	Quadratic
Dasytricha x 10^3/ml	0.17	0.1	0.68	0.23	0.14	0.16
Isotricha x 10^3/ml	1.05	1.96	3.39	0.87	**0.04**	0.77
Entodinium x 10^3/ml	62.27	61.98	55.61	7.34	0.17	0.45
Diplodinium x 10^3/ml	36.51	36.06	40.33	7.42	0.41	0.55
Total x 10^3/ml	165.4	194.8	142.8	15.38	0.24	**0.02**

[1]Standard error of the mean.

Ruminal papillae histology

The results from Table 7 on ruminal morphometry for rumen-cannulated Angus cattle fed moderate-energy diets supplemented with narasin indicate significant effects on certain parameters. Papillae width increased significantly with higher narasin levels, with values of 0.28, 0.35, and 0.40 mm for the 13, 20, and 27 ppm treatments, respectively ($P < 0.01$). Similarly, the papillae area showed a significant linear increase, with values of 2.80, 3.68, and 4.56 cm² for the same treatments ($P < 0.01$). A significant quadratic effect was observed for keratin layer thickness, with 8.12, 6.40, and 7.27 µm values at 13, 20, and 27 ppm levels, respectively ($P = 0.02$). No significant effects were found for papilla height, mitotic index (n), or mitotic index (%).

Table 7. Ruminal histology for rumen-cannulated Angus cattle fed moderate-energy diets supplemented with narasin.

	Narasin (ppm)				P Value	
Item	13	20	27	SEM[1]	Linear	Quadratic
Papillae height, mm	10.20	10.51	11.15	0.58	0.24	0.81
Papillae width, mm	0.28	0.35	0.40	0.02	**<0.01**	0.44
Papillae area, mm^2	2.80	3.68	4.56	0.35	**<0.01**	0.99
KLT[3], um	8.12	6.40	7.27	0.40	0.16	**0.02**
Mitotic index, n	49.00	45.50	45.50	2.14	0.26	0.51
Mitotic index, %	2.45	2.28	2.28	0.11	0.26	0.51

[1]Standard error of the mean; [2]Keratin layer thickness.

4. DISCUSSION

Narasin supplementation has been extensively researched because of its effects on the rumen microbiota, leading to significant modifications in bacterial composition and fermentative dynamics within the rumen. Studies indicate that narasin favors the selection of Gram-negative bacteria and directly influences microbial diversity and ruminal fermentation profiles (Tajima et al., 2021; Zheng et al., 2022; Callaway et al., 2018). Among the evaluated doses in this study, 20 ppm was the most effective in promoting a balanced microbiome, leading to more efficient fermentation and greater stability of ruminal pH. These findings are consistent with previous studies indicating that ionophores modulate the microbiota by promoting bacteria that utilize lactate and propionate, which reduces excessive lactic acid production and, subsequently, the risk of ruminal acidosis (Tedeschi et al 2022).

The influence of narasin on bacterial composition is notable, particularly regarding the increased abundance of *Lachnospiraceae*, a family of butyrate-producing bacteria that generate volatile fatty acids essential for maintaining the integrity of the ruminal epithelium and stabilizing pH levels (Chai et al., 2020). Butyrate is recognized for stimulating the growth and differentiation of ruminal epithelial cells, which enhances VFA absorption and feed efficiency (Górka et al., 2018; Malmuthuge & Guan, 2017). Furthermore, higher doses of narasin significantly reduced the population of lactic acid-producing bacteria, such as *Lactobacillus* and *Pediococcus*, which are known to contribute to ruminal acidosis when present in excess (Nagaraja et al., 1987; Plaizier et al., 2018). This reduction, along with the rise in butyrate-producing bacteria, indicates a beneficial effect of narasin in preventing acidosis and enhancing fermentation efficiency.

The 13 ppm dose promoted increased total VFA production and propionate. Propionate is a vital precursor of hepatic gluconeogenesis and is directly associated with feed efficiency in ruminants (Allen, 2020; Reynolds & Kristensen, 2019). On the other hand, 20 ppm dose increased the acetate and lactate production and increased the acetate:propionate ratio. These findings complement previous research and enhance our understanding of the effects of narasin on feedlot performance and carcass characteristics in Nellore cattle, as noted in the companion study by Silva et al. (2025; unpublished data). Furthermore, cattle consuming 20 ppm of narasin exhibited higher fat deposition at the 12th rib, which may result from greater ruminal stability due to slower DMI. This greater stability supports the findings of the present study, in which animals fed 20 ppm of narasin exhibited increased concentrations of acetate, butyrate, and NH3, along with a decrease in propionate concentration and a smaller area of ruminal pH < 5.6. The increased fat deposition contributes to meat quality and carcass weight, making fat accumulation an essential factor for improving growth performance.

The dose of 27 ppm of narasin led to reduced ammonia and total VFA production. This effect may be related to the selection of less efficient microorganisms in VFA production and the inhibition of proteolytic microorganisms responsible for protein degradation and NH3 release (Costa et al., 2023; Chen et al., 2021). The diversity of bacterial communities increased when the narasin dose rose from 13 to 20 ppm. Additionally, based on the Bray-Curtis dissimilarity test, the bacterial communities of 27-ppm cattle were significantly different from the microbiota of Nellore cattle fed 13 ppm of narasin.

Rumen pH stability is crucial for cattle health and performance. Doses of 20 and 27 ppm of narasin provided enhanced pH stability over time, reducing episodes of excessive acidification and promoting the survival of ruminal protozoa, such as *Isotricha*, which play a vital role in fiber degradation (Dehority, 1993; Williams & Coleman, 2021). The pH stability was also demonstrated by a reduction in the duration the pH remained below 5.2, indicating a

lower risk of ruminal acidosis. Maintaining pH within the ideal range enhances fiber digestibility and fermentation efficiency, as shown in recent studies on ionophores in ruminant nutrition (Beauchemin et al., 2022; Patra et al 2023).

The relationship between ruminal pH and protozoa is also evident. The 20 ppm dose resulted in the highest total number of protozoa, while the 27 ppm dose specifically increased *Isotricha*, contributing to enhanced acid-detergent fiber (ADF) digestibility. The increased DMI was also associated with better ADF digestibility, as efficient fermentation accelerates the ruminal passage rate, promoting greater DMI. Studies indicate that a more balanced microbiome is related to improved fiber utilization, resulting in enhanced feed efficiency (Huws et al., 2021; Plaizier et al., 2019). Although the modulation of the microbiota and fermentation profile showed significant impacts from the use of different narasin doses, ruminal degradability was not influenced by the dosages evaluated in this study.

It's important to note that the impact of narasin on the ruminal epithelium also warrants attention. The increased dosage was linked to wider papillae and, as a result, a larger absorption area. The 27 ppm dose exhibited the greatest width and area of papillae, likely due to the increased presence of butyrate-producing bacteria, as this fatty acid directly promotes papillae growth. However, cattle that were fed 27 ppm of narasin showed a reduction in SCFA production, which allowed the rumen pH to remain above 5.2 throughout the study and contributed to the development of the papillae. These findings emphasize the significance of a stable ruminal environment for the proper development of ruminal morphology (Penner et al., 2019; Steele et al., 2022).

Due to the higher amount of antimicrobial substances, the 20 ppm dose appears to promote greater ruminal stability than the 13 ppm dose, although it results in lower ruminal VFA and propionate concentrations. In intensive beef systems with high-quality feeding management, the 13 ppm dose may be more suitable. However, if the nutritionist seeks greater assurance to counteract poor feeding management, the 20 ppm dose appears to be the better option.

In summary, supplementing with narasin significantly affects the fermentative dynamics and structure of the ruminal microbial ecosystem, influencing the production of SCFA and maintaining pH stability.

5. CONCLUSION

Narasin supplementation effectively enhances ruminal pH stability, microbial diversity, and fermentation efficiency in Angus cattle on feedlot diets. The findings of this study recommend a narasin dose between 13 and 20 ppm for such cattle. Specifically, 20 ppm has been shown to provide optimal ruminal stability and protect against acidification, all while maintaining a balanced microbiome and enhancing fermentation efficiency. For nutritionists looking to offer additional safeguards against ruminal acidosis, 20 ppm remains the preferred choice. In conclusion, a carefully controlled narasin dose of 13 or 20 ppm is essential for maximizing rumen health and performance in feedlot systems.

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CHAPTER 3

Addition of rumen-protected methionine into typical supplementation protocols to finish beef heifers in pasture systems in Brazi

Addition of rumen-protected methionine into typical supplementation protocols to finish beef heifers in pasture systems in Brazil

ABSTRACT

This study evaluated the effects of rumen-protected methionine (RPM) supplementation on performance, carcass traits, ruminal morphometry, cecal histology, and meat quality in pasture-finished crossbred beef heifers. Forty-eight 18-month-old ½ Nellore × ½ Angus heifers (380 ± 44 kg) were randomly assigned to three supplementation strategies: a control group (T1) received increasing levels of an energy-protein supplement without methionine; T2 received the same supplement plus a constant dose of 4 g/animal/day of RPM; and T3 received the same methionine dose, but with a more gradual increase in energy supplementation across the 140-day trial. The study was conducted under tropical pasture conditions, with progressive seasonal decline in forage quality and environmental parameters. Average daily gain and final body weight did not differ significantly among treatments. However, carcass ultrasound revealed that methionine-supplemented heifers (T2 and T3) had greater rib eye area and reduced subcutaneous fat in the Biceps femoris muscle, suggesting enhanced lean tissue deposition. Ruminal morphometry showed a higher number of papillae in the RPM groups, potentially increasing absorptive surface area. Histological evaluation of the cecum indicated that T3 animals had lower lesion scores and fewer goblet cells and cell disruptions, demonstrating better epithelial integrity, likely supported by the antioxidant role of methionine and improved dietary adaptation. Meat quality was also positively affected. Marbling scores were higher in both RPM-supplemented groups, without negative effects on pH or tenderness. All meat samples were aged for 14 days, ensuring consistent evaluation of postmortem traits. These findings indicate that a fixed, low-dose RPM supplementation (4 g/day) can improve carcass characteristics, gastrointestinal health, and meat quality, even without altering growth performance. The benefits were more evident when energy supply increased gradually and forage conditions were favorable, highlighting the importance of aligning amino acid supply with overall diet adequacy in pasture-based beef systems.

Keywords: amino acids, livestock nutrition, performance.

1. INTRODUCTION

The global beef industry is increasingly focused on enhancing the efficiency and sustainability of production systems, particularly in pasture-based operations, where nutritional optimization plays a crucial role. Brazil, one of the world's leading beef producers, predominantly relies on pasture-based finishing systems due to their lower cost and the country's extensive grazing lands. However, these systems often struggle to meet the nutritional requirements of cattle, particularly concerning essential amino acids such as methionine, which is frequently limiting in forage-based diets (Nasem, 2016).

Methionine is a sulfur-containing essential amino acid that plays a vital role in protein synthesis, metabolism, and overall animal growth. It is particularly important for muscle development and the synthesis of key compounds such as creatine and carnitine (Resende Júnior et al., 2006). However, in ruminants, methionine is highly susceptible to degradation by rumen microbes, which reduces its availability for absorption in the small intestine and, consequently, its effectiveness (Cooke and Arthington, 2013). To address this limitation, rumen-protected methionine (RPM) has been developed to bypass ruminal degradation and deliver methionine directly to the small intestine, where it can be more efficiently absorbed and utilized (Odongo et al., 2006).

The inclusion of RPM in the diets of pasture-finished cattle has the potential to improve growth performance by enhancing nitrogen retention and protein synthesis, leading to better muscle development and overall carcass quality (Zanton et al., 2014). Additionally, RPM supplementation may positively influence rumen morphometrics by promoting healthier rumen papillae, which are essential for nutrient absorption and overall digestive efficiency (Resende Júnior et al., 2006).

Among pasture-finished cattle, heifers represent a particularly important category due to their role in beef production systems. Heifers generally exhibit lower growth rates and feed efficiency compared to steers, making their nutritional management a key factor in improving productivity. Optimizing the diet of heifers is crucial not only for achieving desirable carcass traits but also for ensuring reproductive efficiency in replacement heifers within breeding herds. The use of RPM could be especially beneficial for heifers, as improving amino acid balance may enhance their growth performance and overall physiological development, contributing to both short-term production goals and long-term herd sustainability.

Given these potential benefits, this study aims to evaluate the effects of adding RPM to conventional supplementation protocols for pasture-finished crossbred heifers. By assessing key performance indicators such as growth performance, carcass characteristics, and rumen morphometrics, this research seeks to provide critical insights into optimizing nutritional strategies for pasture-based beef production.

The justification for this study lies in the urgent need to enhance the nutritional efficiency of pasture-based beef production systems. As global demand for beef continues to rise, producers face increasing pressure to maximize productivity while maintaining environmental sustainability. Rumen-protected methionine represents a promising nutritional

intervention that could significantly improve nitrogen utilization efficiency and animal growth in pasture-finished cattle. By ensuring a more consistent supply of methionine to the small intestine, RPM has the potential to enhance both cattle performance and health, leading to improved economic returns for producers and more sustainable beef production practices.

This research is both timely and relevant, as it addresses the critical need for innovations in beef cattle nutrition that align with global sustainability goals. The findings from this study could contribute to the development of more effective supplementation strategies, ultimately supporting the competitiveness and sustainability of pasture-based beef production systems, particularly in heifers, whose optimal nutritional management is essential for improving both meat production and herd development

2. MATERIAL AND METHODS

Animals and Experimental Facility

The study was carried out at the Faculty of Agricultural and Veterinary Sciences (FCAV), São Paulo State University (UNESP), Jaboticabal Campus, Brazil, within the experimental facilities of the beef cattle sector. The project received approval from the Animal Ethics Committee (CEUA) of FCAV/UNESP under protocol number 406/2023. Forty-eight 18-month-old heifers (½ Nellore × ½ Angus), averaging approximately 380 ± 44 kg body weight, were randomly assigned to 12 paddocks (4 animals per paddock), with each paddock serving as an experimental unit in this study

Experimental Design

The experimental area encompassed approximately 32 hectares, divided into 12 paddocks of about 2.6 hectares each, composed of *Urochloa brizantha* cv. Marandu (Marandu grass). The experiment was conducted from February to June and followed a randomized block design, with each treatment replicated three times (paddocks). The heifers were assigned to one of three supplementation protocols. In Treatment 1 (T1), they received a supplement without methionine, starting at 0.1% of body weight (BW) from day 0 to day 56, increasing to 0.3% BW from day 57 to day 84, and reaching 1% BW from day 85 to day 140. Treatment 2 (T2) followed the same supplementation schedule but included methionine. Treatment 3 (T3), involved a gradual increase in supplementation with methionine, starting at 0.05% BW from day 0 to day 28, increasing to 0.1% BW from day 29 to day 56, then 0.3% BW from day 57 to day 84, and finally 1% BW from day 85 to day 140.

Management, Feeding, and Animal Handling

For the receiving program, the heifers were acclimated in paddocks, where they were allowed to graze and consume a mineral supplement to standardize the ruminal microbiota. Before the study began, the heifers were weighed, dewormed, and vaccinated against viral and bacterial diseases.

The heifers consumed forage and water ad libitum, and the supplement containing corn, soybean meal, rice meal, cottonseed cake, wheat meal, soybean hulls, and minerals was

formulated according to the Nasem (2016) guidelines and offered to the heifers at 8 a.m. The quantities provided in each paddock depended on the treatment. An additional 20 grams of methionine was provided on top of the basal supplementation, distributed in collective bunks for the treatments that received it. Table 1 presents the composition of the diet, including the supplement, and Table 2 shows the forage consumed on pasture.

Tabela 1. Composition of the supplement provided to ½ blood Nellore × Angus heifers finished on pasture with or without methionine supplementation.

Diets (%BW)	0.05	0.1	0.3	1.0
Ingredients (% DM)				
Rice meal	.	0.088	0.356	.
Soybean hulls	.	0.018	0.014	.
Finely-ground corn	.	3.588	9.000	3.750
Soybean meal	.	0.014	.	.
Wheat meal	.	0.001	0.004	.
Cottonseed meal	.	3.500	8.045	1.000
Urea	.	0.002	0.009	0.030
Antixodant BHT	.	50.000	21.255	.
Calcium Carbonate	38.266	14.630	15.000	.
Calcium iodate	0.012	8.000	12.200	.
Cobalt sulphate	0.027	6.999	11.000	.
Copper sulphate	0.393	5.935	10.288	.
Dicalcium phosphate	31.285	3.000	5.301	.
Flower of Sulphur	6.134	3.000	4.565	.
Manganese sulphate	0.194	0.749	2.000	.
Monensin 20%	0.500	0.403	0.614	.
Rumen-protected urea	.	.	0.150	.
Sodium cloride	22.148	0.040	0.136	0.200
Sodium selenite	0.004	0.034	0.063	.
Zinc sulphate	1.037	0.000	0.001	.
Supplement Nutritional Content (% DM)				
Dry matter	.	90.01	92.63	90.00
TDN1	.	65.00	45.00	65.20
Crude protein	.	25.00	30.00	10.20
Calcium	21.26	2.52	3.50	3.00
Phosphorus	6.00	0.60	6.46	6.00
Sodium	8.60	1.40	2.00	2.00

[1]Total digestible nutrients.

Tabela 2. Quantitative and qualitative components of forage grazed by ½ blood Nellore × Angus heifers finished on pasture.

Variables	Period														
	0-28			28-56			56-84			84-112			112-140		
	Trat 1	Trat 2	Trat 3	Trat 1	Trat 2	Trat 3	Trat 1	Trat 2	Trat 3	Trat 1	Trat 2	Trat 3	Trat 1	Trat 2	Trat 3
Available forage mass, kg/ha	6036.00	6924.00	6325.00	5036.00	5868.00	5554.00	4774.00	4886.00	4957.00	3999.00	3385.00	3742.00	2393.00	2298.00	2291.00
Leaf,%	36.00	34.90	37.10	36.40	34.50	37.50	30.30	33.40	27.40	33.00	32.40	33.00	31.70	18.50	23.00
Stem, %	27.80	31.20	29.90	27.30	26.60	26.60	31.10	31.50	37.00	36.60	33.00	26.10	24.70	30.40	23.60
Dead Material, %	36.20	34.00	33.00	36.20	38.90	35.90	38.70	35.10	35.70	30.40	34.60	40.90	43.60	51.10	53.40
Canopy height, cm	74.00	68.00	75.00	57.00	58.00	52.00	47.00	48.00	48.00	44.00	47.00	44.00	35.00	33.00	35.00
Dry matter, %	24.00	23.50	23.80	24.00	24.20	23.70	22.50	22.90	22.30	23.60	22.10	21.20	22.50	21.50	24.10
Crude protein, %	11.70	10.30	10.70	11.40	11.60	13.60	12.00	10.50	11.80	10.10	7.40	10.30	9.20	10.00	10.70
Neutral detergent fiber, %	66.70	65.50	66.00	66.50	67.00	65.00	66.80	65.70	67.20	65.50	68.30	64.90	68.30	67.50	65.10
Acid detergent fiber, %	37.00	35.10	36.00	36.80	37.00	35.00	36.50	35.80	37.20	35.50	40.00	35.40	33.60	32.80	37.90
Crude Fiber, %	30.60	29.30	30.00	30.50	31.00	29.00	30.50	29.80	31.20	29.50	32.00	29.30	28.50	27.60	30.70
Lignin, %	4.60	4.10	4.30	4.50	4.60	4.00	4.20	4.10	4.70	4.00	5.00	5.00	2.60	2.40	2.20
Ether extract, %	4.50	3.90	4.00	4.40	4.20	3.80	4.00	3.90	4.30	3.80	4.50	5.00	2.80	3.50	4.60
Ash, %	10.20	10.00	10.00	10.10	10.20	9.80	10.10	9.90	10.30	9.80	8.80	10.00	10.20	11.10	9.30
Animal Unit/ha	1.43	1.43	1.44	1.53	1.54	1.54	1.58	1.59	1.58	1.67	1.68	1.68	1.69	1.71	1.68

[1]In Vitro Dry Matter Digestibility.

Forage Components

For the characterization of pasture productive components, forage samples and canopy height were collected from each experimental unit every 28 days throughout the experimental period. Canopy height was measured at 80 points within each paddock using a 1-meter ruler graduated in centimeters. Forage sampling was performed using a 0.25 m² metallic frame, which was randomly thrown in each paddock while avoiding areas contaminated with feces. At each sampling point, all forage within the frame was collected.

Each sample was then divided into two subsamples: the first for determining forage dry matter yield per paddock, and the second for evaluating morphological composition. Both subsamples were placed in paper bags and dried in a forced-air oven at 65 °C for 72 hours. After drying, the bags were weighed on a precision scale, and the dried material was used for chemical analysis. From these values, the total available forage mass per hectare was estimated. Morphological composition was assessed by manually separating the dried material into green leaf blades, green stems (including true stems and leaf sheaths), and dead material. Dead material was visually identified as leaves or stems with 50% or more of their area yellowed or dry. Each component was placed in paper bags and again dried in a forced-air oven at 65 °C for 72 hours. After drying, the bags were weighed, and the relative proportions of each morphological component were calculated.

For the chemical characterization of dry forage samples, the material was ground in a Willey mill (1 mm mesh) and grouped by period. The following components were determined: crude fiber (CF), mineral matter (MM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), and in vitro dry matter digestibility (IVDMD). The analyses were performed by near-infrared reflectance spectroscopy (NIRS), using the wavelength range of 1100 to 2500 nm, with an NR5000 spectrometer coupled to a microcomputer.

Table 3 presents the rainfall data recorded during the experimental period, obtained from the Agroclimatological Station of the University of Jaboticabal. These data provide a climatic characterization of the study period, which is essential for interpreting pasture conditions and animal performance.

Tabela 3. Monthly pluviometric data and rainfall distribution during the experimental period obtained from the agroclimatological station of the university of Jaboticabal.

Month	Pressure (hPa)	Maximum temperature (°C)	Minimum temperature (°C)	Mean temperature (°C)	Relative humidity (%)	Precipitation (mm)	Number of days with rainfall (days)
February	943.00	30.80	20.10	24.10	80.00	338.00	19
March	943.60	31.40	20.20	24.50	75.90	159.90	16
April	943.60	28.40	17.70	22.10	77.60	93.70	10
May	943.20	27.80	14.20	20.20	67.90	39.50	2
Jun	948.30	25.80	12.60	18.40	71.00	61.50	2

Animal performance and carcass ultrasound

Heifers were weighed on day 0 and every 28 days after fasting for 16 hours, except for water. The 12th rib fat thickness, *Biceps femoris* fat thickness, LM area and marbling were measured via ultrasound at days 0, 84 and 140 of the experimental period, following the method described by Perkins et al. (1992). The 12th rib fat thickness daily gain, *Biceps femoris* fat daily gain and LM area daily gain were calculated as the difference between the two measurements divided by days on feed. Images were collected using an Aloka SSD-1100 Flexus RTU unit (Aloka Co. Ltd., Tokyo, Japan) with a 17.2-cm, 3.5-MHz probe. Based on the final hot carcass weight from each animal obtained at the abattoir, dressing percentage was calculated.

Evaluation of ruminal papillae morphometry and histology

Rumenitis was assessed after heifers were eviscerated and the entire rumen contents were washed. The rumen epithelium was classified based on the incidence of lesions (rumenitis) and abnormalities (e.g., clumped papillae) as described by Bigham and McManus (1975), using a scale from 0 (no lesions or abnormalities) to 10 (severe ulcerative lesions). All rumens were scored by two trained individuals who were blinded to the treatments, and the final data represented the average of the two scores. Regarding rumen morphometry, a 1 cm² fragment of each rumen was collected from the dorsal cranial sac and placed in a PBS solution for future morphometric measurements according to Resende Júnior et al. (2006). The number of papillae per square centimeter of rumen wall (NOP) was manually determined. Twelve papillae were randomly collected from each fragment and scanned, and the mean papillae area (MPA) was determined using an image analysis system (Image Tool, version 2.01 alpha 4, UTHSCSA Dental Diagnostic Science, San Antonio, TX). The rumen wall absorptive surface area (ASA) in square centimeters was calculated as follows:

$$ASA = 1 + (NOP \times MPA) - (NOP \times 0.002),$$

where 1 represents the 1 cm² fragment collected, and 0.002 is the estimated basal area of papillae in square centimeters.

Similarly, a 1 cm² fragment of each rumen was collected from the ventral cranial sac for histological assessment. Histological sections were stained with hematoxylin and eosin, embedded in paraffin wax, and sectioned (Odongo et al., 2006). Histological measurements, including papillae height (A-H), papillae width (A-W), papillae surface area (B-PSA), and keratinized layer thickness (B-KS), were performed on four papillae per animal using a computer-aided light microscope image analysis system.

Evaluation of cecal lesions and histology

After evisceration and washing of the cecal compartment, cecal lesion assessments were performed. The cecal epithelium was classified based on the presence of inflammation, lesions, and petechiae on the cecal wall using a scale from 0 (no observed lesions) to 10 (severe lesions), adapted from Bigham and McManus (1975). The entire cecum was scored by two trained individuals, and the final data represent the average of the two scores. For histological evaluation, a 1 cm² fragment from the center of the cecal epithelium was collected and preserved in a 4% paraformaldehyde buffered solution until further histological analyses

(Devant et al., 2016). Tissue samples were dehydrated, embedded in paraffin, sectioned at 8 μm, and stained with hematoxylin and eosin. Histological measurements such as crypt depth (A), the number of enterocytes (B), and Goblet cells (C) were determined in 10% of the total number of crypts per animal using a Leica Qwin Image Analyzer within a Leica light microscope.

Meat quality

For qualitative analyses, a rib eye steak (2.54 cm thick) was collected between the 12th and 13th ribs of each carcass. Samples were vacuum-packed and aged under refrigeration (4 °C) for 14 days before analysis, following standard postmortem maturation procedures. After the aging period, samples were frozen at −20 °C until further analysis. The assessments included meat color, marbling score, and shear force. Meat color was evaluated at three different points of the Longissimus thoracis muscle after a 30-minute bloom time at 4 °C, allowing for myoglobin oxygenation. A Minolta colorimeter (Model CM2500d, Minolta Camera Co. Ltd., Osaka, Japan) was used to measure lightness (L*), redness (a*), and yellowness (b*), following the CIE Lab color space system with a 0°/45° geometry. The device was calibrated using a black and white standard plate, as described by Houben et al. (2000). Marbling score was determined visually by a trained evaluator, following standardized reference images adapted from USDA grading criteria, and expressed as a score indicating the amount of intramuscular fat visible in the Longissimus muscle cross-section. For shear force analysis, after 24 hours of chilling, six cylindrical cores (2.5 cm diameter) were extracted from each steak using a mechanical punch. A Brookfield® CT-3 Texture Analyzer (Brookfield, USA) was used to determine the peak force required to shear each core transversely. The average of these measurements was used to represent the shear force of each sample, according to the methodology described by Wheeler et al. (2001).

Statistical analysis

The paddocks were considered the experimental units for data obtained. Data were statistically analyzed using the SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Before the actual analysis, the data were analyzed for the presence of disparate information (“outliers”) and normality of residuals (Shapiro-Wilk). When the normality assumption is not accepted, the logarithmic or the square root transformation will be tested. Data were analyzed according to the mixed procedure (PROC MIXED). Among the 15 different covariance structures tested, the chosen one was based on the lower value of the Corrected Akaike Information Criterion (AICC) (Wang & Goonewardene, 2004). The model included the effects of treatment. The effects of the block were considered as random factors. All means were presented as least squares means and the treatment effects were separated by the PDIFF option of SAS. Effects were considered significant at $P \leq 0.05$.

3. RESULTS

The heifers initiated the trial with no significant differences in initial body weight ($P > 0.17$). However, initial body weight was incorporated as a covariate in the statistical analysis

of other evaluated variables when deemed appropriate. No statistical differences were observed in average daily gain between treatments (Table 4).

Tabela 4. Body weight and average daily gain of 1/2 blood Nellore/Angus heifers finished on pasture supplemented with methionine.

	Treatments				P value	
Variables	T1	T2	T3	SEM[1]	T2xT3	T1xT2 T3
Initial body weight, kg	385.92	380.83	377.44	3.87	0.55	0.18
Body weight, kg						
28 days	418.63	417.64	419.93	6.84	0.52	0.98
56 days	446.90	449.87	449.73	8.12	0.98	0.53
84 days	463.20	464.13	461.73	8.71	0.44	0.92
112 days	489.14	491.37	490.30	8.85	0.79	0.65
140 days	493.73	501.27	492.64	9.80	0.13	0.51
Average daily gain, kg						
0-28 days	1.31	1.40	1.38	0.09	0.75	0.14
0-56 days	1.20	1.23	1.23	0.04	0.99	0.71
0-84 days	1.00	0.99	0.96	0.03	0.39	0.46
0-112 days	0.98	0.99	0.97	0.04	0.66	0.96
0-140 days	0.82	0.84	0.81	0.04	0.40	0.93
28-56 days	0.99	1.16	1.08	0.18	0.70	0.53
56-84 days	0.56	0.51	0.45	0.09	0.64	0.53
84-112 days	0.91	0.97	1.05	0.09	0.51	0.37
112-140 days	0.13	0.20	0.09	0.08	0.35	0.88

[1]Standard error of the mean; T1: Supplementation without methionine, starting at 0.1% of BW from day 0 to 56, increasing to 0.3% from day 57 to 84, and reaching 1% from day 85 to 140; T2: Supplementation with methionine, following the same schedule as T1; T3: Supplementation with methionine, starting at 0.05% of BW from day 0 to 28, increasing to 0.1% from day 29 to 56, then 0.3% from day 57 to 84, and reaching 1% from day 85 to 140.

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 (Table 5). It is noteworthy that heifers consuming methionine with a reduced supplementation level in the first 28 days of the study tended to have a decreased hot carcass weight (P = 0.07).

Tabela 5. Carcass characteristics of 1/2 blood Nellore/Angus heifers finished on pasture supplemented with methionine.

	Treatments				P value	
Variables	T1	T2	T3	SEM[1]	T2xT3	T1xT2 T3
Hot carcass weight, kg	257.84	265.58	258.08	5.23	**0.07**	0.26
Carcass yield, %	52.26	52.96	52.34	0.35	0.13	0.28
Rib eye area, cm²						
0 day	53.95	54.59	53.74	0.92	0.37	0.78
84 days	56.71	57.68	58.44	0.75	0.50	0.17
140 days	59.45	60.56	62.86	0.74	**0.05**	**0.03**
Rib eye area gain, cm² per day						
0-84 days	0.03	0.04	0.05	0.01	0.43	0.18
84-140 days	0.05	0.05	0.08	0.02	0.33	0.49
0-140 days	0.04	0.05	0.06	0.01	**0.04**	**0.03**
Subcutaneous fat thickness, mm						
0 day	6.38	6.52	6.17	0.21	0.26	0.89
84 days	7.80	7.66	7.54	0.26	0.72	0.49
140 days	9.67	9.71	9.87	0.38	0.60	0.63
Subcutaneous fat thickness gain, mm per day						
0-84 days	0.02	0.01	0.02	0.003	0.86	0.59
84-140 days	0.03	0.04	0.04	0.004	0.22	0.14
0-140 days	0.02	0.02	0.03	0.003	0.31	0.64
Biceps femoris, mm						
0 days	6.91	6.11	6.80	0.29	0.11	0.22
84 days	8.20	7.85	7.91	0.17	0.79	0.10
140 days	9.61	9.13	9.38	0.21	0.17	**0.03**
Biceps femoris gain, mm per day						
0-84 days	0.02	0.02	0.02	0.002	0.71	0.25
84-140 days	0.02	0.02	0.03	0.003	0.83	0.72
0-140 days	0.02	0.02	0.02	0.001	0.87	0.44

[1]Standard error of mean; T1: Supplementation without methionine, starting at 0.1% of BW from day 0 to 56, increasing to 0.3% from day 57 to 84, and reaching 1% from day 85 to 140; T2: Supplementation with methionine, following the same schedule as T1; T3: Supplementation with methionine, starting at 0.05% of BW from day 0 to 28, increasing to 0.1% from day 29 to 56, then 0.3% from day 57 to 84, and reaching 1% from day 85 to 140.

Regarding papillae morphometry (Table 6), heifers supplemented with methionine exhibited a significant increase in papillae number (P = 0.03) without alterations in absorptive surface area (ASA, P = 0.58).

Tabela 6. Ruminal morphometry of 1/2 blood Nellore/Angus heifers finished on pasture supplemented with methionine.

Variables	Treatments			SEM[1]	P value	
	T1	T2	T3		T2xT3	T1xT2 T3
Ruminal Morphometry						
Ruminitis score	0.77	0.96	1.19	0.14	0.21	0.07
Macroscopic Variables						
Papillae average number, n	53.16	64.62	63.56	3.95	0.84	**0.03**
Papillae average area, cm^2	0.53	0.48	0.47	0.03	0.95	0.21
ASA^2, cm^2	29.14	31.19	31.08	2.57	0.97	0.59
RPSA[3], %	96.43	96.86	96.65	0.28	0.56	0.29

[1]Standard error of mean; [2]Absorptive surface area; [3]Representativeness of papillae in the absorptive surface area; T1: Supplementation without methionine, starting at 0.1% of BW from day 0 to 56, increasing to 0.3% from day 57 to 84, and reaching 1% from day 85 to 140; T2: Supplementation with methionine, following the same schedule as T1; T3: Supplementation with methionine, starting at 0.05% of BW from day 0 to 28, increasing to 0.1% from day 29 to 56, then 0.3% from day 57 to 84, and reaching 1% from day 85 to 140.

The cecal histology of ½ blood Nellore/Angus heifers supplemented with methionine is presented in Table 7. Significant effects were observed in response to methionine supplementation protocols, particularly with regard to epithelial integrity.

Heifers receiving the gradually increased methionine supplementation (T3) exhibited a markedly lower lesion score (0.52) compared to those in both the T1 (1.77) and T2 (2.00) treatments (P = 0.04 and P = 0.01, respectively), indicating improved epithelial integrity under the T3 protocol. Additionally, the number of goblet cells was significantly reduced in T3 (1.30) compared to T1 (2.51) and T2 (2.39), with P values of 0.01 for both comparisons. A similar pattern was observed for disrupted goblet cells, which were notably lower in T3 (0.15) relative to T1 (1.36) and T2 (1.40), with statistical significance (P = 0.03 and P = 0.02, respectively). No significant differences were detected among treatments for crypt depth or enterocyte count (P > 0.05), although numerically, crypt depth and enterocyte numbers were lower in T3.

Tabela 7. Cecal histology of 1/2 blood Nellore/Angus heifers finished on pasture supplemented with methionine.

Variables	Treatments			SEM[1]	P value	
	T1	T2	T3		T2xT3	T1xT2 T3
Cecal Epithelium						
Lesion score	1.77	2.00	0.52	0.43	**0.01**	**0.04**
Crypt depth, µm	57.08	49.88	41.07	6.39	0.60	0.22

Enterocytes, n	18.58	22.61	15.40	2.40	0.12	0.47
Goblet cells, n	2.51	2.39	1.30	0.26	**0.01**	**0.01**
Disrupted goblet cells, n	1.36	1.40	0.15	0.32	**0.02**	**0.03**

[1]Standard error of mean; T1: Supplementation without methionine, starting at 0.1% of BW from day 0 to 56, increasing to 0.3% from day 57 to 84, and reaching 1% from day 85 to 140; T2: Supplementation with methionine, following the same schedule as T1; T3: Supplementation with methionine, starting at 0.05% of BW from day 0 to 28, increasing to 0.1% from day 29 to 56, then 0.3% from day 57 to 84, and reaching 1% from day 85 to 140.

The results presented in Table 8 indicate that there were no statistically significant differences among treatments for any of the rumen histological variables evaluated in ½ blood Nellore/Angus heifers finished on pasture and supplemented with methionine. Papillae height, width, and area, as well as keratin layer thickness, were not significantly affected by the treatments (P > 0.05).

Tabela 8. Rumen histology of 1/2 blood Nellore/Angus heifers finished on pasture supplemented with methionine.

Variables	Treatments			SEM[1]	P value	
	T1	T2	T3		T2xT3	T1xT2 T3
Papillae height, mm	10.01	10.71	11.48	0.71	0.37	0.26
Papillae width, mm	0.37	0.38	0.43	0.05	0.78	0.95
Papillae area, mm^2	3.89	4.68	4.70	0.60	0.50	0.46
KLT[2], um	8.10	7.36	7.34	0.36	0.28	0.18

[1]Standard error of the mean; [2]Keratin layer thickness.

Table 9 presents the results of meat quality analyses of ½ blood Nellore/Angus heifers finished on pasture and supplemented with rumen-protected methionine. No significant differences (P>0.05) were observed among treatments for L* (lightness), a* (redness), and b* (yellowness) values, indicating that methionine supplementation did not affect meat color. The average values were consistent with those reported in the literature for crossbred animals finished on pasture.

Meat pH differed between treatments T2 and T3 (P=0.03), although all means remained within the normal physiological range (<5.8), suggesting an appropriate postmortem acidification process and the absence of DFD (dark, firm, and dry) meat condition.

Cooking loss (%) and shear force (expressed in both kgf and Newtons) were not significantly affected by treatments (P>0.05), indicating that rumen-protected methionine supplementation did not impair water-holding capacity or meat tenderness.

On the other hand, a significant difference (P=0.03) was observed in marbling scores between the control group (T1) and the methionine-supplemented treatments (T2 and T3), with higher marbling values in the supplemented groups. This result suggests that supplementation with rumen-protected methionine may enhance intramuscular fat deposition, a desirable trait in terms of palatability and commercial value of the meat.

Tabela 9. Meat quality of 1/2 blood Nellore/Angus heifers finished on pasture supplemented with methionine.

	Treatments				P value	
Variables	T1	T2	T3	SEM[1]	T2xT3	T1xT2 T3
L*	40.42	39.87	36.64	1.23	0.33	0.39
a*	17.81	18.15	17.67	0.31	0.27	0.52
b*	16.04	15.95	17.93	0.80	0.41	0.50
pH	5.60	5.68	5.57	0.03	**0.03**	0.15
Cooking loss (%)	19.46	20.33	20.30	1.54	0.99	0.69
Shear Force (kgf)	3.45	3.79	3.57	0.25	0.42	0.76
Shear Force (N)	33.78	37.19	34.97	1.36	0.69	0.86
Marbling	3.68	4.22	4.27	0.12	0.95	**0.03**

[1]Standard error of the mean; L* = lightness (0 = black, 100 = white); a* = red-green coordinate (positive values indicate redness); b* = yellow-blue coordinate (positive values indicate yellowness).

4. DISCUSSION

Although supplementation with rumen-protected methionine (RPM) did not promote significant differences in average daily gain (ADG) or final body weight, important metabolic responses were observed in carcass characteristics, ruminal morphometry, cecal histology, and meat quality. These results suggest that the benefits of RPM go beyond weight gain and are influenced by the basal nutritional level of the diet, environmental conditions, and the supplementation strategy applied.

At the beginning of the experiment, when forage quality was still satisfactory, crude protein levels ranging from 10.3% to 13.6% and lower proportions of dead material, methionine appeared to exert a positive effect, particularly on protein synthesis and muscle metabolism. The greater longissimus muscle area (rib eye area) observed in the methionine treatments (T2 and T3), especially T3, supports this hypothesis. Methionine acts as a limiting amino acid in protein synthesis and is also a precursor for compounds such as creatine and carnitine, which are essential for lean tissue development and nutrient utilization (Ma et al., 2021; Wang et al., 2023). This efficiency likely explains the reduced subcutaneous fat deposition in the Biceps femoris muscle, reflecting a nutrient repartitioning that favors lean tissue over superficial fat.

Additionally, ruminal morphometry data showed an increase in papillae number in animals receiving methionine, without changes in total absorptive surface area. This suggests a stimulus for epithelial renewal, possibly driven by the systemic action of methionine after intestinal absorption, modulating cellular proliferation in ruminal tissue (Wu et al., 2010). Although the methionine used was protected from ruminal degradation, its indirect effect on the rumen may stem from improved overall nutritional status or systemic metabolic signaling. Increased papillae density may enhance absorption of volatile fatty acids (VFAs), improving fermentative efficiency.

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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IMPLICATIONS

The present study will bring significant contributions to the advancement of knowledge on the metabolism of feedlot beef cattle. The optimal dosage of narasin, identified and validated in this experiment, will be officially registered with the Ministry of Agriculture, Livestock, and Supply (MAPA), providing a solid foundation for its practical use and future regulation.

Similarly, the experiment involving rumen-protected methionine evaluated not only its inclusion in the diet but also different levels of supplementation, allowing the identification of the most economically viable level. The results will contribute to a better understanding of the strategic use of methionine in beef cattle nutrition, providing valuable insights for both the scientific community and the production sector.

Furthermore, the comprehensive investigation of ruminal parameters, nutrient absorption, ruminal microbiota, animal performance, carcass traits, and meat quality offers a holistic view of the impact of these supplements on animal health and productivity. The data generated serve as a basis for future research aimed at improving zootechnical indexes and advancing our understanding of ruminant metabolism.

Upon completion of this study, it will be possible to draw meaningful conclusions across various aspects of ruminant nutrition, offering critical information to optimize nutritional management and support decision-making by professionals and researchers committed to the efficiency and sustainability of beef production.