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Exogenous Enzyme Supplementation for Beef Cattle under Dry-Season Nutritional Strategies: Effects on Growth Performance, Nutrient Utilization and Ruminal Fermentation

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Exogenous Enzyme Supplementation for Beef Cattle under Dry-Season Nutritional Strategies: Effects on Growth Performance, Nutrient Utilization and Ruminal Fermentation

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ABSTRACT: This study evaluated whether the response of grazing beef cattle to exogenous fibrolytic enzymes depends on the nutritional supplementation strategy adopted during the tropical dry season. Two complementary experiments were conducted with Nellore steers grazing *Urochloa brizantha* cv. Xaraés. In Experiment 1, 102 steers were assigned to a completely randomized 2 × 3 factorial design with two supplementation strategies [protein supplement (PRO; 0.1% BW) or protein–energy supplement (PPE; 0.3% BW)] and three levels of a fibrolytic enzyme blend (0, 4.75, or 9.5 g/day). Experiment 2 used five rumen-cannulated steers in a 5 × 5 Latin square to evaluate nutrient intake and digestibility, ruminal fermentation, microbial protein synthesis, and nitrogen utilization. Protein–energy supplementation increased final body weight, average daily gain, nutrient digestibility, ruminal volatile fatty acid production, microbial protein synthesis, and nitrogen retention compared with PRO ($P < 0.05$). Enzyme supplementation produced dose-dependent responses, with quadratic effects on average daily gain and feeding behavior and greatest productive responses at intermediate inclusion levels. Under PPE, enzyme supplementation increased dry matter and neutral detergent fiber digestibility and enhanced acetate and propionate production, whereas microbial protein synthesis and nitrogen utilization were mainly influenced by supplementation strategy. Overall, the benefits of fibrolytic enzymes were associated primarily with improved digestive efficiency and ruminal fermentation rather than increased feed intake. These findings indicate that the efficacy of exogenous fibrolytic enzymes under tropical dry-season grazing depends on the nutritional environment and is enhanced when enzymes are incorporated into protein–energy supplementation strategies.

Keywords: fibrolytic enzymes; dry season; tropical pasture; digestibility; rumen fermentation

INTRODUCTION

Beef cattle production in tropical regions relies largely on pasture-based systems, in which forage availability and nutritive value vary markedly throughout the year because of climatic seasonality (Boval et al., 2015). During the dry season, reduced rainfall and progressive forage maturation increase the proportion of senescent tissues and structural carbohydrates, reducing the supply of fermentable energy and rumen-degradable nitrogen and, consequently, limiting animal performance (Carvalho et al., 2023; Beigh et al., 2023). Under these conditions, nutritional supplementation becomes essential to compensate for pasture deficiencies, with protein and protein–energy supplements differing in both nutrient density and feeding level according to production objectives (Boval et al., 2015; Barbero et al., 2015).

The efficiency of nutrient utilization in dry-season tropical pastures depends largely on the interaction between forage characteristics and ruminal microbial activity. In mature tropical grasses, the close association among cellulose, hemicellulose, lignin, and other structural components reduces the extent of fiber degradation and limits ruminal fermentation (Alvarez et al., 2009). Adequate synchronization between fermentable energy and rumen-degradable nitrogen is therefore important for sustaining microbial growth, microbial protein synthesis, and overall nutrient utilization efficiency (Dias et al., 2023).

Exogenous fibrolytic enzymes have been proposed as a nutritional strategy to improve the degradation of structural carbohydrates by increasing microbial access to plant cell wall polysaccharides (Meale et al., 2014). However, responses in beef cattle remain inconsistent. Meta-analytical evidence indicates modest average improvements in animal performance, with greater benefits observed under specific dietary conditions (Ferreira et al., 2025). Likewise, studies under tropical grazing have reported contrasting responses. Positive effects of xylanase supplementation have been observed during the dry season (Miorin et al., 2022), whereas other studies found no consistent improvements in intake, digestibility, or ruminal fermentation under different supplementation conditions (Damasceno et al., 2024). These findings suggest that the effectiveness of fibrolytic enzymes is strongly influenced by the nutritional context in which they are supplied.

Supplementation strategy may therefore be a key determinant of enzyme efficacy. Although fibrolytic enzymes can increase the availability of potentially digestible fiber fractions, the effective utilization of released substrates depends on an adequate supply of fermentable energy and rumen-degradable nitrogen to support microbial activity and fermentation. Consequently, differences between

protein and protein–energy supplementation may alter the extent to which enzymatically released nutrients are converted into fermentation end-products and microbial biomass (Pedrini et al., 2025).

Despite increasing interest in exogenous fibrolytic enzymes, limited information is available on their dose-response relationships under dry-season tropical grazing, particularly when combined with supplementation strategies differing in nutrient supply. Most previous studies have evaluated enzymes under a single nutritional strategy or have focused separately on productive or metabolic responses, limiting the interpretation of the biological basis for variability in animal response.

We hypothesized that productive and metabolic responses to increasing levels of an exogenous fibrolytic enzyme blend would depend on the nutritional environment created by the supplementation strategy. Therefore, this study evaluated the effects of increasing enzyme inclusion under protein and protein–energy supplementation on growth performance, ingestive behavior, nutrient digestibility, ruminal fermentation, microbial protein synthesis, and nitrogen utilization in Nellore steers grazing *Urochloa brizantha* cv. Xaraés during the tropical dry season.

MATERIALS AND METHODS

Experiment 1: Growth performance and ingestive behavior

Animals, experimental design, and management

The experiment was conducted at the DSM Experimental Center, Caçadinha Farm, Rio Brilhante, Mato Grosso do Sul, Brazil, from July 1 to November 6, 2019, totaling 129 experimental days.

One hundred and two Nellore steers, 12 ± 2.2 months of age and with an initial body weight (BW) of 295 ± 18 kg (mean ± SD), were used. Animals were assigned to a completely randomized design in a 2 × 3 factorial arrangements, with two supplementation strategies and three enzyme inclusion levels. Supplementation strategies consisted of a protein supplement (PRO) supplied at 0.1% of BW or a protein–energy supplement (PPE) supplied at 0.3% of BW. Enzyme treatments consisted of no enzyme supplementation; 1 g/day of Ronozyme® WX combined with 4.75 g/day of Ronozyme® VP; or 2 g/day of Ronozyme® WX combined with 9.5 g/day of Ronozyme® VP. According to the manufacturer, the commercial fibrolytic enzyme complex supplied xylanase, cellulase, β-glucanase, pectinase, hemicellulase, and amylase activities. Enzyme products and supplements were manufactured by DSM Nutritional Products Brazil.

Nutritional plans were formulated to support an expected average daily gain (ADG) of approximately 450 g/day according to the BR-CORTE system (Valadares Filho et al., 2016). For steers

with an initial BW close to 250 kg and the proposed growth target, estimated daily requirements were approximately 5.0 kg dry matter intake (DMI), 2.8 kg total digestible nutrients (TDN), 600 g crude protein (CP), 85 g metabolizable protein, and 4.0 Mcal net energy for gain.

The protein supplement contained, per kilogram of product, 350 g CP, 297 g non-protein nitrogen, 68.0 g Ca, 18 g P, 8 g S, 47 g Na, 2.3 mg Co, 200 mg Cu, 150 mg Fe, 10 mg I, 400 mg Mn, 2 mg Se, 1,000 mg Zn, 40,000 IU vitamin A, and 180 mg F. The protein–energy supplement contained 675 g TDN, 250 g CP, 169 g non-protein nitrogen, 28 g Ca, 8 g P, 4 g S, 19.5 g Na, 3 mg Co, 800 mg Cu, 150 mg Fe, 8 mg I, 100 mg Mn, 1.5 mg Se, 400 mg Zn, 40,000 IU vitamin A, and 80 mg F per kilogram of product.

The experimental area consisted of established *Urochloa brizantha* cv. Xaraés pasture fertilized annually with 150 kg N, 80 kg P, and 60 kg K ha^{-1} $year^{-1}$. Pastures were managed under continuous grazing with variable stocking rate to maintain forage availability throughout the experimental period (Table 1).

Animals were distributed among six paddocks, with 17 steers per paddock, and were rotated among paddocks every 7 days to minimize potential paddock effects. Paddock was included as a random effect in the statistical model. Forage availability and chemical composition were evaluated at the beginning of the study and every 30 days thereafter according to Hodgson et al. (2000). Forage samples were collected by hand-plucking to simulate the diet selected by grazing animals, with subsamples obtained from multiple locations within each paddock and composited within sampling period. Samples were analyzed for dry matter (method 930.15) and crude protein (Kjeldahl N × 6.25; method 984.13) according to AOAC (2000), and neutral detergent fiber according to Van Soest et al. (1991).

Performance and supplement intake

Individual BW was recorded automatically using the INTERGADO® electronic monitoring system (Intergado Ltda., Contagem, Minas Gerais, Brazil) integrated into the water troughs. Steers visited the waterers 3.55 ± 0.75 times/day on average. Daily BW records were obtained throughout 17 consecutive weeks and summarized as weekly means.

Individual BW gain was estimated by linear regression of weekly mean BW against time using PROC REG in SAS 9.4. The slope of the regression equation was used as the individual rate of BW gain.

Supplements were offered ad libitum through an automated feed bunk system capable of continuously recording individual intake. Individual supplement intake was summarized as weekly means, resulting in 17 repeated observations per animal.

Ingestive behavior

Behavioral data were recorded daily throughout the 17-week experimental period using the INTERGADO® automated monitoring system. Variables included frequency of waterer visits, frequency of feed bunk visits, time spent at the waterer, and time spent consuming supplement. Daily observations were summarized as weekly means before statistical analysis.

Statistical analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Residual normality and homogeneity of variances were assessed using the Shapiro-Wilk and Levene tests, respectively.

Final BW and ADG were analyzed according to the model:

$$Y_{ijkl} = \mu + S_i + E_j + (S \times E)_{ij} + P_k + \varepsilon_{ijkl}$$

where μ is the overall mean; Si is the fixed effect of supplementation strategy; Ej is the fixed effect of enzyme inclusion level; (S × E)ij is their interaction; Pk is the random effect of paddock; and εijkl is the residual error.

Variables measured weekly were analyzed as repeated measures using PROC MIXED according to the model:

$$Y_{ijklt} = \mu + S_i + E_j + (S \times E)_{ij} + Tt + (S \times T)_{it} + (E \times T)_{jt} + P_k + Al_{(k)} + \varepsilon_{ijklt}$$

where Tt is the fixed effect of week; (S × T)it and (E × T)jt are the interactions of supplementation strategy and enzyme inclusion level with time, respectively; and Al(k) is the random effect of animal nested within paddock. The remaining terms were defined as above.

Animal nested within paddock was used as the subject for repeated measurements. The covariance structure with the lowest corrected Akaike information criterion (AICc) was selected, and degrees of freedom were adjusted using the Kenward-Roger method. Orthogonal polynomial contrasts were used to test linear and quadratic responses to enzyme inclusion. Statistical significance was declared at $P \leq 0.05$.

Experiment 2: Digestibility and metabolism trial

Experimental design and treatments

Five rumen-cannulated Nellore steers, 10 ± 1.8 months of age and with an average body weight (BW) of 250 ± 16 kg (mean ± SD), were assigned to a 5 × 5 Latin square design. The experiment was conducted from July to October 2019 during the dry season and consisted of five 21-day periods, with 14 days for adaptation and 7 days for sample collection.

The five treatments were: (1) protein–energy supplement (PPE) supplied at 0.3% BW; (2) PPE plus 1 g/day Ronozyme® WX and 4.75 g/day Ronozyme® VP; (3) PPE plus 2 g/day Ronozyme® WX and 9.5 g/day Ronozyme® VP; (4) protein supplement (PRO) supplied at 0.1% BW; and (5) PRO plus 1 g/day Ronozyme® WX and 4.75 g/day Ronozyme® VP. Supplement composition and enzyme inclusion followed those described for Experiment 1 (Table 2). Supplements were provided daily at a fixed time. Representative supplement samples were collected weekly, composited by experimental period, and stored for chemical analysis. Animals grazed established *Urochloa brizantha* cv. Xaraés pastures throughout the experiment.

Forage availability and chemical analysis

Forage mass was estimated on the first day of each experimental period by harvesting herbage at ground level from ten randomly distributed 0.25-m² quadrats, according to McMeniman (1997). Subsamples were dried to determine dry matter (DM) availability and manually separated into leaf, stem, and dead material.

Forage and supplement samples were analyzed for DM, organic matter, crude protein (CP), ether extract, neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, and ash according to AOAC (2000). Total digestible nutrients (TDN) of forage were estimated as TDN (%) = 74.49 - 0.5635 × ADF ($r^2 = 0.82$), whereas TDN of concentrate was estimated as TDN (%) = 9.6134 + 0.829 × in vitro DM digestibility ($r^2 = 0.98$), according to Cappelle et al. (2001).

Intake and digestibility

Dry matter intake and apparent digestibility were estimated using titanium dioxide (TiO_2) as an external marker and an indigestible fiber fraction as an internal marker. Titanium dioxide was administered directly into the rumen at 10 g/day, divided into two equal doses at 08:00 and 17:00 h, from day 2 of each experimental period until completion of fecal sampling, according to Ferreira et al. (2009).

The indigestible fiber fraction was determined by in situ ruminal incubation of feed and fecal samples in non-woven textile bags for 288 h, according to Valente et al. (2011). Daily fecal excretion was calculated from TiO_2 intake and fecal TiO_2 concentration. Pasture intake was estimated from fecal

output and the concentration of the indigestible fiber marker in forage, supplement, and feces, according to Penning and Johnson (1983), as adapted by Detmann et al. (2001).

Ruminal fermentation

Ruminal fluid was collected on the last day of each experimental period at 0 h, immediately before supplementation, and at 2, 4, 6, and 8 h after supplement delivery. Samples were obtained from the cranial, ventral, caudal, and dorsal regions of the rumen, composited, and filtered through four layers of cheesecloth. Ruminal pH was measured immediately after collection.

Volatile fatty acid concentrations were determined by gas chromatography (GC-2014, Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector, following Palmquist and Conrad (1971). Ammonia nitrogen (NH_3-N) was determined colorimetrically according to Chaney and Marbach (1962), using a UV-visible spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan).

Microbial protein synthesis and nitrogen balance

Spot urine samples were collected by spontaneous urination approximately 4 h after supplementation on days 18, 19, 20, and 21 of each experimental period. Samples were immediately diluted in 0.036 N sulfuric acid and stored at -20 °C until analysis.

Allantoin was determined according to Fujihara et al. (1987) and Chen and Gomes (1992). Uric acid and creatinine concentrations were determined using commercial colorimetric kits (Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil). Total urinary purine derivative excretion (PD, mmol/day) was calculated as the sum of allantoin and uric acid. Absorbed purines (absP, mmol/day) were estimated as:

$$PD = 0.85 \times absP + 0.385 \times BW^{0.75}$$

where 0.85 represents the urinary recovery of absorbed purines and $0.385 \times BW^{0.75}$ represents endogenous purine excretion (Verbic et al., 1990).

Nitrogen intake was calculated as CP intake divided by 6.25. Absorbed nitrogen was calculated as nitrogen intake minus fecal nitrogen excretion, and retained nitrogen as absorbed nitrogen minus urinary nitrogen excretion. Daily urinary volume was estimated as:

$$\text{Urinary volume (L/day)} = (27.36 \times BW) / \text{urinary creatinine concentration (mg/L)}$$

where 27.36 represents the estimated daily creatinine excretion per unit of BW according to Rennó et al. (2000).

Statistical analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Residual normality and homogeneity of variances were assessed using the Shapiro-Wilk and Levene tests, respectively.

Variables measured once within each experimental period were analyzed using PROC MIXED according to the model:

$$Y_{ijk} = \mu + T_i + P_j + A_k + \varepsilon_{ijk}$$

where μ is the overall mean; Ti is the fixed effect of treatment (i = 1, ..., 5); Pj is the fixed effect of experimental period (j = 1, ..., 5); Ak is the random effect of animal (k = 1, ..., 5); and εijk is the residual error.

Ruminal variables measured repeatedly after supplementation were analyzed according to the model:

$$Y_{ijk}l = \mu + T_i + P_j + A_k + H_l + (T \times H)_{il} + \varepsilon_{ijkl}$$

where Hl is the fixed effect of sampling time (0, 2, 4, 6, and 8 h); (T × H)il is the treatment × sampling time interaction; and the remaining terms were defined as above. Repeated observations were modeled with animal within experimental period as the subject. Alternative covariance structures were evaluated, and the first-order autoregressive structure [AR(1)] was selected based on the lowest corrected Akaike information criterion (AICc). Degrees of freedom were adjusted using the Kenward-Roger approximation.

Treatment effects were decomposed using pre-planned orthogonal contrasts: C1, PRO versus PPE supplementation; C2, linear effect of enzyme inclusion within PPE; and C3, quadratic effect of enzyme inclusion within PPE. Least-squares means were compared using the PDIFF option when appropriate. Statistical significance was declared at $P \leq 0.05$.

Results

Experiment 1: Growth performance and ingestive behavior

Performance

Supplementation strategy affected final body weight (BW), average daily gain (ADG), and supplement intake ($P \leq 0.004$; Table 3). Steers receiving the protein–energy supplement (PPE) had greater final BW and ADG than those receiving the protein supplement (PRO). A supplementation strategy × enzyme interaction was detected for final BW ($P = 0.043$). Under PPE supplementation, final BW showed a quadratic response to enzyme inclusion ($Y = 340.54 + 1.465X - 0.167X^2$; $r^2 = 0.27$), with a predicted maximum at approximately 4.39 g/day.

Average daily gain was also affected by the supplementation strategy × enzyme interaction (P = 0.017). Under PRO, ADG responded quadratically to enzyme inclusion ($Y = 0.188 + 0.0374X - 0.0037X^2$; $r^2 = 0.18$), with a predicted maximum at 5.05 g/day. Under PPE, ADG also showed a quadratic response ($Y = 0.290 + 0.051X - 0.0046X^2$; $r^2 = 0.32$), with a predicted maximum at 5.54 g/day.

Supplement intake was influenced by the supplementation strategy × enzyme interaction (P = 0.037). Enzyme inclusion linearly increased supplement intake under both PRO ($Y = 0.254 + 0.0091X$; $r^2 = 0.12$) and PPE ($Y = 0.852 + 0.0197X$; $r^2 = 0.15$). Similar linear responses were observed when intake was expressed as percentage of BW.

Supplement intake also changed over time (P = 0.029), with a supplementation strategy × time interaction (P < 0.0001; Figure 1). Differences between PRO and PPE became evident from day 21 and persisted throughout the experimental period.

Ingestive behavior

Supplementation strategy affected feed bunk visitation and time spent at the bunk (P < 0.05; Table 4), with greater values generally observed in PPE-supplemented steers. Feed bunk visits with intake showed quadratic responses to enzyme inclusion under both PPE ($Y = 15.35 - 0.682X + 0.0606X^2$; $r^2 = 0.29$) and PRO ($Y = 6.54 - 0.389X + 0.028X^2$; $r^2 = 0.24$), with predicted minimum values at approximately 5.63 and 6.95 g/day, respectively.

Within PPE, quadratic responses were also observed for visits without supplement intake ($Y = 5.54 - 0.382X + 0.045X^2$; $r^2 = 0.28$), total feed bunk visits ($Y = 20.89 - 1.064X + 0.105X^2$; $r^2 = 0.32$), and total time spent at the bunk ($Y = 1617.17 - 75.36X + 6.619X^2$; $r^2 = 0.31$), with predicted minimum values at approximately 4.24, 5.07, and 5.69 g/day, respectively. PPE-supplemented steers had a greater frequency of feed bunk visits than PRO steers from day 21 onward (Figure 2). Time spent consuming supplement was also greater under PPE, with differences becoming evident from day 35 onward (Figure 3).

Experiment 2: Digestibility and metabolism trial

Intake and digestibility

Steers receiving the protein–energy supplement (PPE) had greater pasture intake, supplement intake, total dry matter intake (DMI), crude protein (CP) intake, and total digestible nutrient (TDN) intake than those receiving the protein supplement (PRO) (P ≤ 0.024; Table 5). Mean pasture intake was 4.69 and 4.59 kg DM/day, whereas total DMI averaged 5.73 and 4.84 kg/day for PPE and PRO, respectively.

Apparent digestibility of DM and CP was greater under PPE than PRO supplementation ($P \leq 0.045$; Table 5). Within PPE, enzyme inclusion linearly increased DM digestibility ($Y = 537.56 + 12.875X$; $r^2 = 0.16$) and NDF digestibility ($Y = 468.85 + 11.547X$; $r^2 = 0.15$).

Ruminal fermentation

Ruminal pH and ammonia nitrogen (NH_3-N) did not differ between supplementation strategies (Table 6). Within PPE, NH_3-N showed a quadratic response to enzyme inclusion ($Y = 32.206 - 2.827X + 0.237X^2$; $r^2 = 0.22$), with a predicted minimum at 5.96 g/day.

PPE supplementation increased ruminal acetate, propionate, and butyrate concentrations compared with PRO ($P \leq 0.049$; Table 6). Mean acetate concentrations were 61.6 and 58.8 mmol/L, and propionate concentrations were 15.2 and 13.7 mmol/L for PPE and PRO, respectively. Within PPE, enzyme inclusion linearly increased acetate concentration ($Y = 58.437 + 0.6738X$; $r^2 = 0.18$).

The acetate-to-propionate ratio was lower under PPE than PRO supplementation ($P = 0.028$). Under PRO, enzyme supplementation increased ruminal acetate concentration and the acetate-to-propionate ratio ($P < 0.005$).

Microbial protein synthesis and nitrogen utilization

PPE supplementation increased urinary allantoin, uric acid, total purine derivatives, absorbed purines, and microbial protein synthesis compared with PRO ($P \leq 0.045$; Table 7). No linear or quadratic responses to enzyme inclusion were detected for microbial protein synthesis within PPE. Under PRO, enzyme supplementation increased allantoin, total purine derivatives, absorbed purines, and microbial protein synthesis relative to the control treatment.

Nitrogen intake, absorption, and retention were greater under PPE than PRO supplementation. Mean absorbed nitrogen was 76.8 and 46.5 g/day, whereas nitrogen retention averaged 54.5 and 26.0 g/day for PPE and PRO, respectively (Table 7).

Discussion

The response to exogenous fibrolytic enzymes was influenced by the nutritional strategy adopted during the dry season. Under these conditions, when tropical forages generally contain greater proportions of structural and poorly digestible fiber, enzyme supplementation interacted with the nutritional environment rather than producing a uniform response. This was evident from the supplementation strategy × enzyme interactions for final BW and ADG. Although PPE consistently supported greater animal performance than PRO, the quadratic responses in ADG under both strategies

indicate that increasing enzyme inclusion was beneficial only up to a predicted maximum, reinforcing that enzyme efficacy cannot be considered independently of the diet in which the enzymes are supplied.

The metabolic responses observed in Experiment 2 help explain this pattern. PPE increased nutrient intake, DM digestibility, ruminal volatile fatty acid concentrations, microbial protein synthesis, and nitrogen retention. More importantly, increasing enzyme inclusion within PPE linearly increased DM and NDF digestibility and acetate concentration. Thus, the productive advantage observed in Experiment 1 was accompanied by a ruminal environment characterized by greater nutrient utilization, while the additional digestive response to fibrolytic enzymes was particularly evident when they were associated with protein–energy supplementation.

These two experiments, however, should not be interpreted as providing direct mechanistic confirmation of one another. Experiment 2 was designed to complement the performance trial but did not include the highest enzyme level under PRO. Therefore, its metabolic responses provide biological support for the performance results without fully explaining the dose-response pattern observed under protein supplementation. In particular, the metabolic basis of the response to the highest enzyme inclusion under PRO remains unresolved.

Taken together, the results indicate that fibrolytic enzymes can contribute to dry-season supplementation programs, but their response depends on the nutritional context in which they are supplied. Although enzyme effects were also detected under PRO, improvements in digestive and fermentative responses were more consistently evident within PPE. This supports considering fibrolytic enzymes as part of an integrated nutritional strategy rather than as an isolated feed additive.

Performance and intake

Supplementation strategy was the main driver of productive performance during the dry season. Steers receiving PPE had greater final BW, ADG, and supplement intake than those receiving PRO, resulting in an approximately 20-kg advantage in final BW. This response is consistent with the nutritional limitations of mature tropical pastures, in which reduced protein availability and increased cell-wall recalcitrance can constrain nutrient utilization and animal performance (Detmann et al., 2014). Similar benefits of protein–energy supplementation during the dry season have been reported by Barbizan et al. (2020) and Tambara et al. (2021), particularly when the additional nutrient supply improves the balance between energy and protein available to the animal.

The response to fibrolytic enzymes, however, was not uniform across supplementation strategies. The supplementation × enzyme interaction for ADG and its quadratic response indicate that increasing enzyme inclusion was beneficial only within a specific range, with intermediate inclusion

producing the greatest performance. Such dose-dependent responses are consistent with the variable effects reported for fibrolytic enzymes, which depend on diet composition, enzyme dose, and feeding conditions (Beauchemin et al., 2003, 2014; Arriola et al., 2017).

This variability is particularly relevant under grazing conditions. Ferreira et al. (2025) reported modest average effects of exogenous enzymes on growth performance but considerable heterogeneity among production systems. Under tropical grazing, Miorin et al. (2022) observed benefits of xylanase supplementation for carcass gain despite limited changes in ADG, whereas Damasceno et al. (2024) found no consistent effects on intake or digestibility at low supplementation levels. Together with the present findings, this evidence suggests that the productive response to fibrolytic enzymes depends not simply on enzyme inclusion, but on the nutritional conditions under which the enzymes are supplied.

Therefore, our results support integrating fibrolytic enzymes into the overall supplementation strategy. Although enzyme responses were detected under both nutritional plans, PPE provided greater overall performance and a nutritional context in which the productive response to enzyme supplementation was more consistently expressed.

Feeding behaviour

Feeding behaviour followed the differences observed in supplement intake and productive performance. Steers receiving PPE visited the feed bunk more frequently and spent more time consuming supplement than those receiving PRO, with these differences becoming evident during the first weeks of supplementation and persisting thereafter. This pattern is consistent with previous studies showing that supplementation level and composition can modify feeding activity and nutrient utilization in grazing cattle (Barbizan et al., 2020; Carvalho et al., 2023).

Enzyme inclusion also modified feeding behaviour, with quadratic responses for feed bunk visits and time spent at the bunk. Intermediate enzyme inclusion generally reduced feeding activity while coinciding with the range associated with greater ADG. This pattern suggests that greater productive performance did not require a proportional increase in feeding activity and may instead reflect improved utilization of consumed nutrients. However, because feeding behaviour alone cannot establish digestive efficiency, this relationship should be interpreted together with the digestibility and ruminal responses observed in Experiment 2.

Overall, behavioural responses were consistent with the productive pattern observed in Experiment 1: PPE promoted greater supplement intake and performance, while enzyme inclusion altered feeding activity in a dose-dependent manner. Considering feeding behaviour alongside

performance therefore provides complementary evidence that the response to fibrolytic enzymes under tropical grazing depends on both enzyme dose and the nutritional strategy in which they are supplied.

Intake

Nutrient intake was primarily influenced by supplementation strategy. Steers receiving PPE had greater pasture, supplement, total DM, CP, and TDN intake than those receiving PRO. This response is particularly relevant during the dry season, when the greater proportion of structural and poorly digestible fiber and the lower protein content of tropical forages can constrain voluntary intake and nutrient supply (Detmann et al., 2014; Dias et al., 2023). Under these conditions, a more balanced supply of energy and protein can improve forage utilization and increase the amount of nutrients available to the animal (Detmann et al., 2014; Carvalho et al., 2023).

In contrast, enzyme supplementation did not consistently increase pasture or total DM intake. This agrees with the variable responses reported in the literature. Damasceno et al. (2024) found no consistent effect of fibrolytic enzymes on intake under low supplementation levels, whereas Pedrini et al. (2025) reported increased nutrient intake with enzyme supplementation. Similarly, Miorin et al. (2022) observed clearer responses in carcass performance than in intake, and the meta-analysis of Ferreira et al. (2025) highlighted the considerable variability in animal responses to exogenous enzymes across feeding conditions.

The absence of a consistent increase in intake is important for interpreting the present findings. Because enzyme supplementation improved digestive and ruminal responses without a corresponding increase in total feed intake, its contribution appears to have been related more to the utilization of consumed nutrients than to stimulation of voluntary intake. This distinction provides a direct link between the intake results and the digestibility responses observed in Experiment 2.

Digestibility

Digestibility responses further support this interpretation. PPE increased DM and CP digestibility, while increasing enzyme inclusion within PPE linearly improved DM and NDF digestibility. Thus, the response to fibrolytic enzymes was expressed primarily through improved utilization of the consumed diet rather than through greater intake. This pattern is consistent with previous reports of improved DM and fiber digestibility following enzyme supplementation without proportional changes in feed consumption (Arriola et al., 2017; Ferreira et al., 2025).

The improvement in fiber digestibility is biologically relevant under dry-season grazing, when mature tropical forages contain a larger proportion of structural and less digestible cell-wall components (Detmann et al., 2014; Carvalho et al., 2023). Fibrolytic enzymes may increase the accessibility of

potentially digestible structural carbohydrates to ruminal microorganisms, thereby facilitating fiber degradation (Beauchemin et al., 2003; Alvarez et al., 2009; Kondratovich et al., 2019). Although this mechanism was not directly measured in the present study, the linear increase in NDF digestibility within PPE is consistent with this proposed mode of action.

Importantly, this digestive response was evident within the protein–energy strategy, reinforcing that enzyme efficacy cannot be separated from the nutritional environment in which the enzymes are supplied. The greater digestibility observed with PPE also provides a biological basis for the subsequent changes in ruminal fermentation and nutrient metabolism. Overall, the results suggest that, under the conditions of this study, the main contribution of fibrolytic enzymes was to improve nutrient utilization rather than feed intake, with the clearest digestive response occurring when enzymes were combined with protein–energy supplementation.

Ruminal fermentation

Ruminal fermentation reflected the interaction between supplementation strategy and enzyme inclusion. Although overall NH_3-N concentrations did not differ between PRO and PPE, enzyme supplementation produced different responses within each nutritional strategy. Within PPE, NH_3-N responded quadratically to enzyme inclusion, whereas the intermediate enzyme level under PRO resulted in greater NH_3-N concentration. This pattern indicates that the ruminal response to fibrolytic enzymes depended on the nutritional environment in which they were supplied.

The fermentation profile also differed between supplementation strategies. PPE increased acetate, propionate, and butyrate concentrations and reduced the acetate-to-propionate ratio compared with PRO. In addition, enzyme inclusion within PPE increased acetate concentration, consistent with the improvement in NDF digestibility observed under this strategy. Increased VFA production following fibrolytic enzyme supplementation has also been associated with improved nutrient digestibility in previous studies (Salem et al., 2013), supporting a link between fiber utilization and ruminal fermentation.

The response under PRO was less consistent. The greater NH_3-N concentration observed at the intermediate enzyme inclusion was not accompanied by a comparable overall improvement in the fermentation profile. Fibrolytic enzymes can modify ruminal ammonia and individual VFA concentrations depending on substrate availability and dietary conditions (Dawson and Tricarico, 1999; Alvarez et al., 2009). Thus, rather than indicating a uniform effect of enzymes on ruminal fermentation, the contrasting responses observed here reinforce the importance of the nutritional matrix in determining their activity.

Overall, the fermentation results extend the digestibility findings. The combination of greater VFA concentrations under PPE and improved fiber digestibility with increasing enzyme inclusion within this strategy suggests that the digestive response was accompanied by changes in ruminal fermentation. These responses provide a logical link between improved nutrient utilization and the productive advantage observed with PPE, while avoiding the assumption that changes in NH_3-N alone represent greater nitrogen-use efficiency.

Nitrogen utilization and microbial protein synthesis

Nitrogen metabolism was influenced primarily by supplementation strategy. PPE increased urinary allantoin, uric acid, total purine derivatives, absorbed purines, and estimated microbial protein synthesis compared with PRO. This response was consistent with the greater nutrient intake, digestibility, and ruminal fermentation observed under PPE and supports the importance of an adequate energy and nitrogen supply for ruminal microbial growth (Dias et al., 2023; Damasceno et al., 2024).

In contrast, enzyme inclusion did not produce linear or quadratic responses in microbial protein synthesis within PPE. Under PRO, however, the intermediate enzyme treatment resulted in greater purine derivatives and estimated microbial protein synthesis than the control. This response occurred without a corresponding increase in total feed intake, suggesting that changes in microbial metabolism may occur independently of increased consumption. Similar responses to fibrolytic enzymes have been reported by Salem et al. (2013). Nevertheless, because Experiment 2 did not include the highest enzyme level under PRO, this response should be interpreted as evidence specific to the evaluated treatment rather than as a complete dose-response relationship.

PPE also increased nitrogen intake, absorption, and retention, indicating that the greater nutrient supply translated into greater whole-animal nitrogen retention. In contrast, enzyme supplementation did not produce a consistent response in nitrogen balance. This distinction is important: while the nutritional strategy clearly influenced nitrogen utilization, the contribution of fibrolytic enzymes was expressed more consistently through digestibility and ruminal fermentation than through nitrogen retention.

Taken together, these results complete the biological sequence observed in Experiment 2: greater nutrient supply with PPE was accompanied by greater digestibility, a different ruminal fermentation profile, greater microbial protein synthesis, and greater nitrogen retention, whereas the additional effects of fibrolytic enzymes were more selective and depended on the nutritional context. This supports their role as a complementary component of the supplementation strategy rather than as the primary determinant of nitrogen metabolism.

Taken together, both experiments show that the response to exogenous fibrolytic enzymes under dry-season tropical grazing depends on the nutritional context in which they are supplied. PPE consistently improved animal performance and was accompanied by greater nutrient intake, digestibility, ruminal VFA concentrations, microbial protein synthesis, and nitrogen retention. Within this nutritional strategy, increasing enzyme inclusion further improved DM and NDF digestibility and altered ruminal fermentation. Thus, the productive response appears to reflect coordinated changes in nutrient utilization rather than an isolated effect on intake or a single ruminal variable.

These findings also help explain the variable responses to fibrolytic enzymes reported across production systems. Enzyme efficacy is influenced by substrate characteristics, nutrient availability, and the conditions supporting ruminal microbial activity, which are particularly relevant when cattle graze mature tropical forages during the dry season (Detmann et al., 2014). Therefore, fibrolytic enzymes should be considered within the nutritional strategy in which they are supplied rather than as feed additives expected to produce uniform responses across dietary conditions.

The integration of both experiments should nevertheless be interpreted within the limits of the experimental design. Experiment 2 complemented the performance trial but did not include the highest enzyme level under PRO. Consequently, the metabolic results support, but do not fully explain, the dose-response patterns observed in Experiment 1, particularly under protein supplementation. Further studies evaluating complete enzyme dose-response relationships across contrasting supplementation strategies are warranted.

Conclusion

Protein–energy supplementation improved growth performance of Nellore steers grazing tropical pastures during the dry season and was associated with greater nutrient utilization, ruminal fermentation, microbial protein synthesis, and nitrogen retention. The response to fibrolytic enzymes depended on the nutritional strategy, with the clearest digestive and fermentative responses occurring when enzymes were combined with protein–energy supplementation. Overall, fibrolytic enzymes appear to be more appropriately used as components of integrated dry-season supplementation strategies than as standalone feed additives.

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Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval

All procedures involving animals were conducted in accordance with institutional guidelines for animal care and use and were approved by the Institutional Animal Care and Use Committee (CEUA) of the DSM Experimental Center (protocol no. 0016/2025).

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Experiment 1

Table 1. Forage availability, canopy height, and chemical composition of *Urochloa brizantha* cv. Xaraés during the dry season

Item	July	August	September	October	November
Forage availability (t DM/ha)	3.73	4.43	5.08	4.19	3.27
Canopy height (cm)	31.7	31.0	29.9	28.6	29.3
Crude protein. % DM	4.56	4.13	4.68	5.00	5.12
Neutral detergent fiber. %DM	71.6	74.6	75.7	72.5	74.2
Total digestible nutrients. % DM	52.0	50.0	48.7	50.5	50.6

Experiment 2

Table 2. Chemical composition of supplements and *Urochloa brizantha* cv. Xaraés pasture. (mean±SEM).

Item	Pasture	Supplements	
		Protein[1]	Protein-energy[2]
Dry matter (as-fed)	52.2±0.56	92.6±1.14	88.5±1.75
Crude protein. %DM	6.59±0.75	34.9±1.28	25.4±1.34
Ether extract. %DM	2.54±0.02	3.21±0.15	3.78±0.22
Neutral detergent fiber. %DM	67.6±4.61	19.3±2.89	18.3±3.36
Acid detergent fiber. %DM	40.1±3.92	5.42±2.01	3.79±1.95
Lignin. %DM	7.89±0.98	2.56±1.12	2.89±1.23
Ash. %DM	8.23±0.32	41.05±0.88	38.55±1.22
Total digestible nutrient[3]. %DM	51.9±2.21	55.6±1.26	66.2±1.35

[1]Protein supplement (0.1 % BW). [2] Protein-energy supplement (0.3 % BW).[1] Mineral composition (per kg): Ca 68,0 g/kg, P 18,00 g/kg, Na 47,00 g/kg, Co 2,3 mg/kg, Cu 200,00 mg/kg, Fe 150,00 mg/kg, I 10,00 mg/kg, Mn 400 mg/kg, Se 2,00 mg/kg, Zn 1.000 mg/kg, Vit A 40.000 UI/kg, e Fl 180 mg/kg.[2] Mineral composition (per kg): Ca 28,00 g/kg, P 8,00 g/kg, S 4,00 g/kg, Na 19,5 g/kg, Co 3,00 mg/kg, Cu 800,00 mg/kg, Fe 150,00 mg/kg, I 8,00 mg/kg, Mn 100,00 mg/kg, Se 1,5 mg/kg, Zn 400,00 mg/kg, Vit A 40.000 UI/kg e Fl 80,00 mg/kg.[3] Calculated according to Capelle et al. (2001).

Experiment 1

Table 3. Growth performance and supplement intake of Nellore steers receiving protein or protein–energy supplementation with increasing inclusion of exogenous fibrolytic enzymes during the dry season. (mean±SEM).

Item	Experimental Supplements[1]						*P* - value [2]			
	Protein			Protein-energy			SUP	ENZ		SUP*ENZ
	0	4.75	9.5	0	4.75	9,5		Linear	Quad	
Initial BW (kg)	295±3.99	294±3.35	295±3.35	294±3.20	294±3.02	295±3.10	-	-	-	-
Final BW (kg) [A,B]	317^{d} ±3.96	328^{c} ±5.15	320^{cd} ±6.01	341^{b}±4.06	345^{ab} ±5.68	339^{a} ± 6.02	<0.001	0.905	0.719	0.043
ADG (kg/d) [C,D]	0.188^{d} ±0.03	0.283^{c} ±0.06	0.210^{cd} ±0.05	0.391^{b} ±0.03	0.429^{ab} ±0.03	0.357^{a} ±0.03	0.004	0.308	0.014	0.017
Supplement intake (kg/d) [E, F]	0.242^{e} ±0.04	0.317^{d} ±0.04	0.331^{c}±0.03	0.872^{ab} ±0.01	0.810^{b} ±0.02	0.934^{a} ±0.01	<.0001	0.02	0.178	0.037
Supplement intake (%BW) [G,H]	0.083^{d} ±0.01	0.110^{d} ±0.01	0.113^{c} ±0.01	0.281^{ab}±0.003	0.265^{b}±0.004	0.291^{a} ±0.003	<.0001	0.032	0.283	0.039

[1] PRO = protein supplement supplied at 0.1% of body weight; PPE = protein–energy supplement supplied at 0.3% of body weight. Blend of carbohydrases (xylanase and beta-glucanase) inclusion - 4.75 g/d (1 g Ronozyme® WX and 3.75 g Ronozyme® VP) or 9.5 g/d (2 g Ronozyme® WX and 7.5 g Ronozyme® VP). The enzymatic complex contained xylanase (20,000 U/g), cellulase (10,000 U/g), β-glucanase (5,000 U/g), and amylase (5,000 U/g) [2] P-values for supplement effect (SUP), linear and quadratic enzyme dose effect (ENZ), and interaction effect between supplement and enzyme (SUP*ENZ). [a-e]Values in the same row with a different letter differ significantly at $P \leq 0.05$ according to the PDIFF test. [(A)] $y = 340.54 + 1.465x - 0.167x^2$; $r^2 = 0.27$ (protein-energy supplement); [(B)] $y = 320.67 - 2.710x + 0.3124x^2$; $r^2 = 0.17$ (protein supplement); [(C)] $y = 0.290 + 0.051x - 0.0046x^2$; $r^2 = 0.32$ (protein-energy supplement); [(D)] $y = 0.188 + 0.0374x - 0.0037x^2$; $r^2 = 0.18$ (protein supplement); [(E)] $y = 0.852 + 0.0197x$; $r^2 = 0.15$ (protein-energy supplement); [(F)] $y = 0.254 + 0.0091x$; $r^2 = 0.12$ (protein supplement); [(G)] $y = 0.278 + 0.0055x$; $r^2 = 0.14$ (protein-energy supplement); [(H)] $y = 0.0873 + 0.0030x$; $r^2 = 0.11$ (protein supplement);

Table 4. Feeding behavior of Nellore steers receiving protein or protein–energy supplementation with increasing inclusion of exogenous fibrolytic enzymes. (mean±SEM).

Item	Supplements[1]						*P* - valor [2]			
	Protein			Protein-energy			SUP	ENZ		SUP*ENZ
	0	4.75	9.5	0	4.75	9.5		Linear	Quad	
Feed bunk visits (visits/day)										
With intake [(A. B)]	6.49±0.25	5.30±0.20	5.48±0.17	15.5±0.35	13.3±0.32	14.3±0.36	<.0001	0.134	0.04	0.762
Without intake [(C)]	4.37±0.22	4.18±0.34	4.51±0.18	5.56±0.20	4.43±0.19	6.25±0.21	0.019	0.430	0.029	0.315
Total [(D)]	10.9±0.41	9.48±0.45	10.0±0.30	21.0±0.48	17.6±0.46	20.6±0.53	<.0001	0.557	0.032	0.425
Time at feed bunk (s/day)										
With intake	588±29.97	528±31.88	519±36.37	1,409±46.8	1,303±41.43	1,285±51.19	<.0001	0.268	0.621	0.947
Total [(E)]	592±27.38	452±20.25	441±15.35	1,621±44.3	1,322±40.34	1,552±52.17	<.0001	0.252	0.026	0.341

[1] PRO = protein supplement supplied at 0.1% of body weight; PPE = protein–energy supplement supplied at 0.3% of body weight.Blend of carbohydrases (xylanase and beta-glucanase) inclusion - 4.75 g/d (1 g Ronozyme® WX and 3.75 g Ronozyme® VP) or 9.5 g/d (2 g Ronozyme® WX and 7.5 g Ronozyme® VP). The enzymatic complex contained xylanase (20,000 U/g), cellulase (10,000 U/g), β-glucanase (5,000 U/g), and amylase (5,000 U/g) [2] P-values for supplement effect (SUP), linear and quadratic enzyme dose effect (ENZ), and interaction effect between supplement and enzyme (SUP*ENZ).). [ae] values in the same row with a different letter differ significantly at $P \leq 0.05$ according to the PDIFF test. [(A)] $y = 15.35 - 0.682x + 0.0606x^2$; $r^2 = 0.29$ (protein-energy supplement); [(B)] $y = 6.54 - 0.389x + 0.028x^2$; $r^2 = 0.24$ (protein suplement); [(C)] $y = 5.54 - 0.382x + 0.045x^2$; $r^2 = 0.28$ (protein-energy supplement); [(D)] $y = 20.89 - 1.064x + 0.105x^2$; $r^2 = 0.32$ (protein-energy supplement); [(E)] $y = 1617.17 - 75.36x + 6.619x^2$; $r^2 = 0.31$ (protein-energy supplement).

Experiment 2

Table 5. Nutrient intake and apparent digestibility of Nellore steers receiving protein or protein–energy supplementation with exogenous fibrolytic enzymes.

Item	Supplements[1]					SEM[2]	P-value[3]		
	Protein-energy			Protein					
	0	4.75	9.5	0	4.75		C1	C2	C3
Intake (kg/d)									
Pasture	4.85	4.54	4.70	4.76	4.42	0.397	0.024	0.871	0.348
Suplement	1.15	0.92	1.04	0.245	0.251	0.1000	0.001	0.514	0.232
Dry matter	6.00	5.46	5.75	5.01	4.67	0.398	0.021	0.795	0.286
Crude protein	0.619	0.501	0.572	0.375	0.340	0.032	0.001	0.536	0.165
NDF	3.51	2.87	3.39	3.25	3.14	0.282	0.91	0.862	0.339
ADF	1.99	1.63	1.95	1.91	1.87	0.174	0.858	0.914	0.355
TDN	3.17	2.83	3.03	2.62	2.38	0.200	0.014	0.762	0.28
Intake (% BW)									
Dry matter	1.95	1.66	1.83	1.62	1.50	0.13	0.022	0.739	0.274
NDF	1.03	0.833	0.969	0.848	0.768	0.065	0.187	0.711	0.263
Apparent digestibility (g/kg)									
Dry matter	509	525	554	505	501	4.213	0.045	0.021	0.485
Crude protein	831	822	818	787	792	2.547	0.001	0.178	0.769
NDF	448	454	486	434	418	3.024	0.547	0.012	0.365

[1] PRO = protein supplement supplied at 0.1% of body weight; PPE = protein–energy supplement supplied at 0.3% of body weight). Blend of carbohydrases (xylanase and beta-glucanase) inclusion - 4.75 g/d (1 g Ronozyme® WX and 3.75 g Ronozyme® VP) or 9.5 g/d (2 g Ronozyme® WX and 7.5 g Ronozyme® VP). The enzymatic complex contained xylanase (20,000 U/g), cellulase (10,000 U/g), β-glucanase (5,000 U/g), and amylase (5,000 U/g)[2] SEM = standard error of the mean. [3]Contrasts: C1 = protein vs. protein-energy, C2 =linear effect of carbohydrase inclusion in protein-energy supplement, and C3 = quadratic effect of carbohydrase inclusion in protein-energy supplement.).

Table 6. Ruminal fermentation of Nellore steers receiving protein or protein–energy supplementation with exogenous fibrolytic enzymes.

Item	Supplements[1]					SEM [2]	P-value[3]		
	Protein-energy			Protein					
	0	4.75	9.5	0	4.75		C1	C2	C3
pH	6.06	6.1	6.01	6.14	6.12	0.037	0.089	0.504	0.261
NH3 -N (mg / dL) *(mmol/L)*	32.20	24.13	26.80	22.80^{b}	34.20^{a}	1.885	0.874	0.083	0.048
Acetate	58.60	61.30	65.00	52.50^{b}	65.10^{a}	2.354	0.013	0.002	0.901
Propionate	14.80	14.60	16.20	15.10^{a}	12.30^{b}	0.781	0.041	0.021	0.453
Butyrate	8.31	8.37	9.38	8.19	7.06	0.430	0.008	0.224	0.512
Isobutyrate	0.777	0.843	0.842	0.805	0.699	0.039	0.485	0.179	0.385
Valerate	0.907	0.883	0.829	1.07	0.944	0.069	0.183	0.556	0.896
Isovalerate	0.577	0.624	0.684	0.533	0.532	0.045	0.198	0.31	0.941
Total	83.9	86.6	92.9	78.1^{b}	86.6^{a}	3.632	0.546	0.213	0.753
Acetate:propionate	4.02	4.23	4.04	3.48^{b}	5.31^{a}	0.072	0.028	0.909	0.236

[1] PRO = protein supplement supplied at 0.1% of body weight; PPE = protein–energy supplement supplied at 0.3% of body weight). Blend of carbohydrases (xylanase and beta-glucanase) inclusion - 4.75 g/d (1 g Ronozyme® WX and 3.75 g Ronozyme® VP) or 9.5 g/d (2 g Ronozyme® WX and 7.5 g Ronozyme® VP). The enzymatic complex contained xylanase (20,000 U/g), cellulase (10,000 U/g), β-glucanase (5,000 U/g), and amylase (5,000 U/g)[2] SEM = standard error of the mean. [3]Contrasts: C1 = protein vs. protein-energy, C2 =linear effect of carbohydrase inclusion in protein-energy supplement, and C3 = quadratic effect of carbohydrase inclusion in protein-energy supplement.). [a,b] Means within the PRO treatments with different superscripts differ at $P \leq 0.05$ according to the PDIFF option

Table 7. Purine derivatives, microbial protein synthesis, and nitrogen utilization of Nellore steers receiving protein or protein–energy supplementation with exogenous fibrolytic enzymes

Item	Supplements[1]					SEM [2]	P-value[3]		
	Protein-energy			Protein					
	0	4.75	9.5	0	4.75		C1	C2	C3
mmol/d									
Allantoin	295	186	189	94.1[b]	200[a]	6.547	0.032	0.254	0.247
Uric acid	38.4	19.9	19.9	32.3	24.15	2.547	0.045	0.145	0.198
Total purines	333	206	209	126[b]	224[a]	6.874	0.001	0.308	0.487
Purines absorbible	381	230	234	135[b]	252[a]	5.987	0.003	0.341	0.412
g/d									
N mic	277	167	170	98.5[b]	183.54[a]	8.547	0.021	0.307	0.523
Microbial crude protein	1,733	1,044	1,062	616[b]	1,147[a]	12.55	0.021	0.307	0.523
N utilization (g/d)									
N intake	99.0	80.2	91.6	60.0	54.5	5.23	0.001	0.536	0.165
Fecal N	16.73	14.28	16.67	12.78	11.34	1.74	0.785	0.448	0.335
Urine N	25.0	17.4	24.1	17.1	23.6	1.66	0.547	0.881	0.243
Absorbed	82.27	65.92	74.93	47.22	43.16	4.66	0.015	0.258	0.377
Retained	57.27	48.52	50.83	30.12	19.56	1.26	0.016	0.098	0.896

[1] PRO = protein supplement supplied at 0.1% of body weight; PPE = protein–energy supplement supplied at 0.3% of body weight). Blend of carbohydrases (xylanase and beta-glucanase) inclusion - 4.75 g/d (1 g Ronozyme® WX and 4.75 g Ronozyme® VP) or 9.5 g/d (2 g Ronozyme® WX and 7.5 g Ronozyme® VP). The enzymatic complex contained xylanase (20,000 U/g), cellulase (10,000 U/g), β-glucanase (5,000 U/g), and amylase (5,000 U/g)[2] SEM = standard error of the mean. [3]Contrasts: C1 = protein vs. protein-energy, C2 =linear effect of carbohydrase inclusion in protein-energy supplement, and C3 = quadratic effect of carbohydrase inclusion in protein-energy supplement.). [a,b] Means within the PRO treatments with different superscripts differ at $P \leq 0.05$ according to the PDIFF option.

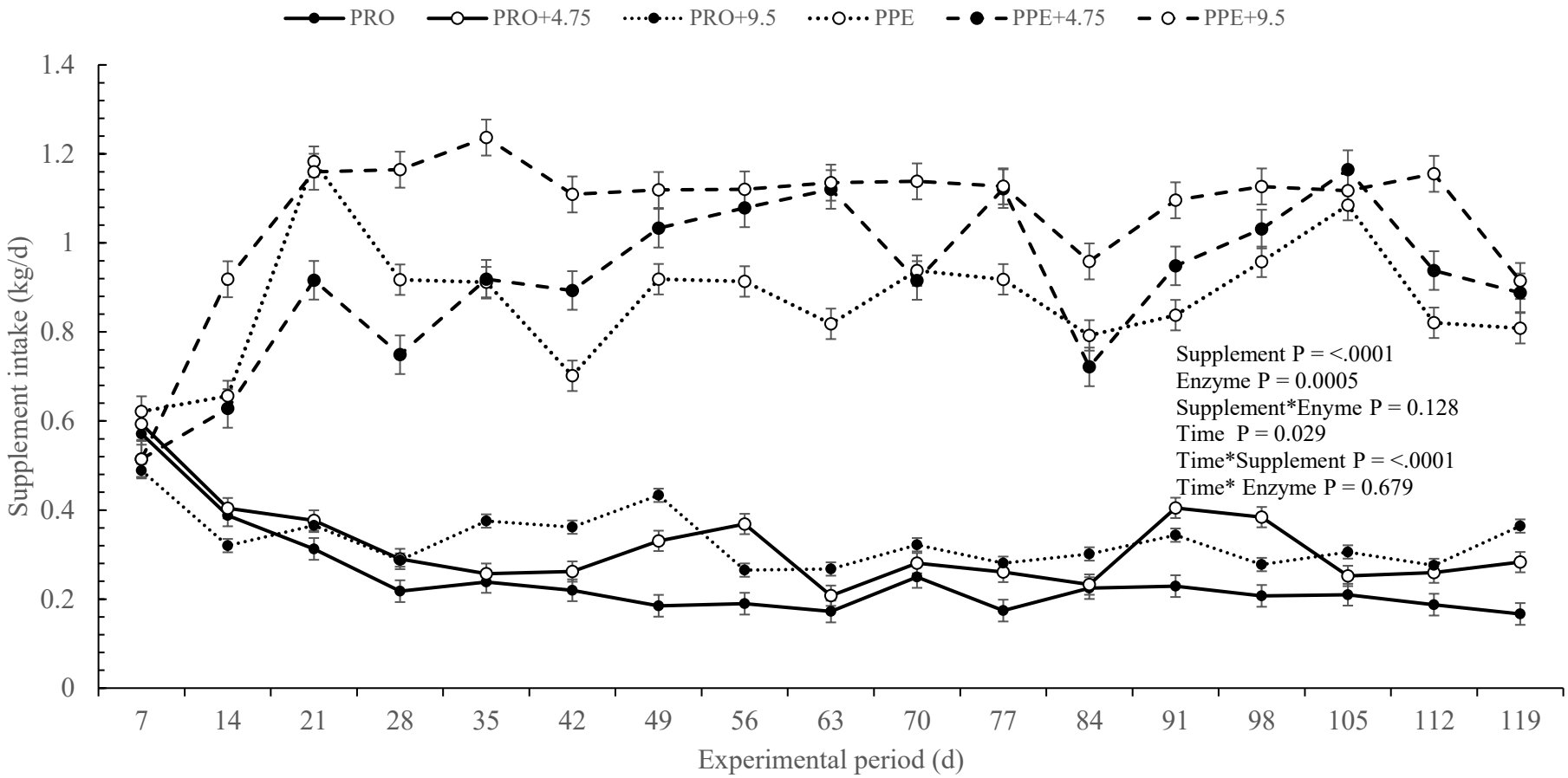


Figure 1. Temporal changes in supplement intake of Nellore steers receiving protein (PRO) or protein–energy (PPE) supplementation with increasing levels of an exogenous fibrolytic enzyme blend (0, 4.75, and 9.5 g/head/day) during the experimental period.

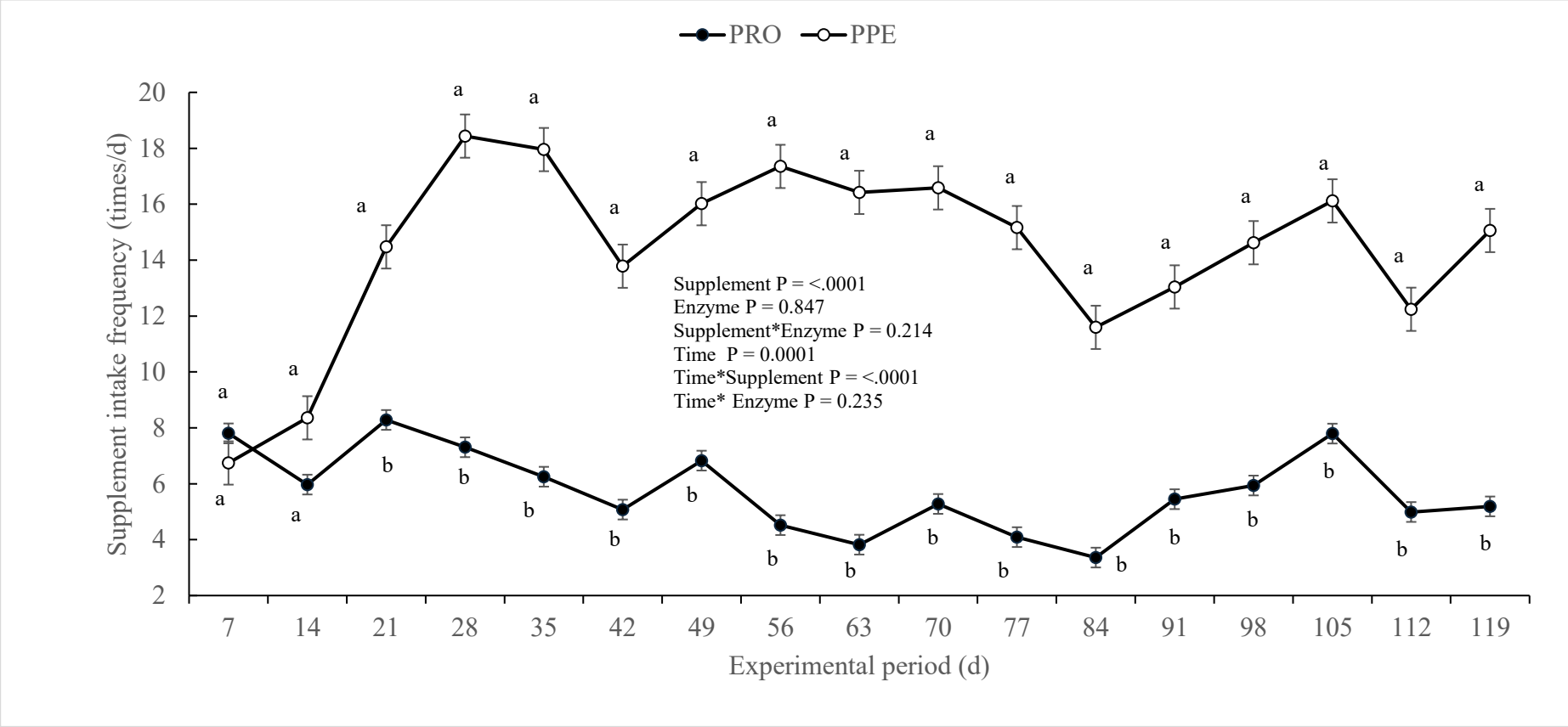


Figure 2. Temporal changes in feed bunk visit frequency of Nellore steers receiving protein (PRO) or protein–energy (PPE) supplementation with increasing levels of an exogenous fibrolytic enzyme blend (0, 4.75, and 9.5 g/head/day) during the experimental period.

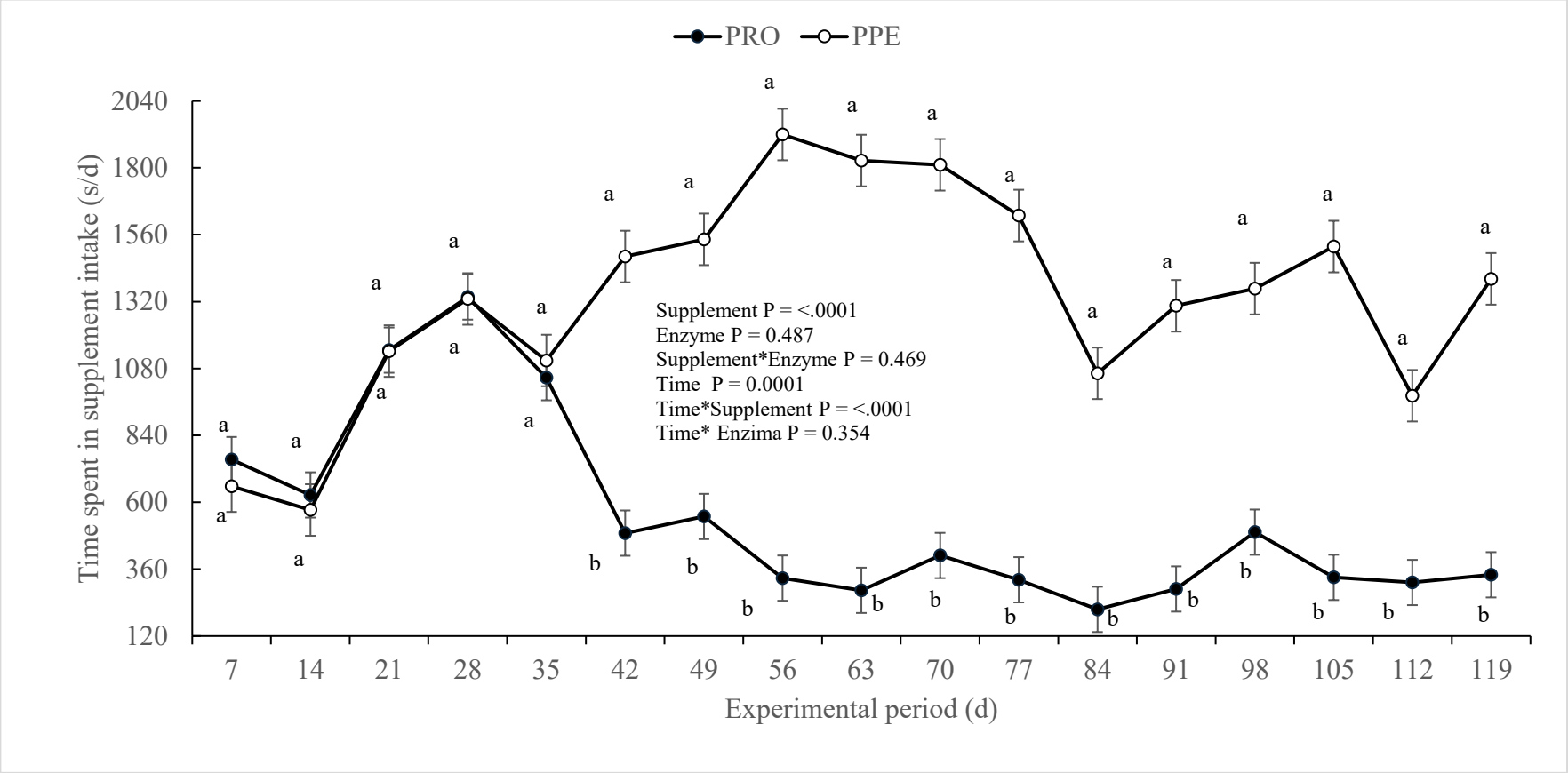


Figure 3. Temporal changes in time spent consuming the supplement by Nellore steers receiving protein (PRO) or protein–energy (PPE) supplementation with increasing levels of an exogenous fibrolytic enzyme blend (0, 4.75, and 9.5 g/head/day) during the experimental period