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Ruminal degradation and fermentation kinetics of the Mulato II grass (Convert HD364) under different sources of nitrogen fertilization

Degradação ruminal e cinética de fermentação do capim Mulato II (Convert HD364) sob diferentes fontes de adubação nitrogenada

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Abstract
It is important to evaluate nutritional value of new grass species, which can be better characterized through rumen fermentation and degradation techniques. The aim was to evaluate the effects of Nitrogen (N) fertilization on the kinetics of fermentation and ruminal degradation of the Mulato II grass. Two distinct sources of N (common and protected urea) were used during two periods of the year (summer - I and autumn - II). A completely randomized experimental design was used, in a 2x4 factorial arrangement with three replicates. Fraction A changed based on N dosage. The passage rate (K) and effective degradability (ED) were influenced by sources and doses of N, while Fraction B and Lag Time were not influenced during period I. Fractions A and B and EDs were all influenced by N sources and doses during period II. Lag time and potential degradability were influenced by N sources only. Among the kinetics parameters of dry matter ruminal fermentation obtained during period I, only K1 was influenced by the interaction between N doses x sources, while the remaining parameters were only influenced by the N doses applied. As for period II, with the exception of K2, all remaining parameters were influenced by the interaction of N doses and sources. Protected urea improved fermentation parameters during period I. The dose equivalent to applying 150kg/ha positively influenced ruminal fermentation parameters of the Mulato II grass mainly in the summer. The use of protected urea as a Nitrogen source improved fermentation parameters in the summer.
Keywords: Brachiaria; digestibility; digestion kinetics; rumen fermentation; gas production

Resumo
É importante avaliar o valor nutricional de novas espécies forrageiras, parâmetro que pode ser melhor caracterizado por meio de técnicas de fermentação e degradação ruminal. Objetivou-se avaliar os efeitos da adubação nitrogenada (N) sobre a cinética de fermentação e degradação ruminal do capim Mulato II. Duas fontes de N (ureia comum e protegida) foram utilizadas em dois períodos do ano (verão - I e outono - II). O delineamento experimental utilizado foi inteiramente casualizado, em esquema fatorial 2x4 com três repetições. A fração A mudou com base na dosagem de N. A taxa de passagem (K) e a degradabilidade efetiva (DE) foram influenciadas pelas fontes e doses de N, enquanto a Fração B e *Lag Time* não foram influenciadas durante o período I. As frações A e B e DE foram influenciadas pelas fontes e doses de N durante o período II. *Lag time* e a degradabilidade potencial foram influenciadas apenas pelas fontes de N. Dentre os parâmetros cinéticos da fermentação ruminal da matéria seca obtidos no período I, apenas K1 foi influenciado pela interação entre doses de N x fontes, enquanto os demais parâmetros foram influenciados apenas pelas doses de N aplicadas. Já para o período II, com exceção do K2, todos os demais parâmetros foram influenciados pela interação das doses e fontes de N. A ureia protegida trouxe melhorias para os parâmetros fermentativos no período I. A dose equivalente à aplicação de 150kg/ha influenciou positivamente os parâmetros fermentativos ruminais do capim Mulato II, principalmente no verão. O uso de ureia protegida como fonte de nitrogênio melhorou os parâmetros de fermentação no verão.
Palavras-chave: Braquiária; cinética da digestão; digestibilidade; fermentação ruminal; produção de gás

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1. Introduction

Ruminant production systems in tropical conditions primarily rely on native or cultivated pastures. These pastures exhibit inherent seasonality in production because of water deficits and other climatic conditions that hinder the mass production of forage plants. Consequently, low productivity is common and can be worsened by improper management, lack of soil correction, and inadequate fertilization, leading to soil degradation and low pasture stocking rates[1].

Efficient soil use is a major challenge in pasture-based cattle raising. This involves improving productivity rates and implementing management strategies tailored to each production system, with the goal of increasing profitability. Thus, choosing a cultivar with desirable and profitable characteristics, including productivity, animal utilization, and grazing resistance, is a crucial factor for success[2].

The most cultivated forage species in tropical regions belong to the genus *Brachiaria*. This is primarily because of the genus' high adaptability to soil and climatic conditions, resistance to grazing, satisfactory productivity, and chemical characteristics[3]. To achieve higher productivity and nutritional quality, species from the genus *Brachiaria* are being used to obtain hybrids like the Mulato II grass. Consequently, it is essential to evaluate and characterize the nutritional quality and animal utilization of these lesser-studied hybrids.

Nutrient repositioning in the soil is another influential factor in forage production quality. This strategy, combined with proper management, is crucial for ensuring soil fertility and conservation while increasing forage production. Within this context, the dosage and source of fertilization will depend on factors such as soil quality and animal load on the pasture. This is essential for soil conservation and promoting better performance for both the forage species and ruminants[3–5].

To ensure optimal ruminant performance, it is crucial to understand the productivity and nutritional value of the forage, as well as ruminal degradation characteristics. This knowledge allows for greater precision in meeting the animal's nutritional requirements. Techniques like *in situ* degradability and *in vitro* gas production are particularly useful for this purpose[6,7].

The *in vitro* gas production technique assesses ruminant food based on gases produced during ruminal microbial fermentation. One of these gases is CO_2, which correlates strongly with the amount of fermented organic matter. This technique is a valuable tool for evaluating food utilization by ruminants[8].

The *in situ* ruminal degradability gravimetric technique, in conjunction with the *in vitro* gas production technique, offers valuable insights into the kinetics and rates of ruminal degradation for food fractions. This technique relies on monitoring food depletion during the ruminal fermentation process, providing valuable nutritional information[9,10].

This study aimed to evaluate the kinetics of ruminal fermentation for Mulato II grass under nitrogen fertilization using the *in vitro* gas production technique and the *in situ* degradability gravimetric technique. Both common and protected urea were examined as nitrogen sources.

2. Materials and methods

2.1 Experimental locality and handling

This study was approved by the Committee on Ethics in the Use of Animals (CEUA/PRPI) of the Federal University of Goiás (UFG) (approval number: 116/15).

The evaluation of *Brachiaria* Mulato II forage cultivar was conducted at the experimental area of the School of Veterinary and Animal Science of the Federal University of Goiás (UFG) (16° 36'S, 49° 16' W and 727 m above sea level) from December 2016 to June 2017. The regional climate classification is Aw, characterized by hot and semi-humid conditions with two distinct seasons: dry (May-October) and rainy (November-April)[11]. During the experimental period, the Meteorological Laboratory of UFG's School of Agronomy and Food Engineering recorded minimum and maximum temperatures of 13 °C and 32 °C, respectively. The soil in the experimental area is classified as a dystric Red-Yellow latosol[12]. Soil samples were collected at a depth of 0-20 cm for the physicochemical characterization. The analysis was conducted on subsamples taken before the establishment of the experimental plots, and the results are presented in Table 1.

A 2 × 4 factorial experimental design was setup, with the treatments comprising two sources (common urea and protected or slow-release urea) and four doses (0, 50, 100, and 150 kg/ha) of N, resulting in 24 experimental units distributed in triplicates. The urea protection used polymers and Kimcoat® technology, a proprietary method developed by Kimberlit, involving the coating of urea granules with layers of additives. These additives, present in the Kimcoat N, safeguard the nitrogen fertilizer (urea) against major losses during the fertilization process, such as NH_3 volatilization, nitrification, and denitrification, while promoting a higher presence of nitrogen in the form of ammonium in the soil[13].

The leveling cut was conducted on December 5, 2016, followed by the administration of treatments. A single nitrogen dose equivalent to 50 kg/ha was administered, whereas the remaining doses were split into

Table 1. Physicochemical characteristics of the soil in the experimental area

pH	S	P*	P**	K	K	Ca	Mg	H + Al	Al	CEC
$CaCl_2$		(mg/dm³)			(cmol/100 dm³)					
5.0	13	2	5	120	0.31	1.7	0.7	2.2	0.0	5.0
Clay	**Silt**	**Sand**	**Base sat.**		**Al sat.**	**OM**	**Ca/CEC**	**Mg/CEC**	**K/CEC**	
					%					
45	19	36	56		0	2.7	34	14	6	

*P-Mehlich 1. ** P-Resin. CEC: cation exchange capacity; Base sat.: base saturation; Al sat.: aluminum saturation

two applications: one on December 24, 2016, and the other on February 25, 2017. The remaining nitrogen was administered to achieve the doses of 100 and 150 kg/N.

For the evaluation of forage production cuts, a 0.15-m tall rectangular metal frame with an area of 0.5 m² was used. The frame was randomly positioned within the experimental unit, and all the material contained within the square was manually cut and immediately weighed. Approximately 500 g of the sample were collected and placed in a forced air circulation oven at 55 °C for 72 h to determine the pre-dry matter. Following the drying process, the samples were ground using a 1-mm sieve in a Wiley mill and subsequently subjected to laboratory analysis.

Analyses of insoluble neutral detergent (NDF) and acid detergent (ADF) fibers were conducted following the methods of Van Soest[14], whereas lignin analyses were performed following the methods recommended by the AOAC[15].

For the *in vitro* gas production and *in situ* degradability analyses, samples were prepared as follows: a pool was created for each unit by combining equal weights of the dry ground sample from each cut and period (summer and fall), resulting in a sample for each experimental unit. The analyzed sample for each treatment comprised a mixture of samples from each unit and treatment, with equal weights. The control sample was prepared by combining all samples from the six units that did not receive nitrogen fertilization.

2.2 In vitro gas production

The Ankom RF Gas Production System® equipment was used for the *in vitro* gas production analysis. Four replicates per treatment were set up to measure gas production in the dry matter. In each 310-mL glass bottle, 1.0 g of sample along with 10 mL of the inoculum and 80 mL of Kansas buffer solution were added[16].

The culture medium was prepared under continuous CO_2 flux and maintained in a water bath at 39 °C following the techniques of Theodorou et al.[17] and Mauricio et al.[18]. Ruminal inoculum was obtained from two crossbred cattle with an average weight of 480 kg and an average age of 60 months, both equipped with ruminal cannulae. The animals were fed a diet based on *Brachiaria brizantha* cv. Marandu. The inoculum was collected manually, combining equal volumes of the solid and liquid phases from the dorsal and ventral rumen sacs of both animals. The collected phases were stored in pre-heated thermic bottles at 39 °C and immediately transported to the laboratory. The inoculum was then filtered through two layers of cheesecloth fabric and maintained in a water bath at 39 °C.

Bottles were maintained at 39 °C in a water bath, and the cumulative pressure of each bottle was automatically measured every 10 min using the Ankom RF Gas Production System equipment. This measurement continued until the 48-h post-incubation period. The volume of gases generated was calculated using the ideal gas law to determine the quantity of moles produced. The Avogadro law was then applied to determine the total gas volume.

The bicompartmental logistic model[19], used to estimate microbial fermentation patterns, was determined based on the average gas production of each sample. The model is expressed as:

$$V = VF1\left[1 - exp^{(-K1(T-g)}\right] + VF2[1 - exp^{(-k2(T-g))}]$$

where V is the accumulated volume of gases produced during the period (T), $VF1$ denotes the volume of gas produced by the fermented soluble fraction, $K1$ represents the rate of gases produced by the fermented soluble fraction, g is the colonization time (lag time), $VF2$ refers to the volume of gas produced by the fermented insoluble fraction, and $K2$ indicates the rate of gases produced by the fermented insoluble fraction.

2.3 In situ degradability

To determine the *in situ* degradability of samples, 100 g/m² nonwoven fabric bags, measuring 20 × 5 cm, were used. The weight of empty bags was obtained by washing them in acetone and placing them in trays lined with ink-free paper in an oven at 55 °C for approximately 72 h. After 30 min in the oven, the weights of the bags were recorded using an analytical digital scale. Two grams of sample was then added, following the ratio of 20 mg dry matter/cm² surface[10]. Finally, the bags were sealed with an electric sealer.

To incubate the bags, two Holstein-zebu crossbred bovine animals weighing approximately 500 kg were used. Both animals had ruminal cannulae and were kept on Mombaça grass paddocks. They had *ad libitum* access

to water and were provided mineral supplementation in covered troughs.

Incubations were conducted using a polypropylene bag, allowing for contact between the nonwoven fabric bags and the rumen fluid. A weight was added to ensure that the bags remained in the rumen's ventral sac. Incubation times were 0, 12, 24, 48, 72, 96, 120, 144, and 240 h, following the descending order as described by Nocek[20]. At the end of the 240 h, all nonwoven fabric bags were simultaneously removed, promoting a uniform lavage of the material.

Once the bags were taken out of the rumen, they were immersed in ice-cold water to halt the degradation process. They were then washed in running water until the water ran clear. Finally, the bags were dried in a forced-ventilation oven at 55 °C for 72 h.

2.4 Statistical analyses

To evaluate *in situ* degradability, data were fitted to the ruminal disappearance model[10]. The results were tested for analysis of variance, and a comparison was made using the Tukey's test at a 5% significance level. For the *in vitro* gas production analysis, data were fitted to the two-compartment logistic model[19]. Equations were compared using the identity test of non-linear models to verify parameter uniformity between models[21]. The R statistical software[22] was used for this analysis at the 5% significance level.

3. Results

Nitrogen doses did not affect NDF, ADF, or lignin contents. In addition, the nitrogen source did not affect the fiber contents of the forage (Table 2).

Table 2. Chemical composition of forage fiber contents of Mulato II during two seasons

	Parameter	Control	Nitrogen dose					
			50		100		150	
			Com	Prot	Com	Prot	Com	Prot
Summer	NDF (%)	55.35	50.93	53.6	57.96	54.93	49.58	50.39
	ADF (%)	28.03	26.96	27.06	30.18	28.29	28.42	25.67
	Lignin (%)	6.95	6.21	5.74	5.79	6.04	7.35	5.56
Fall	NDF (%)	57.67	58.85	57.09	60.03	58.12	56.66	55.46
	ADF (%)	29.69	31.43	30.65	30.05	30.02	28.76	29.00
	Lignin (%)	7.85	8.05	7.93	9.57	7.88	7.78	8.68

Com, common urea; Prot, protected urea. The values showed no statistical difference ($p > 0.05$).

Table 3 presents the ruminal degradability parameters of Mulato II grass dry matter during both seasons: period I and period II (summer and fall, respectively). Fraction A varied significantly ($p < 0.05$) depending on N doses, while K (%/h), ED (K = 2%), and ED (K = 5%) were influenced by N sources and doses. The highest K (%/h) value was observed with an N fertilization dose of 150 kg/ha compared with that of the control and for the source of common urea. However, fraction B and lag time were not affected by N sources or doses during the summer season.

In the fall season, no differences were observed in the soluble fraction for the protected urea source compared with that of the control. However, for the common urea source, N doses of 100 kg/ha and 150 kg/ha showed a smaller quantity of soluble material compared with those of the control and the protected urea source (Table 3). A higher percentage of the potentially degradable fraction was found during this period for the N dose of 150 kg/ha with common urea compared with the N dose of 50 kg/ha and the N dose of 150 kg/ha with the protected urea source.

Similar to that of the summer season, the degradation rate of the potentially degradable fraction was higher with the N fertilization dose of 150 kg/ha in the fall for the common urea source compared with that of the control. No effects were observed regarding N doses for the protected urea source in both seasons.

Nitrogen fertilization at a N dose of 150 kg/ha increased the effective degradability at a passage rate of 2% (ED2%) for both sources of N compared with that of control in the summer. However, this effect was not observed in the fall. ED2% is considered a low ruminal passage rate[23] for forages and indicates the material degradation during a 48-h stay in the rumen. As for ED (k = 5%), it was not influenced by N sources, but a significant difference was found for the N dose of 100 kg/ha, which had the lowest value observed for the protected urea source.

Regarding lag time, a superiority was observed when using the protected urea N dose of 150 kg/ha compared with the common urea (Table 3). A positive effect on potential degradability was observed with doses equivalent to 50 kg/ha and 100 kg/ha of N when using protected urea compared with the same doses of common urea. However, no difference was observed compared with that of control.

Figure 1 illustrates the effect of ruminal disappearance of dry matter in the analyzed treatments, highlighting the slower degradation in the control treatment compared with the application of 150 kg/ha of nitrogen using both sources. The degradation pattern in treatments showed little change in the fall (Figure 2).

Table 4 shows parameters of dry matter ruminal fermentation kinetics obtained by *in vitro* gas production. The dose equivalent to applying 50 kg/ha of nitrogen in the summer with protected urea as a source increased the final volume of gas produced by the soluble fraction fermentation. In the fall, the same effect was observed for higher N doses. The highest volumes of soluble fraction

Table 3. Ruminal degradability of Mulato II grass dry matter evaluated under nitrogen fertilization and two sources of N (common and protected urea) during two seasons

Summer							
Parameter	Source of urea	Control	Nitrogen dose (kg/ha)			MSE	CV (%)
			50	100	150		
Fraction A	Common	35.63^{b*}	38.18^{a}	37.25^{ab}	38.04^{ab}	0.63	3.38
	Protected	35.63^{b}	38.79^{a}	38.22^{a}	38.78^{a}		
Fraction B	Common	49.67^{a}	46.73^{a}	48.11^{a}	48.88^{a}	0.95	3.92
	Protected	49.67^{a}	47.16^{a}	46.91^{a}	49.42^{a}		
K (%/h)	Common	3.00^{b}	4.60^{Aa}	1.90^{c}	4.00^{a}	0.003	16.74
	Protected	3.00^{a}	2.50^{Ba}	2.50^{a}	3.50^{a}		
ED (k = 2%)	Common	65.22^{b}	70.27^{Aa}	60.78^{Bc}	70.63^{a}	0.54	1.62
	Protected	65.22^{b}	64.88 ^{Bb}	64.25^{Ab}	70.15^{a}		
ED (k = 5%)	Common	54.05^{b}	60.18^{Aa}	50.58^{Bc}	59.78^{a}	0.54	1.94
	Protected	54.05^{b}	54.44^{Bb}	53.85^{Ab}	59.05^{a}		
Lag time	Common	1.45^{a}	1.45^{a}	0.84^{a}	0.55^{a}	0.35	66.34
	Protected	1.45^{a}	0.47^{a}	1.10^{a}	1.20^{a}		
DP	Common	85.30^{a}	84.91^{a}	85.36^{a}	86.92^{a}	0.71	1.65
	Protected	85.30^{b}	85.95^{b}	85.13^{b}	88.20^{a}		
Fall							
Parameter	Source of urea	Control	Nitrogen dose (kg/ha)			MSE	CV (%)
			50	100	150		
Fraction A	Common	37.22^{a}	36.24^{ab}	33.93^{Bb}	34.42^{Bb}	0.70	3.88
	Protected	37.22^{a}	37.00^{a}	36.77^{Aa}	36.63^{Aa}		
Fraction B	Common	48.11^{ab}	46.97^{b}	48.94^{ab}	50.19^{Aa}	0.54	2.24
	Protected	48.11^{a}	48.33^{a}	48.33^{a}	46.52^{Ba}		
K (%/h)	Common	2.60^{b}	2.90^{ab}	3.20^{ab}	3.50^{a}	0.002	11.56
	Protected	2.60^{a}	2.70^{a}	3.10^{a}	3.20^{a}		
ED (k = 2%)	Common	64.49^{ab}	63.96^{b}	63.90^{Bb}	66.18^{a}	0.52	1.59
	Protected	64.49^{a}	64.71^{a}	66.12^{Aa}	64.92^{a}		
ED (k = 5%)	Common	53.78^{a}	53.41^{a}	52.89^{Ba}	54.91^{a}	0.53	1.95
	Protected	53.78^{a}	53.90^{a}	55.28^{Aa}	54.53^{a}		
Lag time	Common	0.70^{a}	0.85^{a}	1.27^{a}	0.99^{Aa}	0.19	43.69
	Protected	0.70^{a}	1.02^{a}	0.94^{a}	0.42^{Ba}		
DP	Common	85.33^{a}	83.22^{Ba}	82.86^{Ba}	84.62^{a}	0.67	1.59
	Protected	85.33^{a}	85.34^{Aa}	85.09^{Aa}	83.15^{a}		

Fraction A, Soluble fraction; Fraction B, Potentially degradable fraction; K, Degradation rate of fraction B; ED (k = 2%), Effective degradability at a 2% passage rate; ED (k = 5%), Effective degradability at a 5% passage rate; Lag time, Colonization time; DP, Potential degradability. MSE, Mean standard error; CV, Coefficient of variation.

* indicates that values with the same lowercase letter in the same row and uppercase in the column do not differ from each other ($p > 0.05$).

fermentation were obtained with N doses of 100 kg/ha and 150 kg/ha using protected urea as a source, and with an N dose of 100 kg/ha using common urea as the N source. Along with the gas volume produced, the soluble fraction degradation rate was higher with the N dose of 150 kg/ha using protected urea as a source in the fall.

Nitrogen fertilization with common urea as a source favored the colonization of matter by ruminal bacteria in both summer and fall. This colonization was faster with the highest N dose (150 kg/ha) and only in the summer for the protected urea source. The N dose of 150 kg/ha from the protected urea source promoted a smaller gas volume from the potentially degradable fraction in both seasons.

4. Discussion

The absence of significant influence due to N rates in NDF and ADF is associated with the evaluation period, particularly during the growing season (Table 2). However, higher growth rates can lead to stem accumulation and increased NDF. The adoption of the 95% LI herbage management criterion reduces stem elongation due to grazing. This pattern may explain the results obtained for the fibrous portions.

Neutral detergent fiber contents influence ruminal disappearance kinetics and are inversely related to the involuntary ingestion of food, similar to ADF and lignin contents, which are inversely proportional to the food's potential digestibility[24,25]. As plants grow, cell wall development produces cellulose and hemicellulose, providing firmness to the plant's structure and supporting it. Lignin is deposited later during development, acting as a cementing agent for the cell wall[26]. The NDF and ADF values obtained in this experiment (Table 2) are similar to those found by Delavatti et al.[27] for *Brachiaria* forage, showing lower lignin values compared with the current experiment.

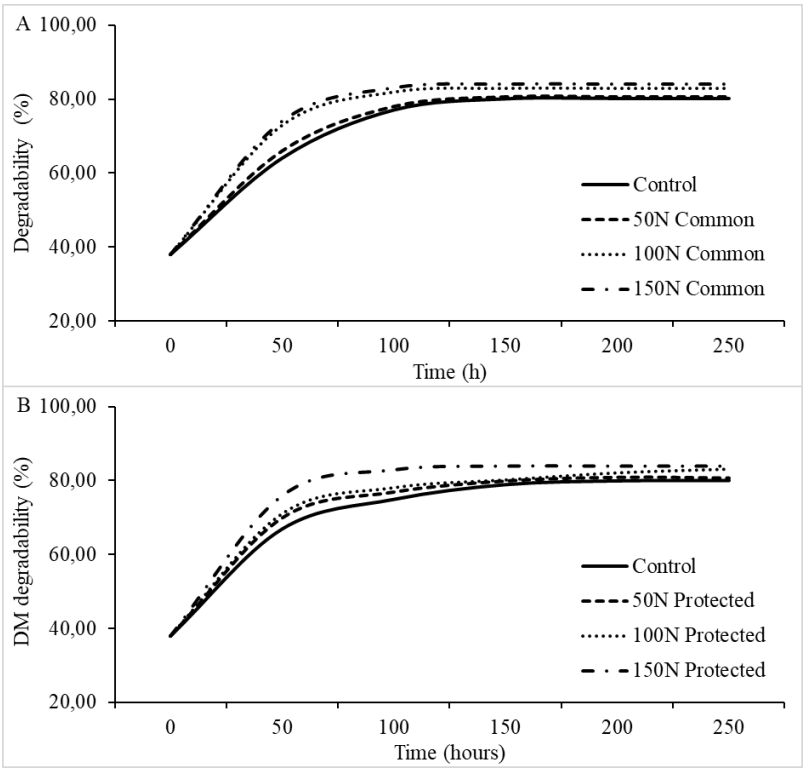

Figure 1. Ruminal degradability curves of the dry matter (DM) of Mulato II grass evaluated in the summer under nitrogen (N) fertilization and two N sources, common urea (A) and protected urea (B), using the *in situ* degradability technique

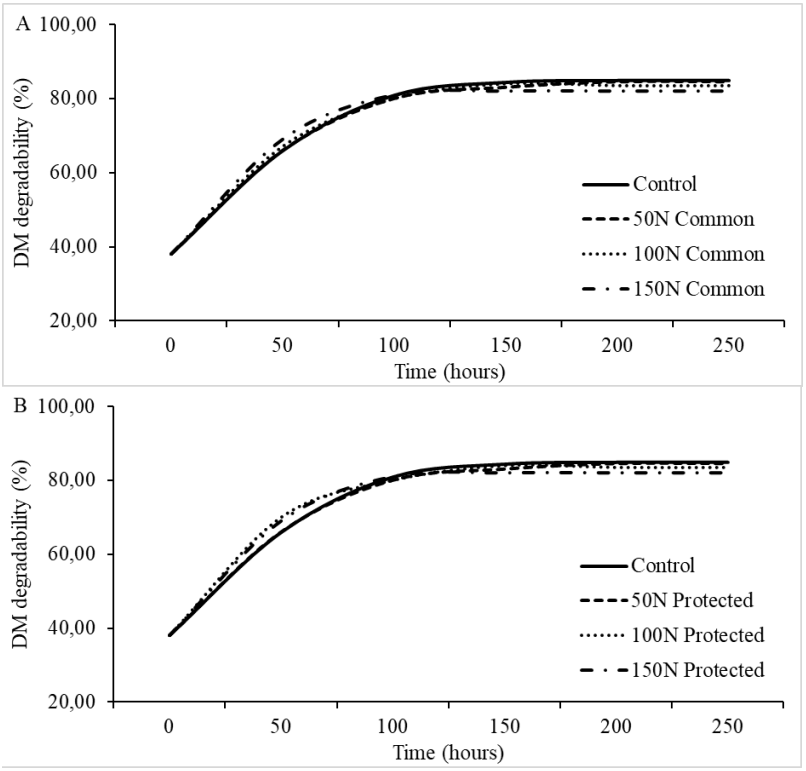

Figure 2. Ruminal degradability curves of dry matter (DM) of Mullato II grass analyzed in the fall under nitrogen (N) fertilization and two N sources, common urea (A) and protected urea (B), using the *in situ* degradability technique

Table 4. *In vitro* gas production of dry matter of Mulato II grass evaluated under nitrogen fertilization and two sources of N (common and protected urea) during two seasons

Summer					
Parameter	Source of urea	Control	Nitrogen dose (kg/ha)		
			50	100	150
VF1 (mL/g DM)	Common	7.86^{a*}	7.66^{a}	8.29^{a}	7.97^{a}
	Protected	7.86^{b}	8.96^{a}	7.80^{b}	7.89^{b}
K1 (%/h)	Common	3.90^{a}	4.40^{a}	4.00^{Aa}	3.90^{Ba}
	Protected	3.90^{b}	4.10^{b}	2.90^{Bc}	4.90^{Aa}
Lag time (h)	Common	9.51^{a}	7.57^{b}	7.65^{b}	7.58^{Ab}
	Protected	9.51^{a}	8.14^{b}	8.20^{b}	6.83^{Bc}
VF2 (mL/g DM)	Common	4.21^{a}	3.31^{a}	3.96^{a}	4.77^{a}
	Protected	4.21^{ab}	3.13^{b}	4.55^{a}	3.21^{b}
K2 (%/h)	Common	9.00^{a}	12.60^{a}	11.10^{a}	10.60^{a}
	Protected	9.00^{b}	14.80^{a}	9.70^{b}	17.60^{a}
Fall					
Parameter	Source of urea	Control	Nitrogen dose (kg/ha)		
			50	100	150
VF1 (mL/g DM)	Common	7.70^{b*}	6.16^{b}	9.63^{a}	7.18^{Bb}
	Protected	7.70^{b}	7.64^{b}	8.43^{ab}	9.50^{Aa}
K1 (%/h)	Common	3.60^{a}	3.80^{a}	4.10^{a}	3.80^{Ba}
	Protected	3.60^{b}	4.40^{a}	4.10^{ab}	4.70^{Aa}
Lag time (h)	Common	7.73^{b}	8.53^{a}	7.94^{b}	6.73^{Bc}
	Protected	7.73^{a}	7.60^{a}	8.29^{a}	8.08^{Aa}
VF2 (mL/g DM)	Common	3.85^{a}	4.63^{a}	1.83^{b}	3.63^{Aa}
	Protected	3.85^{a}	3.22^{a}	2.58^{ab}	1.48^{Bb}
K2 (%/h)	Common	9.70^{a}	10.6^{a}	12.00^{a}	12.30^{a}
	Protected	9.70^{b}	12.9^{b}	14.40^{ab}	24.10^{a}

VF1, Volume of gas produced by soluble fraction; K1, soluble fraction degradation rate; Lag time, Colonization time; VF2, Volume of gas produced by potentially degradable fraction; K2, potentially degradable fraction rate. * indicates that values with the same lowercase letter in the same row and uppercase in the column do not significantly differ from each other ($p > 0.05$) by the Model Identity test.

An increase in the readily soluble fraction of the summer material was observed with the fertilization of 50 kg/ha of N using protected urea compared with that of the control (Table 3). This fraction contains cell contents, soluble minerals, and carbohydrates in the cell wall. This observation is linked to the plant's growth pattern, as fertilized plants have higher amounts of soluble material due to increased cell activity related to the growth process[27]. This finding aligns with the results reported by Leite et al.[28], who demonstrated lower values of the soluble fraction in growing forage plants with a greater proportion of green leaves of *Brachiaria brizantha* cv. Marandu.

However, Araújo et al.[29] reported lower values in a study on the composition of leaves and stems using *Brachiaria brizantha* cv. Marandu in a monoculture without nitrogen fertilization. The lower content of soluble material with common urea doses can be attributed to the plant's growth, as it affects the light intensity captured by the plant and subsequently reduces its growth, resulting in a lower proportion of cell content compared with cell wall content.

The similarity in the potentially degradable fraction between different treatments during the summer (Table 3) can be attributed to the availability of soil water and light, which enable the plant's vegetative growth and the development of a less-lignified cell wall. This facilitates degradation by ruminal microorganisms[28,30,31].

Similar degradation rates were reported by Tosta et al.[32] in a study on *Brachiaria brizantha* cv. Marandu grown in an intercrop with the Babaçu palm at different cultivation densities. The build-up of cell walls with higher potential for ruminal degradation is more significant during the plant's vegetative growth before inflorescence formation compared with that during reproductive growth. This contributes to improved forage digestibility during the period of maximum vegetative growth, resulting in increased ruminal emptying and a greater nutrient availability for ruminants. Consequently, better animal performance can be achieved during this period[33,34].

The soluble fraction, which increased in ruminal degradability compared with the control (Table 4), is also more fermentable and readily available in the rumen. While the *in vitro* gas production technique provides an accurate evaluation of fermentable fractions, it complements gravimetric techniques for estimating ruminal degradation[6,35].

Similar values of soluble fraction gas production were reported by Garcez et al.[34] for *Panicum* grasses. The higher N dose resulted in greater fermentation of the soluble fraction, which occurs at a faster rate and delivers nutrients from the forage's dry matter in a shorter time period. This promotes bacterial growth and the production of short-chain fatty acids, providing animals with a greater energy supply within a shorter timeframe[33,36].

In a study on *Cynodon* forages subjected to nitrogen fertilization, Assis et al.[37] also found no effect on ruminal colonization time. Garcez et al.[34], however, reported lower colonization times for *Panicum* grasses, possibly due to harvesting at different ages. Colonization time is influenced by the ability of rumen microorganisms to attach to particles, which increases when there is a higher proportion of lignified cell wall in the forage, making it more difficult for microorganisms to adhere to[34]. Therefore, the observed low lignin levels may explain the shorter colonization time.

The smaller gas volume obtained from the potentially degradable fraction is likely due to its slower degradation compared to the soluble fraction. de Sá et al.[38] reported higher values in a study on different harvest ages of *Brachiaria brizantha* cv. Marandu, using different evaluation techniques, determination equations, and longer evaluation times than the present experiment. Discrepancies between studies can be attributed to the use of different techniques and equations. In addition, these results may also be associated with variations in NDF and cellulose contents in the evaluated forage species, which influence the portion that is potentially degradable by animals.

5. Conclusions

Applying a dose equivalent to 150 kg/ha positively influenced ruminal fermentation parameters of Mulato II grass, particularly during summer. The use of protected urea as a nitrogen source improved fermentation parameters in the summer. Fertilizing with protected urea at a dose of 150 kg/ha is recommended to enhance dry matter utilization of the forage by ruminants.

Declaration of conflict of interest

No conflicts of interest were declared by the authors.

Author contributions

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