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Carcass traits and gastrointestinal morphological responses to rumen-protected methionine supplementation in grazing beef heifers under tropical conditions

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ABSTRACT

This study evaluated the effects of rumen-protected metabolizable methionine (RPM) supplementation on performance, carcass traits, ruminal morphometry, cecal histology, and meat quality of crossbred heifers finished on Urochloa brizantha pastures during the rainy-to-dry seasonal transition. Forty-eight 18-month-old ½ Nellore × ½ Angus heifers (380 ± 44 kg) were assigned to three supplementation strategies: a control treatment (T1) receiving an energy–protein supplement without methionine; T2 receiving the same supplement plus 3 g/animal per day of RPM; and T3 receiving 3

g/day of RPM combined with a more gradual increase in concentrate supplementation over the 140-day grazing period. RPM supplementation did not affect final body weight or average daily gain throughout the experimental period. However, heifers receiving RPM supplementation (T2 and T3) showed greater ribeye area and lower *Biceps femoris* fat thickness than the control treatment. Ruminal morphometry revealed a greater number of papillae in RPM-supplemented animals, although absorptive surface area and other ruminal histological measurements were not affected by treatment. In the cecum, the most pronounced responses were observed in T3, which showed lower lesion scores and fewer goblet and disrupted goblet cells than the other treatments. Meat quality evaluations indicated greater marbling scores in RPM-supplemented heifers without changes in tenderness or cooking loss. In conclusion, RPM supplementation was associated with changes in carcass traits, ruminal morphometric variables, and cecal histological responses in grazing beef heifers under tropical conditions. The most pronounced cecal responses occurred in the treatment combining RPM supplementation with gradual concentrate adaptation, although the experimental design does not allow complete separation of the effects of methionine supplementation and feeding-management strategy.

Keywords: amino acid supplementation; carcass composition; gastrointestinal integrity; marbling development; ruminal epithelium.

1. INTRODUCTION

Pasture-based beef systems dominate production in tropical regions and remain the foundation of Brazil's beef supply chain. Despite their economic relevance, these systems are frequently characterized by seasonal fluctuations in forage availability and nutritive value, which may limit the supply of metabolizable nutrients required to support optimal growth and carcass development. Among essential amino acids, methionine has received particular attention because tropical forage-based diets often provide limited amounts of rumen-undegradable protein, while methionine itself is extensively degraded in the rumen, reducing its postruminal availability [1–3]

Beyond its role in protein synthesis, methionine participates in several metabolic pathways related to methyl-group transfer, antioxidant metabolism, and cellular regulation. Through S-adenosylmethionine synthesis, methionine contributes to one-carbon metabolism and serves as a precursor for compounds involved in oxidative balance and energy metabolism [4,5]. Therefore, responses to rumen-protected metabolizable methionine (RPM) supplementation may extend beyond muscle accretion and include changes in tissue metabolism and carcass composition. Previous studies have associated RPM supplementation with improvements in nitrogen utilization and productive responses in cattle under forage-based conditions [6–8].

Heifers finished on pasture represent a relevant category for evaluating nutritional strategies because productive responses may differ according to nutrient availability, physiological stage, and tissue deposition priorities [9–11].

In addition to potential effects on carcass traits, nutritional management during the adaptation to concentrate supplementation may also influence gastrointestinal morphology and epithelial responses, particularly under grazing systems subjected to seasonal variation in forage quality.

We hypothesized that RPM supplementation could influence carcass traits and morphological responses of the gastrointestinal tract in crossbred heifers finished on tropical pastures. We further hypothesized that the association between RPM supplementation and a gradual concentrate adaptation strategy could affect cecal histological responses during the transition from rainy to dry season. Therefore, this study evaluated the effects of different RPM supplementation protocols on performance, carcass traits, ruminal morphometry, cecal histology, and meat quality in grazing beef heifers under tropical conditions.

2. MATERIAL AND METHODS

All procedures with animals followed the relevant guidelines and regulations approved by the São Paulo State University, Brazil. All the experimental procedures were approved following the Ethical Committee for Animal Research (Protocol Number CEUA 406/2023). We confirm that this study complied with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

Animals and experimental facility

The study was conducted at the Faculty of Agricultural and Veterinary Sciences (FCAV), São Paulo State University (UNESP), Jaboticabal, SP,

Brazil, within the experimental beef cattle facilities. The experimental area comprised approximately 32 ha of Urochloa brizantha cv. Marandu pasture, divided into 12 paddocks of approximately 2.6 ha each.

Forty-eight 18-month-old crossbred heifers (½ Nellore × ½ Angus), averaging 380 ± 44 kg of body weight (BW), were used in the study

Experimental design

The study was conducted from February to June using a randomized complete block design with three treatments and four blocks. Prior to treatment allocation, heifers were stratified according to initial BW and assigned to four blocks. The 12 paddocks were organized into four blocks of three paddocks each. Within each block, the three treatments (T1, T2, and T3) were randomly assigned to the paddocks, resulting in one paddock per treatment within each block and four paddock replicates per treatment across the experiment. Each paddock contained four heifers and was considered the experimental unit.

The paddocks were maintained under continuous stocking throughout the 140-day experimental period. No pasture rotation or stocking-rate adjustments were performed during the study. As forage availability declined during the transition from the rainy to the dry season, supplementation levels were progressively increased according to the experimental protocol to partially compensate for the expected reduction in forage mass and nutritive value while maintaining animal satiety and grazing activity under pasture conditions.

Heifers were assigned to one of three supplementation protocols throughout the 140-day grazing period. The control group (T1) received an energy–protein supplement without methionine, supplied at 0.1% BW from day 0 to 56, 0.3% BW from day 57 to 84, and 1% BW from day 85 to 140. In T2, animals received the same supplementation schedule as in T1, with the addition of 3 g of rumen-protected metabolizable methionine (RPM; Smartamine M, Adisseo Inc., Antony, France) per animal per day. In T3, heifers also received 3 g/day of RPM; however, a more gradual supplementation strategy was adopted during the adaptation phase, consisting of 0.05% BW from day 0 to 28, 0.1% BW from day 29 to 56, 0.3% BW from day 57 to 84, and 1.0% BW from day 85 to 140.

For methionine-supplemented treatments, RPM was top-dressed onto the basal supplement and offered in collective bunks. The RPM used in this study contained 75% DL-methionine, with approximately 80% rumen bypass. Thus, 5 g/head/day of RPM was supplied to provide 3 g/day of metabolizable methionine. The RPM dose was established to achieve an estimated lysine ratio of 2.5.

Management, feeding, and animal handling

Before the experimental period, heifers underwent an acclimation phase in which all animals grazed the same paddocks and received a mineral supplement to facilitate adaptation to the experimental conditions. Animals were subsequently weighed, dewormed, and vaccinated against common viral and bacterial diseases.

Animals were managed by the experimental farm staff according to routine husbandry practices throughout the study, including daily inspection of health status, pasture conditions, water supply, and supplement delivery

Heifers had *ad libitum* access to pasture and water throughout the study. The supplement, composed of corn, soybean meal, rice meal, cottonseed cake, wheat meal, soybean hulls, and minerals, was formulated according to NASEM [1] recommendations and offered daily at 08:00 h. Supplement amounts differed according to treatment. During the initial adaptation phase, animals in T3 received lower amounts of supplement than those in T1 and T2 to better match pasture conditions during the transition from mid to late summer. From day 85 onward, all treatments received the same supplementation level (1.0% BW).

The ingredient and chemical composition of the supplements are presented in Table 1, whereas pasture chemical and morphological characteristics are presented in Supplementary Table S1. Supplement delivery and refusals were monitored daily at the paddock level to verify supplement consumption throughout the experimental period.

Forage components

To characterize pasture conditions and forage availability, forage samples and canopy height were collected from each paddock at 28-day intervals. Canopy height was measured at 80 points using a 1-m graduated ruler [12]. Forage sampling was conducted using a 0.25 m^2 metallic quadrat

placed at representative locations within each paddock while avoiding fecal-contaminated areas. All forage within the quadrat was harvested.

Each sample was divided into two subsamples: one for estimating forage dry matter yield and another for morphological composition. Subsamples were dried at 65 °C for 72 h in a forced-air oven, weighed, and later used for chemical analysis [13]. Available forage mass (kg/ha) was then estimated. Morphological composition was determined by manually separating dried forage into green leaf blades, green stems (true stems + leaf sheaths), and dead material (≥50% yellowed or dry). Components were dried again at 65 °C for 72 h, weighed, and expressed as proportions.

For chemical composition, dried samples were ground through a 1-mm screen (Wiley mill), pooled by period, and analyzed by near-infrared reflectance spectroscopy (NIRS; 1100–2500 nm) using a spectrometer (Model NR5000; NIRSystems, Inc., USA). Determined components included crude protein, crude fiber, mineral matter, ADF, NDF, and in vitro dry matter digestibility (IVDMD), as in Marten et al. [14].

The amino acid profile of feed ingredients and pasture samples was determined to characterize the amino acid composition of the dietary components offered across treatments. Samples of each ingredient were collected at the beginning of the study, ground to 1 mm, and analyzed for their full amino acid composition, including methionine. The proteins present in natural feedstuffs and animal diets were hydrolyzed with 6 N hydrochloric acid for 24 hours [15]. The amino acids released during acid hydrolysis were

derivatized with phenylisothiocyanate (PITC), separated by reversed-phase HPLC, and detected at 254 nm [16–18]. Quantification was performed using a multilevel internal calibration with alpha-aminobutyric acid (AABA) as the internal standard. The resulting values are presented as mean concentrations on a dry matter basis (Supplementary Table S2). Likewise, estimated amino acid balance based on diet formulation is shown in Supplementary Table S3.

Animal performance and carcass ultrasound

Heifers were weighed on day 0 and every 28 days thereafter. The initial and final body weights were obtained after a 16-h withdrawal from solid feed (water available *ad libitum*). Intermediate weighings were conducted without fasting, and shrunk BW was estimated by subtracting 4% from the observed BW. Average daily gain (ADG) was calculated as the cumulative BW change across the experimental period.

Ultrasound measurements of 12th-rib fat thickness, *Biceps femoris* fat thickness, *Longissimus* muscle (LM) area, and marbling were obtained on days 0, 84, and 140 following Perkins et al. [19]. Daily gains for fat thickness and LM area were calculated from measurement differences divided by days on feed. Images were captured using an Aloka SSD-1100 Flexus RTU unit (3.5 MHz, 17.2 cm probe; Aloka Co. Ltd., Tokyo, Japan).

All animals were managed in accordance with established welfare standards, and no invasive procedures or additional experimental interventions were performed before slaughter. Humane slaughter was conducted in accordance with Brazilian legislation, using a penetrating

captive bolt for stunning, followed by immediate exsanguination [20]. Because the animals were not exposed to surgical or painful procedures, anesthesia or analgesia was not required. Handling practices were designed to minimize stress throughout pre-slaughter operations. Hot carcass weight (HCW) was recorded after removal of kidney, pelvic, and heart fat, and dressing percentage was calculated as the ratio of HCW to final body weight.

Rumen papillae morphometry and histology

Immediately after evisceration, rumens were opened, washed, and evaluated for rumenitis lesions according to the scoring system proposed by Bigham and McManus [21] (0 = no lesions; 10 = severe ulcerative lesions). Scoring was performed independently by two trained evaluators, blinded to treatment allocation.

For ruminal morphometry, a 1 cm^2 tissue sample was collected from the dorsal cranial sac immediately after slaughter and maintained in phosphate-buffered saline (PBS) until analysis. The number of papillae per cm^2 (NOP) was manually counted. Twelve representative papillae per animal were scanned, and the mean papilla area (MPA) was determined using ImageTool (v2.01 alpha 4). Absorptive surface area (ASA, cm^2) was calculated as:

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

where 1 represents the sampled area and 0.002 cm^2 is the estimated basal papilla area.

For histological evaluation, an additional 1 cm^2 sample from the ventral cranial sac was collected immediately after slaughter, fixed, and processed

according to Odongo et al. [22], stained with hematoxylin and eosin, and used to measure papilla height (A-H), width (A-W), surface area (B-PSA), and keratinized layer thickness (B-KS) using computer-assisted image analysis. Measurements were averaged per animal before statistical analysis.

Cecal lesions and histology

Cecal lesion scoring was performed using the same 0-to-10 scale adopted for rumenitis evaluation (adapted from Bigham and McManus [21]) by two trained evaluators blinded to treatment allocation.

For histological analyses, a 1 cm^2 fragment from the central cecal region was collected immediately after slaughter and fixed in 4% paraformaldehyde [23], dehydrated, embedded in paraffin, sectioned at 8 µm, and stained with hematoxylin and eosin.

Histological measurements included crypt depth, enterocyte count, and goblet cell number. Representative and well-oriented crypts were selected for evaluation using a Leica Qwin Image Analyzer coupled to a Leica light microscope. Measurements obtained from individual crypts were averaged to generate a single value per animal before statistical analysis.

Meat quality

A ribeye steak (2.54 cm) from between the 12th and 13th ribs was collected from each carcass, vacuum-packed, aged for 14 days at 4 °C, and then frozen at −20 °C until analysis. Meat color, marbling, cooking loss, and shear force were assessed.

Color (L*, a*, b*) was measured at three points on the *Longissimus thoracis* after 30 min bloom at 4 °C using a spectrophotometric colorimeter (CM2500d; Konica Minolta Brasil, São Paulo, Brazil), following CIE Lab standards. Marbling score was visually evaluated using USDA-adapted reference images.

For shear force, six 2.5-cm cores were removed from each steak after 24 h of chilling. Peak shear force was measured using a Texture Analyzer (CT-3/100, Brookfield, USA), and the mean of six values was recorded [24].

Steaks were thawed for 24 h at 2 °C and cooked in an electric oven (Imequi, Brazil) at 170 °C until the internal temperature reached 71 °C, monitored with copper-constantan thermocouples (E5CWL Omron, CSW) inserted into the geometric center of each steak. After cooking, steaks were chilled for 24 h at 4 °C. Cooking loss was calculated as the difference between raw and cooked steak weights [25].

Statistical analysis

Paddocks were considered the experimental units. Data were analyzed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Residual normality and the presence of outliers were evaluated using the Shapiro–Wilk test. When necessary, logarithmic or square-root transformations were tested to satisfy model assumptions. Variables that met normality assumptions were analyzed using parametric procedures.

Data were analyzed using PROC MIXED according to a randomized complete block design. Treatment was included as a fixed effect, whereas

block was included as a random effect. Paddocks were considered the experimental units and were nested within blocks. Degrees of freedom were calculated using the Satterthwaite approximation. Initial measurements (e.g., body weight, ribeye area, subcutaneous fat thickness, and Biceps femoris fat thickness) were included as covariates when significant. Results are presented as least-squares means, and treatment comparisons were performed using the PDIFF option. Significance was declared at $P \leq 0.05$. Lesion scores and count variables were retained in the parametric analysis because residual diagnostics indicated approximate normality after evaluation of model assumptions.

3. RESULTS

No significant differences in initial body weight were observed among treatments at the beginning of the study ($P > 0.17$). Therefore, initial BW was included as a covariate in subsequent analyses when appropriate. No treatment effects on ADG were detected throughout the experimental period (Table 2).

Dry matter intake (DMI) differed only during days 0 to 28, in which T3 presented lower intake than T2 ($P = 0.048$). This difference reflected the lower supplement allowance intentionally adopted in T3 during the initial adaptation phase. Similarly, when expressed as % BW, DMI was lower in T3 during the first 28 days compared with the other treatments ($P = 0.048$). No differences in DMI were observed among treatments during the remaining experimental periods ($P > 0.17$; Table 2).

Heifers receiving methionine supplementation under the conventional supplementation strategy (T2) and gradual supplementation strategy (T3) showed greater rib eye area at the end of the experiment and lower *Biceps femoris* fat thickness compared with the control group (Table 3). Hot carcass weight and carcass yield were not affected by treatment ($P > 0.05$).

Ruminal and cecal histology results are presented in Table 4. In ruminal morphometry, methionine-supplemented treatments showed a greater number of papillae compared with the control group ($P = 0.03$). However, absorptive surface area (ASA; $P = 0.58$), proportion of papillae in total ruminal surface area ($P > 0.29$), rumenitis scores, papilla height, papilla width, papilla area, and keratinized layer thickness were not affected by treatment ($P > 0.05$).

In the cecal epithelium, treatment effects were observed primarily in T3. Heifers receiving the gradual supplementation strategy associated with RPM supplementation (T3) showed lower lesion scores than T1 ($P = 0.04$) and T2 ($P = 0.01$). Likewise, T3 exhibited fewer goblet cells and fewer disrupted goblet cells than T1 and T2 ($P \leq 0.03$). Crypt depth and enterocyte counts were not significantly affected by treatment ($P > 0.05$), although T3 showed numerically lower values.

Meat quality results are summarized in Table 5. No treatment effects were observed for color parameters L*, a*, and b* ($P > 0.05$). Meat pH differed between T2 and T3 ($P = 0.03$), although all values remained within the normal physiological range (<5.8). Cooking loss and shear force were not

affected by treatment ($P > 0.05$). Marbling scores were greater in T2 and T3 compared with T1 ($P = 0.03$).

4. DISCUSSION

In forage-finished cattle, responses to RPM supplementation may depend not only on amino acid supply but also on dietary adaptation dynamics and nutrient availability throughout the production cycle. In the present study, RPM supplementation was not associated with changes in overall weight gain; however, differences were observed in selected carcass characteristics, ruminal morphometric variables, and cecal histological parameters. These findings suggest that responses to RPM supplementation may vary depending on the physiological and nutritional context in which it is provided.

An important limitation of the present study is that individual intake of both RPM and pasture was not directly measured. Supplements were offered in collective bunks at the paddock level, and pasture intake was not estimated using markers or other intake determination techniques. Consequently, actual methionine consumption and total nutrient intake likely varied among animals within the same treatment. This limitation is inherent to many grazing studies conducted under commercial-like conditions and should be considered when interpreting treatment responses, particularly those related to nutrient supply, amino acid balance, and individual animal performance

Growth Performance and Nutritional Context

During the initial phase of the experiment, pasture conditions were characterized by relatively high crude protein concentrations and lower proportions of senescent material. Under these conditions, supplementation with RPM was associated with greater Longissimus muscle area, particularly in T3 animals. Previous studies have suggested that methionine supplementation may influence muscle deposition and protein metabolism in beef cattle through its participation in methyl-group transfer reactions and other metabolic pathways related to protein synthesis and cellular metabolism [26,27].

Despite differences in muscle area, RPM supplementation did not improve overall ADG throughout the experimental period. As pasture quality progressively declined during the seasonal transition, changes in nutrient availability may have altered the efficiency with which supplemental amino acids were utilized for tissue accretion. In grazing systems, productive responses to amino acid supplementation are strongly dependent on the simultaneous availability of fermentable energy and metabolizable nutrients [28,29]. Similar dissociation between carcass-related responses and overall BW gain has been reported in beef cattle receiving rumen-protected amino acid supplementation under forage-based conditions [30,31].

It is important to note that estimates of amino acid balance presented in Supplementary Table S3 were based on diet formulation and pasture characterization rather than direct measurements of individual pasture intake, metabolizable amino acid flow, or actual RPM consumption by each

animal. Consequently, individual variation in nutrient intake cannot be ruled out and may have contributed to the variability of the observed responses.

Ruminal papillae and epithelial dynamics

Heifers supplemented with RPM showed a greater number of ruminal papillae, whereas papilla area, papilla height, papilla width, keratin layer thickness, absorptive surface area, and rumenitis scores were not affected by treatment. Therefore, although changes in papilla number were observed, the present data do not support the conclusion of increased absorptive capacity or major functional alterations in ruminal epithelial morphology.

Previous studies have suggested that methionine may influence epithelial metabolism and tissue turnover through mechanisms associated with methyl-group transfer, oxidative balance, and cellular metabolism [32,33].

In addition, Mauck et al. [34] reported alterations in epithelial barrier-related variables in dairy cows supplemented with rumen-protected methionine under heat stress conditions. However, because ruminal fermentation characteristics, epithelial metabolism, inflammatory biomarkers, and barrier-function measurements were not evaluated in the present study, the mechanisms underlying the observed ruminal morphometric responses remain uncertain.

The lack of changes in rumenitis scores and keratinized layer thickness indicates that the increase in papilla number occurred without detectable alterations in ruminal epithelial integrity. Since RPM supplementation was formulated to increase postruminal methionine availability, any effects on

ruminal morphology were likely indirect and should be interpreted cautiously within the limitations of the present experimental design.

Cecal integrity and adaptation to concentrate

The most pronounced histological responses were observed in the cecum, particularly in T3, which combined RPM supplementation with a more gradual concentrate adaptation strategy. Heifers in this treatment exhibited lower cecal lesion scores and fewer goblet and disrupted goblet cells than animals receiving the other supplementation protocols. However, because T3 simultaneously involved changes in both RPM supplementation and concentrate adaptation dynamics, the present experimental design does not allow these responses to be attributed exclusively to methionine supplementation.

A slower increase in concentrate intake during the adaptation phase may have reduced the intensity of hindgut fermentation challenges commonly associated with abrupt increases in fermentable carbohydrate supply in cattle consuming forage-based diets [35,36]. In parallel, previous studies have suggested that methionine supplementation may influence epithelial metabolism and oxidative balance in gastrointestinal tissues [37–39]. Nevertheless, inflammatory biomarkers, epithelial permeability, oxidative stress indicators, and hindgut fermentation parameters were not evaluated in the present study. Therefore, the mechanisms underlying cecal histological responses remain speculative.

Additionally, because supplement intake was evaluated at the paddock level rather than individually, variation in RPM consumption among animals within the same treatment cannot be excluded. Therefore, the observed histological responses should be interpreted as treatment-associated outcomes at the paddock level rather than direct individual responses to a known methionine intake.

Although crypt depth and enterocyte counts were not statistically affected by treatment, numerically lower values were observed in T3 animals. Collectively, these findings suggest that the gradual supplementation strategy, as used in RPM, was associated with alterations in cecal histological characteristics under the conditions evaluated in the present study.

Carcass traits and meat quality

RPM supplementation was associated with greater marbling scores without detectable negative effects on meat color, cooking loss, or tenderness. Similar responses have been reported in beef cattle supplemented with rumen-protected amino acids under forage-based finishing systems [30]. Although the biological mechanisms were not evaluated in the present study, previous studies have suggested that methionine may influence lipid metabolism through its participation in one-carbon metabolism and other metabolic pathways associated with adipose tissue development [40,41].

Ultimate meat pH remained below 5.8 across all treatments, indicating normal postmortem acidification and low risk of dark, firm, and dry (DFD)

meat. Although a statistical difference in pH was detected between T2 and T3, the magnitude of this difference was small and remained within the normal physiological range reported for beef cattle [42,43]. In the absence of accompanying changes in meat color, cooking loss, or tenderness, this response is likely to have limited biological relevance.

Shear force values remained below 4 kgf across treatments, indicating acceptable meat tenderness under all supplementation strategies. Collectively, the results suggest that RPM supplementation was associated with changes in marbling deposition without major alterations in other meat-quality parameters evaluated in the present study.

System-level interpretation of responses

Among the evaluated treatments, T3 was associated with the most consistent combination of cecal histological responses and carcass-related outcomes. However, because this treatment simultaneously combined RPM supplementation with a more gradual concentrate adaptation strategy, the present experimental design does not allow complete separation of the individual contributions of methionine supplementation and feeding-management effects.

The absence of differences in ADG, despite observed changes in selected carcass and histological variables, suggests that responses to RPM supplementation under grazing conditions may occur independently of overall BW gain. Previous studies have reported that productive responses to protected amino acid supplementation in forage-based systems can vary

according to nutrient availability, dietary adaptation, and energy supply [6,30,44]. Nevertheless, because nutrient intake, ruminal fermentation, inflammatory responses, and amino acid metabolism were not directly measured in the present study, mechanistic interpretations should be made cautiously. Furthermore, because neither individual pasture intake nor individual RPM consumption were directly quantified, the relationships between nutrient supply and biological responses cannot be established with certainty. Future studies combining individual intake measurements with gastrointestinal and metabolic assessments would help clarify the contribution of methionine supplementation under grazing conditions

Collectively, the present findings suggest that the combination of RPM supplementation and gradual concentrate adaptation was associated with alterations in cecal histological characteristics and selected carcass traits in grazing beef heifers under tropical conditions.

CONCLUSION

Rumen-protected metabolizable methionine (RPM) supplementation was not associated with improvements in the overall growth performance of pasture-finished heifers under tropical conditions. However, RPM supplementation was associated with changes in selected carcass characteristics, ruminal morphometric variables, and cecal histological responses. The most pronounced cecal responses were observed in T3, which combined RPM supplementation with a more gradual concentrate adaptation strategy. Because T3 simultaneously involved modifications in both

methionine supplementation and concentrate adaptation dynamics, the present experimental design does not allow complete separation of the individual effects of RPM and feeding-management strategy on the observed gastrointestinal responses. Nevertheless, the findings suggest that the association between RPM supplementation and gradual concentrate adaptation may influence carcass traits and cecal histological characteristics in grazing beef heifers.

Further studies evaluating nutrient intake, ruminal fermentation, epithelial function, and inflammatory and metabolic markers are necessary to clarify the biological mechanisms underlying these responses.

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Conceptualization: Ana Laura J. Lelis; Danilo D. Millen; Methodology: Ana Laura J. Lelis; Fernanda Lopes; Ricardo A. Reis; Danilo D. Millen; Funding Acquisition: Fernanda Lopes; Danilo D. Millen; Investigation (Data and Sample Collection): Leandro A. F. da Silva; Daniel M. Casali; Rodrigo J. de Oliveira; Jorge Augusto A. Campos; Beatriz O. da Silva; Ana Laura J. Lelis; Data Curation: Danilo D. Millen; Writing – Original Draft: Ana Laura J. Lelis; Writing – Review & Editing: Ana Laura J. Lelis; Gercino F. Virgínio Júnior; Danilo D. Millen; Supervision: Ricardo A. Reis; Danilo D. Millen. All authors read and approved the final version of the manuscript.

COMPETING INTERESTS

The authors declare no competing interests. Fernanda Lopes is an employee of Adisseo Animal Nutrition (the funding source for this study), but confirms

that this affiliation did not influence the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

DATA AVAILABILITY STATEMENT

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

TABLES

Table 1. Composition of the supplement provided to ½ blood Nellore × Angus heifers finished on pasture with or without rumen-protected metabolizable methionine supplementation.

Diets (%BW)	**0.05**	**0.1**	**0.3**	**1.0**
Ingredients (% DM)				
Finely-ground corn	.	21.255	50.000	75.301
Cottonseed meal	.	15.000	14.630	20.080
Rice meal	.	11.000	6.999	.
Wheat meal	.	9.000	8.000	.
Soybean hulls	.	5.301	3.000	.
Urea	.	4.565	3.500	0.602
Soybean meal	.	.	3.000	.
Supplement	100.000	33.879	10.871	4.017
Supplement Nutritional Content (% DM)				
Dry matter	.	92.63	90.01	88.00
Total digestible nutrients	.	45.00	65.00	78.20
Crude protein	.	30.00	25.00	17.34
Calcium	21.3	6.46	2.52	0.88
Phosphorus	6.00	2.00	0.60	0.69
Sodium	8.60	4.00	1.40	0.40

The supplementation levels (0.05, 0.10, 0.30, and 1.00% BW) correspond to the common feeding schedule applied to all treatments throughout the 140-day grazing period. T1 heifers received only the basal supplement at these levels (0.10, 0.30, and, 1.00%), whereas T2 received the same levels plus 5 g/day of rumen-protected methionine (RPM). T2 received RPM from the beginning of supplementation, whereas in T3 the inclusion of RPM followed the gradual supplementation (0.05, 0.10, 0.30, and, 1.00%) strategy described for each period. Minor ingredients (values shown as % of supplement DM for each supplementation level, 0.05, 0.10, 0.30, and 1.00% BW, respectively): calcium carbonate (38.266, 12.200, 5.935, 38.266); dicalcium phosphate (31.285, 8.045, 0.749, 31.285); sodium chloride (22.148, 10.288, 3.588, 22.148); flowers of sulfur (6.134, 0.614, 0.403, 6.134); rumen-protected urea (–, 2.000, –, –); monensin 20% (0.500, 0.150, –, 0.500); copper sulfate (0.393, 0.136, 0.034, 0.393); manganese sulfate (0.194, 0.063, 0.018, 0.194); cobalt sulfate (0.027, 0.009, 0.002,

0.027); antioxidant BHT (–, 0.014, 0.014, –); calcium iodate (0.012, 0.004, 0.001, 0.012); sodium selenite (0.004, 0.001, –, 0.004).

Table 2. Body weight and average daily gain of 1/2 blood Nellore/Angus heifers finished on pasture consuming rumen-protected metabolizable methionine.

Variables	Treatments			SEM [1]	P value	
	T1	T2	T3		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
Supplement intake, kg/d						
0-28 days	0.39	0.33	0.24	0.03	**0.048**	**0.023**
28-56 days	0.44	0.44	0.42	0.01	0.203	0.710
56-84 days	1.70	1.62	1.83	0.21	0.191	0.628
84-112 days	5.07	5.04	5.52	0.60	0.117	0.359
112-140 days	5.56	5.45	5.63	0.63	0.109	0.397
Supplement intake, % BW						
0-28 days	0.10	0.10	0.05	0.01	**0.048**	**0.034**
28-56 days	0.10	0.10	0.10	0.00	0.283	0.973
56-84 days	0.37	0.36	0.37	0.05	0.174	0.575
84-112 days	1.08	1.08	1.09	0.15	0.094	0.309
112-140 days	1.12	1.10	1.12	0.15	0.088	0.361

[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.

Table 3. Carcass characteristics of 1/2 blood Nellore/Angus heifers finished on pasture consuming rumen-protected metabolizable methionine.

Variables	Treatments			SEM[1]	P value	
	T1	T2	T3		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 fat, 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 fat 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.

Table 4. Ruminal morphometry, ruminal histology, and cecal epithelial characteristics of 1/2 blood Nellore × Angus heifers finished on pasture and consuming rumen-protected metabolizable methionine.

Variables	Treatments			SEM[1]	P value	
	T1	T2	T3		T2xT3	T1xT2+T3
Ruminal macroscopic morphometry						
Ruminitis score	0.77	0.96	1.19	0.14	0.21	0.07
Macroscopic Variables						
Papillae number, n	53.16	64.62	63.56	3.95	0.84	**0.03**
Papillae 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
Ruminal microscopic histology						
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[4], um	8.10	7.36	7.34	0.36	0.28	0.18
Cecal epithelial histology						
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; [2] Absorptive surface area; [3] Representativeness of papillae in the absorptive surface area; [4] Keratin layer thickness.
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.

Table 5. Meat quality of 1/2 blood Nellore/Angus heifers finished on pasture consuming rumen-protected metabolizable methionine.

Variables	Treatments			SEM[1]	P value	
	T1	T2	T3		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 score	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).
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.