

Does an Excess of Rumen Degradable Protein Improve Performance of Growing Zebu Heifers in Tropical Pasture?

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Abstract

Although concentrations exceeding theoretical requirements may enhance microbial activity, their potential effects on cattle grazing tropical pastures remain to be clarified. The objective of this study is to assess the influence of excess rumen degradable protein (RDP) on feed intake, digestibility and weight gain of Nellore heifers grazing tropical pastures. Forty crossbred heifers (1/2 Nellore x 1/2 Brahman) with 12 ± 2 months old and 210 ± 27.8 kg were used in completely randomized design. The heifers were allocated to eight 1-hectare paddocks covered with *Urochloa brizantha* cv. Xaraés. The treatment consisted of concentrate supplementation formulated to provide dietary 100% of RDP requirement (RDP100) or 150% of RDP requirement (RDP150). Increasing RDP over 50% of the requirement did not affect ($P > 0.05$) dry matter intake and digestibility. Rumen ammonia nitrogen, serum urea nitrogen and urine urea were higher ($P < 0.05$) in RDP150 than RDP100. However, no differences ($P > 0.05$) were observed between treatments for average daily gain or final body condition score. Increasing RDP above requirement does not improve intake, digestibility and performance of heifers in tropical pasture.

Introduction

Grass constitutes the primary dietary component of grazing beef cattle in tropical regions (Poppi et al. 2018). However, tropical grasses are often characterized by suboptimal nutritional value, including inadequate crude protein concentrations, elevated proportions of indigestible fiber, and pronounced seasonal variability in forage quality, which usually constrain animal performance (Barbero et al., 2021).

Rumen degradable protein (RDP) is essential to supply nitrogen to microbial synthesis sustaining optimum ruminal fermentation (Soliva et al., 2015). RDP concentration exceeding theoretical requirements may enhance microbial activity, thereby improving intake and digestibility, particularly in high-fiber diets with sufficient energy to support nitrogen assimilation (McDonald et al., 2011). Tropical C4 grasses have lower effective rumen degradable protein content compared with other forages (Bowen et al. 2008); however, nitrogen supplementation may enhance the fiber degradation rate (Souza et al. 2010).

Although feedlot beef cattle requirement has been extensively evaluated, data on the requirements of grazing cattle remain limited. Only recently were the nutrient requirements for grazing Zebu cattle compiled (Valadares Filho et al. 2023), and the existing database still requires expansion. In a metanalysis, Detmann et al. (2014) reported that beef cattle grazing tropical pastures exhibit a positive weight gain response to supplemental protein, with the response theoretically corresponding to diets containing up to 225 g/kg DM, which is considerably higher than predicted requirement (Valadares Filho et al. 2023). However, excess dietary CP can increase nitrogen excretion and energy cost in urea cycle (Araújo et al. 2020). Requirement data is still limited for grazing cattle in tropical pasture and the effect of potentially high dietary CP on beef cattle performance is not well understood.

The hypothesis is that a relative excess of RDP compared to conventional recommendations to grazing beef cattle enhance animal performance. Therefore, the objective of this study is to assess the influence of excess RDP on feed intake, digestibility and weight gain of Nellore heifers grazing tropical pastures.

Material and Methods

Local and animals

The experiment was carried out in Entre Rios do Oeste – PR, Brazil (latitude 24°40'34" S e 54°16'39" W; 261 m above sea level) during the rainy season. The climate of the region, according to the Köppen classification, is type Cfa - humid subtropical climate - with an average temperature of 22°C (Alvares et al. 2013).

The experimental period lasted 98 d, comprising 14 d for acclimation and 84 d for data collection. Forty crossbred heifers (1/2 Nellore x ½ Brahman) with 12 ± 2 months old and 210 ± 27.8 kg were used in completely randomized design.

The heifers were allocated to eight 1-hectare paddocks covered with *Urochloa brizantha* cv. Xaraés. Each paddock was subdivided into eight sections and managed under an intermittent grazing system with three days of occupation. Parasite control was performed prior to the beginning of the experiment and whenever infestations were detected.

The treatment consisted of concentrate supplementation formulated to provide dietary 100% of RDP requirement (RDP100) or 150% of RDP requirement (RDP150). The RDP requirement was estimated according to the BR-Corte (Valadares Filho et al. 2016) considering growing Zebu Heifers with an expected average daily gain of 0.6 kg/d (Table 1).

Table 1
Dietary Chemical composition

Item	Treatment ¹	
	RDP100	RDP 150
Organic matter, g/kg	921.9	913.1
Crude protein, g/kg	122.2	165.3
Rumen degradable protein, g/kg	60.0	104.8
Rumen non degradable protein, g/kg	62.1	60.5
Neutral detergent fiber, g/kg	618.7	634.1
Ether extract, g/kg	24.9	25.7
¹ Diet containing 100% (RDP100) or 150% (RDP150) of rumen degradable protein requirement.		

The protein content of the concentrate supplement was adjusted based on hand-plucked forage samples collected at the beginning of the experiment and every 28 days (Table 2). A mineral mixture was offered *ad libitum*, and the supplements were provided at a rate of 6 g/kg of body weight, adjusted every 28 days according to the heifers' average weight. Supplements were offered daily at 1000 h in each paddock, ensuring simultaneous access by all heifers within each group. Water was available *ad libitum* throughout the entire experimental period.

Table 2
Ingredients and chemical composition of the concentrate supplements and pasture

Item	Supplement ¹		Forage ²
	RDP100	RDP150	
Ingredients in supplement			
Grounded corn, g/kg	864.0	573.0	
Soybean meal, g/kg	110.0	350.0	
Urea, g/kg	23.4	69.3	
Ammonium sulphate, g/kg	2.60	7.70	
Chemical composition			
Dry matter, g/kg	877.1	882.5	260.8
Organic matter, g/kg	955.1	890.9	915.8
Crude protein, g/kg	205.1	428.0	96.7
Rumen degradable protein, g/kg	141.5	338.1	59.2
Rumen undegradable protein, g/kg	63.6	89.9	39.4
Neutral detergent fiber, g/kg	291.8	268.5	730.7
Ether extract, g/kg	25.6	21.6	25.9
¹ Concentrate supplements used to reach 100% (RDP100) or 150% (RDP150) of rumen degradable protein requirement.			
² Sample obtained by hand-plucked method.			

Intake and Digestibility

Intake and digestibility were estimated using markers between days 37 and 45. Fecal excretion was estimated using chromium oxide (CrO) as an external marker, and individual supplement intake was estimated using titanium dioxide (TiO). Chromium oxide was e

The digestibility trial lasted eight days, with the first five days used for marker stabilization in the gastrointestinal tract and the remaining three days for fecal collection. Fecal samples were collected on days 43, 44, and 45, at 0700 h, 1200 h, and

Fecal dry matter excretion (kg/day) was estimated based on the ratio between the amount of chromium oxide administered and its concentration in feces, using the following equation:

$$\text{Fecal excretion (kg)} = \frac{\text{Administrated marker (g)}}{\text{fecal marker (g/kg)}}$$

Supplement intake was estimated by the following equation:

$$\text{SI} = \frac{\text{FE} * \text{FM}}{\text{SM}}$$

Where: SI is the individual supplement intake (kg/day); FE is the fecal excretion (kg/day); FM = concentration of marker the feces (TiO₂, kg/kg); SM is the concentration of marker in the supplement (TiO₂, kg/kg).

The forage dry matter intake (FDMI) was estimated using the internal marker iNDF:

$$\text{FDMI (kg/day)} = \left\{ \frac{[(\text{FE} * \text{FM}) - (\text{SI} * \text{SM})]}{\text{FOM}} \right\}$$

Where: FE is the fecal excretion (kg); FM the concentration of marker in feces (iNDF, kg/kg); SI is the supplement intake (kg); SM the concentration of marker in the supplement (iNDF, kg/kg); FOM is the concentration of marker in the forage (iNDF, kg/kg).

Individual total dry matter intake was estimated as the sum of forage and supplement intake.

Collection of ruminal fluid, urine and blood

On day 46, feces, ruminal fluid, urine, and blood samples were collected from the same heifers used in intake and digestibility trial. Ruminal fluid, urine, and blood were collected 4 h before supplement feeding and 4 hours after supplement feeding.

Urine samples were obtained as spot samples during spontaneous urination. Aliquots of 6 mL of urine were diluted in 24 mL of H₂SO₄ (0.036 N; dilution ratio 1:4) and stored at – 20°C until laboratory analysis. Ruminal fluid was collected using an esophageal probe, a silicone hose, and a vacuum pump. The first 100 mL of rumen fluid were discarded, and the following 50 mL were collected with pH measured immediately after sampling. Aliquots of 25 mL of ruminal fluid were mixed with 0.5 mL of H₂SO₄ (1:1) and stored at – 20°C until laboratory analysis.

Immediately after ruminal fluid collection, blood samples were obtained by jugular venipuncture using vacuum tubes. Samples were centrifuged at 3,000 × g for 15 minutes, and serum aliquots were stored in microtubes at – 20°C until analysis.

Chemical analysis

Forage, feces, and supplement ingredient samples were dried in forced-air ovens at 55°C for 72 h and then processed using a Wiley knife mill. The samples were sieved through a 1-mm mesh, except for indigestible neutral detergent fiber (iNDF) analysis, which used a 2-mm mesh.

The analyses included dry matter (DM, method no. 930.15), crude protein (CP, method no. 954.01), and organic matter (OM, method no. 942.05) following AOAC (1990) protocols, as well as starch analysis according to Hall (2009). Neutral detergent fiber (NDF) was analyzed using thermostable α -amylase without sodium sulfite (Mertens et al. 2002). The iNDF content was determined using F57 filter bags (Ankom, Macedon, NY, USA) after a 288-h rumen incubation (Valente et al. 2011). Fecal samples were analyzed for chromium concentration using atomic absorption (Williams et al. 1962) and for titanium dioxide concentration using colorimetric method (Myers et al. 2004).

Rumen fluid was analyzed for ruminal ammonia nitrogen (RAN) using a modified colorimetric technique (Chaney and Marbach 1962), replacing phenol with sodium salicylate (Felix and Cardoso 2004). Serum and urine samples were analyzed for urea concentration using an enzymatic method with a commercial kit (Analisa[®], Belo Horizonte, MG, Brazil).

Productive performance

Productive performance was assessed by measuring the body weight (BW) of the animals after a 14-h fast from both feed and water. Weight gain was determined as the difference between the final and initial body weights. Average daily gain (ADG) was calculated by dividing the weight difference by the duration of the experiment.

Statistical analysis

The experimental unit was group of heifers in each paddock (four replicates). All data were analyzed as completely randomized design using the MIXED procedure of SAS OnDemand for Academics (SAS Institute Inc., Cary, NC, USA). The statistical model adopted was as follows:

$$Y_i = \mu + \text{RDP}_i + e_i$$

where Y_i = dependent variable; μ = overall mean; RDP_i = fixed effect of RDP; and e_{ijk} = random error associated with Y_i normally distributed $e_i \sim N(0, \sigma_e^2)$.

Initial body weight was included as a covariate in the analysis of final body weight. A significant level of 5% was adopted for all statistical tests.

Results

Intake and digestibility

Forage dry matter intake, DM intake, NDF intake, EE intake, OM intake, and digestible organic matter intake (DOMI) did not differ significantly between treatments ($P > 0.05$). In contrast, CP intake was higher in the RDP150 treatment (0.81 kg/day) compared with RDP100 (0.47 kg/day; $P < 0.01$). Conversely, starch intake was greater in RDP100 (0.77 kg/day) than in RDP150 (0.56 kg/day; $P < 0.01$) (Table 3).

Table 3

Feed intake of heifers fed diets with varying rumen degradable protein levels in tropical pasture

Item	Treatment ¹			
	RDP100	RDP150	SEM	P-value
Forage, kg/d	4.76	5.00	0.43	0.584
Total dry matter, kg/d	6.21	6.49	0.49	0.584
Organic matter, kg/d	5.72	5.93	0.43	0.664
Crude protein, kg/d	0.76	1.07	0.09	< 0.001
Neutral detergent fiber, kg/d	3.84	4.12	0.33	0.427
Ether extract, kg/d	0.15	0.17	0.01	0.354
Digestible organic matter, kg/d	4.01	4.49	0.44	0.322
Starch, kg/d	0.77	0.56	0.06	< 0.001
¹ Diet containing 100% (RDP100) or 150% (RDP150) of rumen degradable protein requirement.				

Crude protein digestibility was significantly higher in RPD150 (74.13%) compared with RPD100 (54.84%; $P < 0.01$). No significant differences were observed for DM digestibility, NDF digestibility, OM digestibility, EE digestibility, or starch digestibility (Table 4).

Table 4

Digestibility of heifers fed diets with varying rumen degradable protein levels in tropical pasture

Item	Treatment ¹		SEM	P-value
	RDP100	RDP150		
Dry matter, %	70.02	72.72	1.45	0.121
Organic matter, %	73.05	75.41	1.28	0.116
Crude protein, %	54.84	74.13	4.17	< 0.001
Neutral detergent fiber, %	66.93	68.92	2.61	0.474
Ether extract, %	40.94	47.33	4.68	0.220
Starch, %	86.36	89.95	1.77	0.089

¹Diet containing 100% (RDP100) or 150% (RDP150) of rumen degradable protein requirement.

Rumen, blood and urine parameters

No interaction was observed ($P > 0.05$) between RAN and sampling time (Table 5). RAN was higher in RDP150 than RDP100 in both 4 h before and 4 h after supplementation ($P < 0.05$).

Table 5

Nitrogen metabolism throughout the day in heifers fed diets with varying rumen degradable protein levels in tropical pasture

Item	Treatment ¹			P-value ²		
	RDP100	RDP150	SEM	T	P	TxP
Rumen ammonia nitrogen, mg/dL						
Morning	9.64	13.42	1.88	0.006	< 0.001	0.419
Afternoon	22.97	29.71				
Serum urea nitrogen, mg/dL						
Morning	15.48	15.16	1.76	0.056	0.43	0.037
Afternoon	13.16	20.19				
Urine urea nitrogen, mg/dL	9.40	17.86	2.35	0.025		

¹Diet containing 100% (RDP100) or 150% (RDP150) of rumen degradable protein requirement.

²T is the effect of treatment, P is the effect of sampling period, TxP in the interaction between these main effects.

Serum urea nitrogen (SUN) showed a treatment × time interaction. Before supplementation NUS were similar ($P > 0.05$) between RDP100 and RDP 150. However, 4 hours after supplementation, the RDP150 group exhibited higher serum urea nitrogen (SUN; $P < 0.05$) than the RDP100 group, reflecting a 33.2% increase from pre-supplementation values, whereas SUN decreased by 16.9% in heifers from the RDP100 group. Urinary urea excretion (UUE) was higher ($P < 0.05$) in RDP150 than in RDP 100 (17.86 mg/dL vs. 9.40 mg/dL).

Productive performance

No significant differences were observed between treatments for final body weight, average daily gain, total weight gain, or final body condition score (Table 6).

Table 6
Effects of different rumen-degradable protein levels on the performance of heifers grazing tropical pasture

Item	Treatment ¹			
	RDP100	RDP150	SEM	P-value
Final body weight, kg	278.1	276.9	6.06	0.844
Average daily gain, kg/d	0.440	0.420	0.07	0.861
Final weight gain, kg	36.7	35.6	6.25	0.861
Final body condition score	7.07	7.06	0.25	0.967
¹ Diet containing 100% (RDP100) or 150% (RDP150) of rumen degradable protein requirement.				

Discussion

This study aimed to assess the effectiveness of relative excess of RDP to nutritional response and productive performance grazing beef cattle. Tropical pastures often contain low CP, and supplementation is a common strategy to improve their nutritional quality (Sampaio et al. 2010; Figueiras et al. 2010; Poppi et al. 2018; Barbizan et al. 2020). Dietary protein plays a key role in modulating feed intake, as both excess and deficiency can affect dry matter intake. Protein excess may lead to toxicity due to increased ammonia production and higher energy expenditure associated with urea synthesis, whereas protein deficiency can limit intake because of insufficient nitrogen availability for ruminal microorganisms (Cavalcante et al. 2006). Protein requirements are determined by the animal's production level and the balance of dietary energy and other nutrients (Valadares Filho et al. 2023). Therefore, a relative excess of dietary protein does not necessarily represent a high-protein diet. In the present study, dietary CP ranged from 122 to 165 g/kg DM, values similar to those commonly used for growing beef cattle under tropical conditions during the rainy season.

Excessive supplementation of RDP did not improve feed intake. Although increasing crude protein in low-quality pastures has been shown to enhance intake (Lazzarini et al. 2009; Souza et al. 2010), in tropical pasture of moderate quality the benefits of additional protein appear to be limited. Adequate supply of RDP is critical to microbial growth as well as the energy to protein balance (Chen et al. 2022). However, increasing the RDP-to-digestible organic matter ratio may enhance microbial protein synthesis rather than alter rumen particle kinetics (Bowen et al. 2018), indicating a limited potential to affect feed intake.

Dietary crude protein concentrations above 10% of DM do not typically influence dry matter intake in cattle (Sampaio et al. 2010; Menezes et al. 2016). Conversely, increasing dietary protein can enhance the efficiency of ruminal microbial CP synthesis, which may reach a maximum in diets containing 14.5% CP in growing cattle in tropical pasture (Detmann et al. 2014). Nevertheless, the similarity in dry matter and fiber digestibility observed among RDP treatments indicates that protein supply was not a limiting factor to microbial activity.

The increase in RDP did not improve DM digestibility, likely due to energy limitations constraining microbial growth. The protein-to-energy ratio exerts a greater influence on digestibility than the absolute protein concentration in the diet (Lazzarini et al. 2009; Valente et al. 2014). The increase in RDP did not improve DM digestibility, likely due to energy limitations constraining microbial growth. The protein-to-energy ratio exerts a greater influence on digestibility than the absolute protein concentration in the diet (Souza et al. 2010).

The treatment RDP150 likely presented an unbalanced protein-to-energy ratio, which may have limited the efficiency of nitrogen utilization. The protein-to-digestible OM ratios were 190 and 238 g/kg for RDP100 and RDP150, respectively. When CP content exceeds approximately 210 g of CP/ kg of digestible OM, losses of protein or incomplete net transfer may occur (Poppi and McLennan 1995).

The observed increase in apparent protein digestibility in the RDP150 treatment was likely associated with a reduction in the proportion of endogenous nitrogen excreted in the feces, a response commonly observed with higher protein intake (Silva et al. 2016). Although starch digestibility did not differ significantly among treatments, a tendency toward increased values was noted for RDP150. The potential contribution of high-RDP supplementation to starch digestion may be attributed to an improved protein-to-energy balance, as well as to a reduction in starch intake associated with the higher-CP supplement.

The availability of RAN is critical for ruminants' production, and a minimal concentration is required for microbial growth, while a much greater concentration is required for optimization of feed degradability (Dewhurst and Newbold 2022). Nitrogen availability for microbial growth and the efficiency of nitrogen utilization have been commonly assessed through RAN measurements (Calsamiglia et al. 2010; Jin et al. 2018). However, a portion of ruminal ammonia is absorbed across ruminal wall (Hailemariam et al. 2021), and its absorption rate is positively related to its concentration (Xu et al. 2019). Blood urea represents the main product of nitrogen metabolism before excretion in ruminants (Kessler et al. 2024). The increase in RDP enhanced nitrogen availability to microorganisms, particularly from non-protein

nitrogen (NPN) sources that are rapidly degraded to ammonia (Jin et al. 2018). Nevertheless, the additional RDP was not efficiently assimilated due to limited energy availability for microbial protein synthesis.

The increase in serum urea nitrogen (SUN) concentration observed only 4 h after supplementation evidence a transient excess of RDP, leading to elevated urinary nitrogen excretion. Concentrate supplementation promotes fluctuations in the supply of protein and other nutrients to the rumen throughout the day. While ruminal ammonia concentrations typically peak a few hours after supplementation, most of the daily nitrogen supply depends on forage intake and nitrogen recycling in grazing cattle. When the rate of NPN degradation exceeds microbial utilization, excess ammonia is absorbed into the bloodstream and converted to urea by the liver, resulting in elevated SUN levels (Rocha et al. 2016). The similarity in SUN concentrations before supplementation suggests that most of the excess nitrogen was excreted, with minimal contribution to improving ruminal nitrogen availability through recycling.

The 90% increase in urinary urea observed in the RDP150 treatment indicates an excess of dietary protein and greater energy expenditure associated with urea synthesis and excretion (Pessoa et al. 2009). Elevated urinary urea levels confirm that dietary nitrogen supply exceeded the animals' requirements or that an imbalance existed between nitrogen and the energy provided by fermentable carbohydrates, thereby reducing the efficiency of nitrogen utilization. Relative excesses of metabolizable protein in relation to metabolizable energy are known to increase urinary nitrogen excretion (Reed et al. 2017).

Animal productive performance did not differ significantly among treatments, indicating that the higher crude protein supply did not result in additional weight gain. The productive response to protein supplementation has been inconsistent across studies. The magnitude of the response to increasing supplemental protein is generally associated with the animal's potential for weight gain and the nutritional composition of the pasture (Detmann et al. 2004; Barros et al. 2014; Martins et al. 2015; Fernandes et al. 2016). In a metanalysis, Detmann et al. (2014) reported that beef cattle grazing tropical pastures exhibit a positive weight gain response to supplemental protein, with the response theoretically corresponding to diets containing up to 225 g/kg DM of CP. However, the response of grazing cattle to supplemental protein also depends on the dietary protein-to-energy ratio, which is critical for efficient protein utilization (Detmann et al., 2014a; Poppi & McLennan, 1995; Valente et al., 2013).

Conclusion

Increasing RDP above requirement does not improve intake, digestibility and performance of heifers in tropical pasture. Optimum RDP is associated with energy availability to microbial growth, whereas excessive nitrogen results in excretion rather than contributing to microbial fermentation of dietary substrates.

Declarations

Conflict Of Interest

The authors declare no conflict of interest.

Ethics approval

This study received approval from Animal Care and Use Committee at Western Paraná State University (Protocol 21 – 17).

Conflict of interest

The author declares no conflict of interest.

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Author Contributions

Conceptualization and design: Dresch, A; Valente, EEL. Data curation, Investigation: Dresch, A; Soares, AA; Diniz, L.C; Markmann, RC. Data analysis: Dresch, A; Valente, EEL. Writing – original draft: Dresch, A; Valente, EEL. Writing – review & editing. Alfai, SCA; Lopes, SA; Valente, EEL.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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