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Intake, digestibility and rumen fermentation in Nellore bulls grazing degraded or recovered pastures of *Urochloa* ssp. or the grass intercropped with pigeon pea

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ABSTRACT

With global population growth, livestock production must be intensified with reduced environmental impact. The benefits of legume-grass intercropping are well-documented; however, no studies have evaluated ruminal fermentation and digestive kinetics. Pigeon pea, a legume intercropped with tropical grasses, can improve the nutritional value of forage, increasing digestive efficiency and mitigating methane emissions, while also reducing the need for nitrogen fertilization. Over two years, nine rumen-cannulated Nellore bulls were allocated to nine experimental paddocks (1.25 ha each; total area of 11.25 ha) in a randomized complete block design, with year as the blocking factor. Treatments followed a 3 × 2 factorial arrangement, consisting of three forage systems and two seasons (dry and rainy), with three paddock replicates per treatment: DEG, a degraded pasture; REC, recovered pasture fertilized with 200 kg of N; and MIX, mixture of legume-grass pasture. External and internal markers were used to determine dry matter intake (DMI). Grass and legume intake proportions were estimated using C3 and C4 carbon isotopes. The concentration and production of short-chain fatty acids, methane, and ammonia compounds were calculated using the *ex-situ* fermentation technique. Animals in DEG pasture

Abbreviations: δ13C, stable carbon isotope; A:P, acetate to propionate ruminal ratio; ABW, average body weight; ADF, acid detergent fibre; AICC, Akaike information criterion; BW, body weight; CEUA, Committee for the Use and Care of Animals; CP, crude protein; CT, condensed tannin; DEG, degraded pasture; DM, dry matter; DMD, dry matter digestibility; DMI, dry matter intake; DR, disappearance rate; EE, ethereal extract; ED2, effective degradability for ruminal passage rates of 2 %; ED5, effective degradability for ruminal passage rates of 5 %; ED8, effective degradability for ruminal passage rates of 8 %; GE, gross energy; IND, non-degraded portion; Kt, disappearance rate.; LIG, lignin; NDF, neutral detergent fibre; NDFi, indigestible neutral detergent fiber; NH_3-N, ammonia nitrogen; NPN, non-protein nitrogen; MIX, mixture of legume-grass pasture; PD, potential degradability; REC, recovered pasture; REL, relative energy loss for CH_4 production; SCFA, short-chain fatty acids; SEM, Standard error of the mean.

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presented a higher DMI of the supplement and higher relative energy loss as methane production, while a higher level of DM digestibility was observed in the MIX pasture. Higher ruminal concentrations of NH_3-N were obtained for animals in the REC pasture. The lowest ruminal DM and NDF degradation rates were observed in the MIX pasture.

1. Introduction

The *World Population Prospects* (United Nations (UN),2024) projects that the global population will peak at 10.3 billion people in the 2080 s. Natural resources, especially those exploited without any concern for mitigating environmental impacts, will likely make food production unsustainable and have the potential to cause irreversible damage to the environment. Consequently, the depletion of natural resources has become a major concern. The solution to this challenge is the intensification of agricultural production systems with strategies that do not compromise the environment (Monteiro et al., 2024).

Brazilian livestock production is based on grazing systems with tropical grasses (C4), and improving the seasonal production of forage and nutritional quality is essential. Adopting more sustainable practices, such as intercropping legumes with grasses, could improve these characteristics (Camero et al., 2001; Boddey et al., 2020; Singh et al., 2023).

This alternative has become an extensively studied research topic (Lascano and Thomas, 1988; Menezes et al., 2015; Boddey et al., 2020). Given this, the pigeon pea emerges as a potential species to be evaluated (Tulu et al., 2021) mainly due to its resilience maintaining stable growth in dry seasons and during conditions of water deficit. Additionally, the crude protein (CP) and condensed tannin (CT) contents of pigeon pea may enhance N supplementation efficiency in grazing systems, especially during the dry seasons when the seasonal extremes impact tropical pastures significantly (Corriher et al., 2007; Tenakwa et al., 2022).

A previous study with pigeon pea intercropped with tropical pastures, particularly *Urochloa* spp., showed the potential for reducing the intensity of enteric CH_4 emissions; furthermore, the system eliminated the use of nitrogen fertilization, which is the main emitter of nitrous oxide in agricultural production systems (Furtado et al., 2023). Mitigating CH_4 emissions is particularly important, as it has a global warming potential 27.2 times greater than that of CO_2 (IPCC, 2022), and in ruminants its production can represent an energy loss of up to 12 % from feed consumption (Johnson and Johnson, 1995; Perna Junior et al., 2022). Although the beneficial effects of legume–grass intercropping have been widely documented, there are no studies specifically evaluating the dynamics of ruminal fermentation and digestive kinetics in Nellore bulls grazing on pigeon pea intercropped with *Urochloa* spp. Little is known about how this intercropping system influences ruminal parameters such as pH, ammonia-N concentration, and the *in vivo* rates of passage and degradation. Understanding these aspects is essential to elucidate the mechanisms behind potential reductions in enteric CH_4 emissions and improvements in nutrient utilization efficiency.

The objective of this study was to evaluate the intake, *in situ* digestibility, pH, and rumen fermentation of Nellore bulls grazing

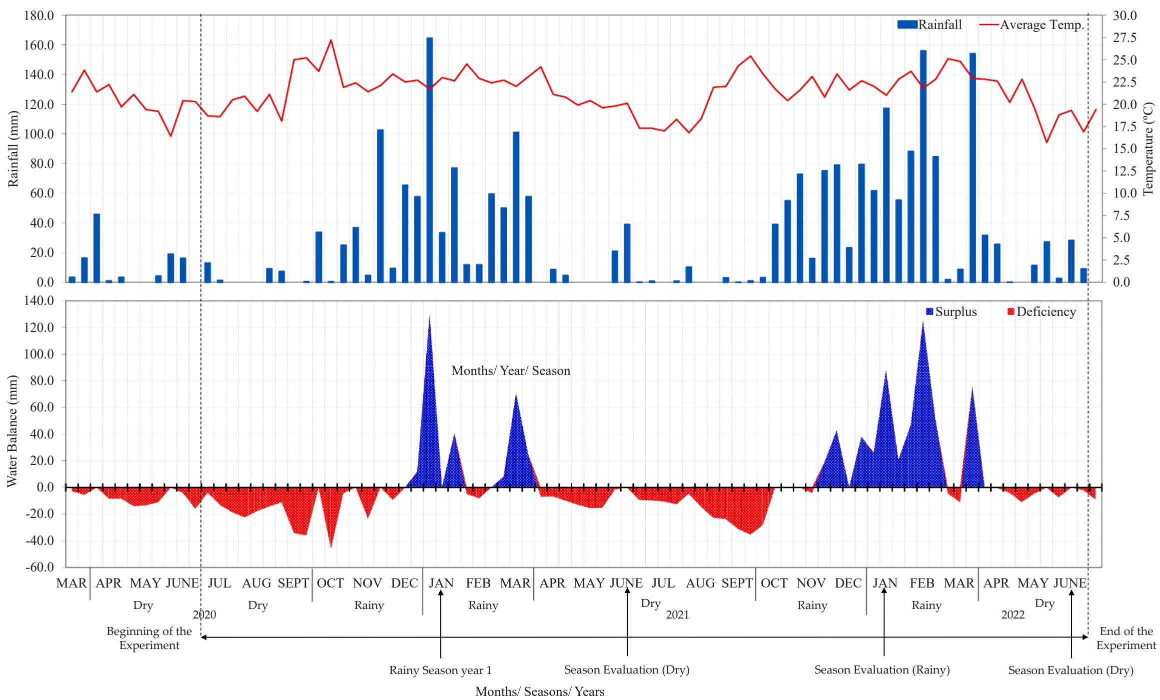

Fig. 1. Climatic conditions and water balance during the experimental period.

pigeon pea intercropped with *Urochloa* spp. pasture. These parameters were compared with those from bulls grazing on a degraded grass-only pasture and a recovered pasture fertilized with 200 kg N-urea ha^{-1} $year^{-1}$. Our hypothesis is that intercropping pigeon pea with *Urochloa* spp. enhances the nutritional value of the diet, thereby improving rumen fermentation, increasing animal efficiency, and reducing enteric CH_4 emissions.

2. Materials and methods

This experiment was approved by the Committee for the Use and Care of Animals (CEUA) Embrapa Livestock Southeast (03/2020 no. 20.19.00.047.00.00) and University of São Paulo (nº. 6228200521).

2.1. Location, treatments, experimental design, and climate conditions

The experiment was realized at Embrapa Pecuaria Sudeste, São Carlos, São Paulo, Brazil in the years 2021 and 2022 during the dry (April-September) and rainy (October-March) seasons. To better represent the distinctions of the seasons, sampling was carried out in January (rainy season) and June (dry season), as shown in Fig. 1. Nine rumen-cannulated (diameter of 11.4 cm) Nellore bulls with an initial body weight of 445 kg (SEM: 44.3 kg) and approximately 18 months (SEM: 1.1 month) were handled for two years, resulting in a total of 18 experimental units across the study. The number of experimental animals used in this study is considered sufficient by the scientific community for metabolism studies involving rumen-cannulated animals. All procedures followed the animal welfare guidelines established by the Ethics Committee on Animal Use of Embrapa Livestock Southeast and the University of São Paulo. Additional animals (regulators) animals were introduced in the pastures to maintain a forage height of 15–30 cm. The animals were distributed into nine paddocks of 1.25 ha each, totaling 11.25 ha, in a randomized complete block design with a 3 × 2 factorial arrangement of treatments (three treatments and two seasons), with year as a blocking factor and three paddock replicates per treatment: *i)* The DEG pasture was degraded pasture of *Urochloa decumbens* Stapf cv. Basilisk and *Urochloa brizantha* cv. Marandu, *ii)* The REC pasture was recovered pasture receiving 200 kg N-urea ha^{-1} $year^{-1}$ with the same grass species as the DEG pasture, and *iii)* The MIX pasture was intercropped pasture composed of the same grasses as the DEG and REC pastures but intercropped pigeon pea legume (*Cajanus cajan* cv. BRS Mandarin). Each animal remained in the same treatment for two years.

The animals were managed under continuous grazing with stocking rate adjustments made through the "put-and-take technique" (Mott and Lucas, 1952), where regulator animals were added or removed as needed. This method provides an indirect assessment of forage mass availability and ensures an intermediate pasture height of 15–30 cm in the REC and MIX treatment pastures (Costa and de Queiroz, 2017). In contrast, the DEG pasture treatment was not subjected to minimum height management because of the low vigor of *Urochloa* spp. due to the lack of nutrients and the large presence of invasive plants, such as grasses of the genus *Paspalum* that are less than 15 cm tall (Oliveira et al., 2007). Detailed climate information is provided in Fig. 1.

The *Urochloa* spp. pastures (DEG, REC and MIX) were established in 1996. The MIX pasture treatment was implemented in 2011 by planting *Cajanus cajan* (for planting, the area with grass was grazed to a height of 15 cm and planting was carried out in rows with a tractor-driven planter). In the present experiment, an initial stand of 180,000 plants per hectare was estimated, which was reduced to approximately 75,000 plants per hectare by the end of the study. Replanting was done every three years due to the decline in plant population over the years from animal trampling and grazing, adverse weather conditions, plant senescence and leaf-cutting ant attack. During the dry season, leaf-cutting ants of the genus *Atta* cause intense defoliation of pigeon pea plants, leading to the death of plants already weakened by drought and grazing (Furtado et al., 2023).

We classified the DEG pasture as a degraded pasture using the criterion proposed by Oliveira (2007), in which pastures can be

Table 1
Formulation and composition of mineral supplements.

	Supplements	
Ingredients (g/kg, as fed)	Mineral[d]	Mineral-Energetic-Protein[c]
	Rainy Season	Dry Season
Ground corn	-	480
NaCl	500	150
Mineral mixture[a]	500	150
Urea[b]	-	220
	Estimated Chemical Composition (g/kg, Dry-matter basis)	
CP	-	654.4
NPN	-	99.0
NDF	-	40.2
Ash	886.9	283.5

[a] Minerthal® quantity per kg of product: 200 g of calcium, 160 g of phosphorus, 60 g of sulfur, 185 g of sodium, 200 mg of cobalt, 2.5 g of copper, 125 mg of iodine, 2.25 g of manganese, 50 mg of selenium, 7.5 g of zinc,

[b] Urea Heringer® (45 % N),

[c] Before receiving this supplement in dry animals received supplement adaptation provided for 14 days at the beginning of the dry season for adaptation of the rumen microbiota,

[d] Mineral formulation for MIX throughout the year and in the rainy season for REC and DEG, CP: crude protein, NPN: non-protein nitrogen, NDF: neutral detergent fiber, Ash: mineral matter.

considered degraded when there are areas larger than 2 m^2 in the canopy that are covered by invasive plants, in this case, *Paspalum notatum* Flüggé. The DEG pasture systems were not corrected and fertilized to represent extensive Brazilian farms.

The nitrogen fertilization was applied only for the REC pasture system (200 kg of N-urea per ha, divided into five applications during the rainy season). REC and MIX pastures were corrected and fertilized with superphosphate and potassium and managed to maintain a specific intermediate height for each grass species, as recommended by Costa and de Queiroz (2017).

The mineral supplement was provided *ad libitum* throughout the experimental period. During the rainy season, all animals received mineral mixture in powder. In the dry season, MIX pasture animals continued to receive mineral mixture, while REC and DEG pasture animals were supplemented with Mineral-Energetic-Protein. Table 1 shows the composition, nutritional quality and season of supply of each type of supplement.

2.2. Forage sampling, dry matter intake measurements and ruminal degradability

Forage sampling was performed after observing the animals grazing, a method developed by Sollenberger and Cherney (1995). This technique consisted of sampling parts of the plants (separating the grass from the legume into different paper bags), based on the observation of the ingestive behavior of the animals. The forages were collected by hand plucking (ca. 150 g of fresh matter) over three consecutive days, observing the animals for 25 min, and using scissors to cut the portion of forages the animals were consuming. Samples were placed in paper bags (18 cm × 44 cm), weighed, and dried in a forced ventilation oven at 65 °C for 72 h. For pigeon pea samples, drying was performed at 40 °C until a constant weight was achieved to preserve the integrity of the tannin analysis. After drying, the samples were milled using a Willey-type mill and analyzed chemically at the Animal Nutrition Laboratory of Embrapa Southeast Livestock.

The samples were analyzed based on the following methodologies: Fiber fractions were analyzed as follows: neutral detergent fiber (NDF) was determined without the use of heat-stable amylase and expressed inclusive of residual ash, according to Mertens (2002); acid detergent fiber (ADF) was also expressed inclusive of residual ash, following AOAC (2005), Method 973.18; and Lignin was determined by solubilization of cellulose with sulfuric acid, as described by Robertson and Van Soest (1981). Dry matter (DM), ash (mineral matter), crude protein (CP), and ether extract (EE) were assessed following the AOAC (1990) methods: 934.01 for DM, 923.03 for ash, 920.87 for CP, and 920.85 for EE. Gross energy (GE) content was determined with an adiabatic bomb calorimeter (IKA WERKE®, model C 5000, Staufen, Germany). Condensed tannin (CT) levels were quantified using the methodology described by Makkar (2003).

The sum of the forage and mineral supplement consumed by the animals was used to estimate the total DMI of the animals (Furtado et al., 2023). Supplement consumption was calculated based on the difference between the amount supplied and the amount consumed after five days. On day zero, the supplement was weighed using a digital scale (1–10000 g) and provided in a covered plastic trough. On the fifth day, the supplement was weighed again. The following equation was used to obtain supplement consumption (Furtado et al., 2023):

$$DMI_s = \frac{\left[\frac{(DMI_{sSupplied} - DMI_{sLeftovers})}{5_{(days)}}\right]}{\text{Total Weight}}$$

Where: DMI_s = Mineral supplement intake (kg/kg of BW.day); $DMI_{sSupplied}$ = Total supplement provided (kg); $DMI_{sLeftovers}$ = mineral supplement leftovers; Total Weight = total weight of animals with access to that trough (kg).

Forage DMI (DMI_f) was calculated using titanium dioxide (TiO_2) as an external marker (Titgemeyer et al., 2001), and indigestible neutral detergent fibre (iNDF; Mertens, 2002) as an internal marker incubated in the rumen for 240 h. The TiO_2 was weighed in small 15 g capsules and supplied via ruminal cannula daily for 10 days. At 6:00 a.m. on the fifth day, fecal samples were collected after spontaneous defecation in the paddocks; this sampling continued until the tenth day. As soon as feces sampling was performed, the samples were frozen in plastic bags and later thawed, homogenized, and dried at 65 °C for 72 h.

To calculate fecal output, the marker concentration in feces was corrected considering a recovery rate of 93 % of TiO_2, as recommended by Titgemeyer et al. (2001). Fecal output was then estimated using the following equation:

$$\text{Fecal Output} = \frac{\text{Dosed}TiO_2}{\text{Concentration}TiO_2}$$

Where: Fecal output: Total amount of feces excreted (kg DM/day); Dosed TiO_2: Amount of TiO_2 fed to the animal (g/day); Concentration TiO_2: Concentration of TiO_2 in feces (g/kg fecal DM);

To calculate DMI_f, the marker concentration in feces was corrected considering a recovery rate of 87.5 % of iNDF, as recommended by Peters et al. (2025). The DMI_f was then estimated using the following equation:

was calculated based on the following equation:

$$DMI_f = \frac{\left[iNDF_{(feces)} \times \text{fecal output}\right]}{iNDF_{(forages)}}$$

Where: DMI_f = Forage dry matter intake (kg DM/day); Fecal Output = Total amount of feces excreted (kg DM/day); $iNDF_{(feces)}$ = feces content of indigestible neutral detergent fiber (%); $iNDF_{(forages)}$ = forages content of indigestible neutral detergent fiber (%).

The dry matter digestibility (DMD) was calculated through an indirect method, using the following equation:

$$DMD = 100 - \left(100 \times \left(\frac{\text{fecal output}}{DMI_{Total}}\right)\right)$$

Where: DMD = Dry matter digestibility (%); DMI_{total} = Total dry matter intake (kg); and fecal output = Total amount of feces excreted (kg DM/day).

The C isotopic composition of the feces samples was analyzed based on previously established methods by Norman et al. (2009) and Ovani et al. (2022). Using the isotopic distinction between C3 plants (e.g., pigeon pea or other legumes) and C4 plants (e.g., tropical grasses), the intake proportion of each forage was estimated. This calculation followed the equation:

$$C4 \quad (\%) = 100 - \left(100 \times \left(\frac{(A - B - D)}{(C - B)}\right)\right)$$

Where: A = δ^{13}C value in feces; B = δ^{13}C value of the C_4 plant; C = δ^{13}C value of the C_3 plant; D = Fraction factor for δ^{13}C in feces.

The *in situ* ruminal degradability technique was used to estimate the rumen digestion rate (Mehrez and Ørskov, 1977; Ørskov et al., 1980; Nocek and Russell, 1988). Forage samples were placed in nylon bags of 50 μm porosity and then incubated in rumen via the cannula. The DEG and REC bags contained only *Urochloa* spp., while the MIX bags contained a mixture of *Urochloa* spp. and pigeon peas. The components in the MIX bags were weighed according to the proportion of grasses and legumes consumed by the animals, calculated based on isotopic analysis of the feces. The samplings were incubated for 0, 8, 16, 24, 48, 72 and 96 h with the bags placed at different times but to be removed all at the same time, thus promoting uniform washing of the material. To obtain solubility at time 0, bags with samples not incubated in the rumen were washed with distilled water at 39°C for five minutes. The degradation of DM was obtained by the difference between initial and final weight of incubation. The degradation of NDF and CP was obtained by the difference according to determination of concentration in forage before and after incubation. The results obtained after processing the samples were subjected to non-linear regression adjustments of the degradation curves according to Ørskov and McDonald (1979). The disappearance of DM from polyester bags on time was fitted to the following first-order kinetic equation:

$$y = a + b\ (1 - e^{-ct)}$$

Where: y, represents degradation at a given incubation time (t); a, is the soluble fraction that is immediately degradable in the rumen; b, is the non-soluble but potentially degradable fraction; c, is the fractional rate of degradation of fraction (b); potential degradability is represented by the sum of a and b.

2.3. Ruminal fermentative parameters

The *ex-situ* ruminal fermentation technique, originally developed by Rodrigues et al. (2012) and Perna Junior et al. (2017), was used to determine the production of short-chain fatty acids (SCFA), ammonia nitrogen (NH_3-N), and CH_4 at different time points. Briefly, this method involves directly sampling rumen contents and incubating in four glass bottles per animal, each sealed and flushed with CO_2. Two bottles were immediately inactivated in an autoclave, while the remaining two were incubated in a water bath for 30 min before also being autoclaved. The differences in SCFA, NH_3-N, and CH_4 concentrations between the 0 and 30 min samples were used to estimate the production, which was then extrapolated to daily values. Rumen contents were manually collected at 06:00 and 14:00 h on one day, and at 10:00 and 18:00 h on the following day, across all seasons.

The ruminal pH was continuously measured using a data logger pH [T7–1 LRCpH, Dascor®, Escondido, CA, USA] during 24 h in each season (Penner et al., 2006).

The total ruminal emptying technique was used to obtain the volume of ruminal content and subsequent calculation of ruminal dynamics. To determine the rate of ruminal disappearance, the contents present in the rumen were manually removed through the cannula of each animal (Dado and Allen, 1995). During the ruminal emptying process, the solid and liquid phases were manually separated using 4-mm mesh sieves and buckets, and then weighed. Homogeneous subsamples were collected from at least six different points within the bucket for both the solid and liquid fractions, and approximately 1 kg of each phase was used to determine the total rumen DM. In a test conducted by the research group, separating the rumen contents into liquid and solid phases significantly improved sampling accuracy. When the contents were left in a bucket without prior separation, particle segregation occurred: the liquid settled at the bottom while the fiber remained on top and became drier-a behavior similar to the "rumen mat." This stratification made it difficult to collect representative samples and increased the risk of sampling errors. The strategy of separating the phases also helped mitigate inconsistencies commonly associated with direct rumen sampling, where the presence of the rumen mat makes it challenging to obtain a truly uniform sample. After the end of the procedure, both the solid and liquid contents were reconstituted in the rumen of the respective animals. According to Robinson et al. (1987), the rate of DM ingestion is similar to the rate of DM disappearance; therefore, we used the following equations to calculate the disappearance rate:

$$\text{DM disappearance rate}(\% / h) = 100 \times \frac{\left(\frac{\text{Daily intake,kg}}{\text{DM Ruminal contents,kg}}\right)}{24}$$

$$\text{DM disappearance rate}(kg / h) = \text{DM Ruminal contents}(kg) \times \left(\frac{DR(\%/h)}{100}\right)$$

Where: DR = Disappearance rate.

2.4. Statistical analyses

For statistical analysis, we considered the animals as experimental units each year. Data was processed using SAS® 9.4 statistical software, developed by SAS Institute Inc. located in Cary, North Carolina, USA. Before the statistical analysis itself, the criterion for outlier removal was based on studentized residuals obtained from the PROC GLM mode, observations with extreme residuals were identified using PROC UNIVARIATE. Outliers were manually removed one by one, until the residuals became normal by the Shapiro-Wilk test. When the normality assumption was not met, logarithmic transformation was applied. Then, the data were analyzed using the mixed procedure (PROC MIXED), exploring different covariance structures. We selected the best-fitting model based on the lowest value of the corrected Akaike information criterion (AICC), as proposed by Wang and Goonewardene (2004). The statistical model included the three grazing systems as fixed effects, the seasons (dry and rainy) as repeated measures over time and the years as a random effect. In addition, we tested the interaction between treatment and seasons. When there were significant interactions, we evaluated the effects of one factor within the other using the SLICE command of PROC MIXED. Finally, we compared all means using the least squares test (LSMEANS) and performed multiple comparisons using the GLIMMIX procedure, applying Fisher's test with the PDIFF LINES option.

The statistical model consists of the following equation:

$$Y_{ijk} = u + t_i + a_k + e_{(1)ik} + s_j + (ts)_{ij} + e_{(2)ij}$$

Where: Y_{ijk}: experimental answer; u: general mean; t_i: effect of treatment (fixed); a_k: effect of year (random); $e_{(1)ik}$: error term associated to experimental unit (treatments); s_j: effect of season (fixed); $(ts)_{ij}$: interaction effect of treatment and season (fixed); $e_{(2)ij}$: random error (seasons).

3. Results

The chemical and isotopic composition of pigeon pea and *Urochloa* spp. from the MIX pasture treatment sampled during the seasons are presented in Table 2.

The C isotopic composition determined in the animals' feces ($\delta^{13}C = -13.79 \pm 0.061$ ‰ and -16.86 ± 0.65 ‰ in the rainy and dry seasons, respectively) and from the different forages (pigeon pea and *Urochloa* spp.) from the MIX pasture treatment allowed the estimation of the proportion of pigeon pea intake in the experiment. In 2021, the animals consumed about 95.91 % of C_4 plants (*Urochloa spp.*) in the rainy season and 59.25 % in the dry season, while in 2022, the proportion consumed was 92.60 % and 86.98 %, respectively. Considering this proportion, Table 3 presents the chemical composition and CT content of the forage in the different pasture-based treatments.

Significant treatment × season interactions were observed for CT, LIG, NDF, ADF, EE, and CP (Fig. 2). In the dry season, MIX pasture presented the highest values of CP, LIG, EE, and CT (Fig. 2a, d, e and f), while DEG and REC pastures presented the highest values of NDF and ADF (Fig. 2b and c). During the rainy season, none of the treatments differed for NDF, ADF or LIG (Fig. 2b, c and d), while the REC pasture presented the highest CP values (Fig. 2a), and the DEG pasture presented the highest EE values (Fig. 2e), and MIX, DEG and REC pastures differed for CT (Fig. 2f). The effect of season was found for GE (Table 3).

The values of DM digestibility, forage DMI, mineral supplement DMI and total DMI, ruminal pH and ruminal dynamic of Nellore bulls in pasture systems and seasons are presented in Table 4, while the decomposition of significant interactions are presented in Fig. 3. The highest DMD was observed in the MIX treatment pasture, while the DEG pasture presented higher supplement intake (Table 4). Except for average body weight (ABW) and ruminal solid mass, effect of season was observed for DM digestibility, forage, mineral supplement and total DMI, ruminal pH and ruminal dynamic parameters (Table 4). During the rainy season, supplemental DMI did not differ between treatments, the REC pasture had the highest forage DMI (Fig. 3b), total DMI and Kt (Fig. 3a, c and d). In the dry

Table 2
Chemical and isotopic composition of *Cajanus cajan* and *Urochloa* spp. in the MIX treatment collected by hand-plucking during the rainy and dry seasons.

Plant species	Seasons	Descriptive Statistics	Variables							
			CP (%)	NDF (%)	ADF (%)	LIG (%)	EE (%)	GE (cal/g)	CT (g/kg)	$\delta^{13}C$
Cajanus cajan	Rainy		21.2	45.3	28.2	12.3	5.47	4815.7	23.7	-29.8
	Dry		18.5	45.3	25.5	26.8	4.82	4254.7	61.3	-26.7
		Average	19.85	45.3	26.9	19.6	5.15	4535.2	42.5	-28.3
		SEM	0.97	0.58	0.56	2.02	0.24	85.27	5.70	0.35
Urochloa spp.	Rainy		10.2	68.2	36.2	2.41	2.40	3683.9	1.01	-12.8
	Dry		7.4	69.9	40.8	4.50	1.64	3690.6	0.28	-13.4
		Average	8.8	69.1	38.5	3.46	2.02	3687.2	0.65	-13.1
		SEM	0.38	0.73	0.65	0.25	0.10	14.37	0.18	0.10

CP, Crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; LIG, lignin; EE, ether extract; GE, gross energy expressed as calorie per gram (cal/g); CT, condensed tannins expressed as eq-g leucocyanidin/kg DM; $\delta^{13}C$, isotopic signature. SEM: Standard error of the mean.

Table 3
Forage chemical composition from the different treatments during the seasons.

Effects		Variables						
Treatment	Seasons	CP (%)	NDF (%)	ADF (%)	LIG (%)	EE (%)	GE (cal/g)	CT (g/kg)
DEG		7.58[B]	71.1 [A]	39.7 [A]	3.71[B]	2.09	3651.3[B]	0.44[B]
REC		9.47 [A]	68.6[B]	39.2 [A]	3.56[B]	1.96	3691.1[B]	0.36[B]
MIX*		9.99 [A]	64.9 [C]	37.5[B]	4.94 [A]	2.34	3837.1 [A]	12.52 [A]
	Rainy	9.79	68.8	37.4	2.95	2.29	3697.9	0.78
	Dry	8.24	67.6	40.2	5.19	1.96	3755.1	8.10
Average		9.01	68.2	38.8	4.07	2.13	3726.5	4.44
SEM		0.40	0.91	0.60	0.30	0.12	22.6	1.87
		Statistical Probabilities (*P* value)						
Trat.		0.0013	< .0001	0.0258	0.0001	NS	< .0001	< .0001
Seasons		0.0042	NS	0.0003	< .0001	0.025	0.0393	< .0001
Treat. × Season		0.0032	0.0108	0.0007	0.0200	< .0001	NS	< .0001

* Values considering the proportion of Pigeon pea intake estimated using C stable isotopes. [A, B, C] Different capital letters in the same column represent treatments that differ from each other (P < 0.05) by Fisher's test. CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; LIG, lignin; EE, ether extract; GE, gross energy expressed as calorie per gram (cal/g); and CT, condensed tannins expressed as eq-g leucocyanidin/kg DM; SEM, Standard error of the mean. DEG, degraded pasture of *Urochloa decumbens* cv. Basilisk, REC, mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu fertilized with 200 kg of N-urea ha^{-1} year, MIX, mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu intercropped with *Cajanus cajan* (L. Millsp.) cv. BRS Mandarim.

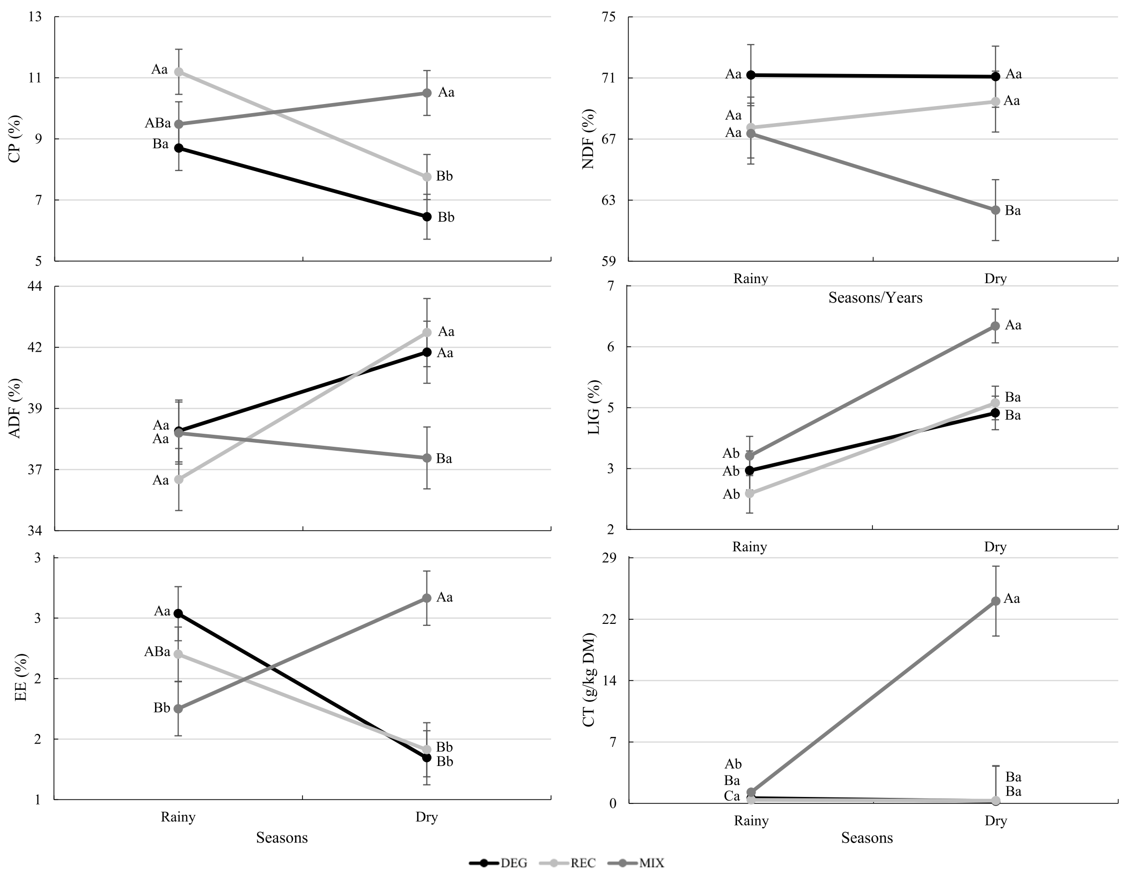

Fig. 2. Decomposition of the treatment×season interaction of nutrient intake estimated via $\delta^{13}C$ for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (LIG), ether extract (EE), condensed tannins (CT) expressed as eq-g leucocyanidin/kg DM in the different pasture-based systems. Different capital letters indicate statistical differences among treatments in the same season, while different lowercase letters indicate statistical differences between seasons for each treatment by Fisher's test (P < 0.05). Vertical bars are the standard error of the mean.

Table 4
Dry matter digestibility, forage, mineral supplement and total DMI, ruminal pH and ruminal dynamic of Nellore bulls in the different pasture-based systems and seasons.

Effects		Variables										
				DMI				Ruminal Kinetics				
Treatment	Seasons	ABW (kg)	forage (kg/d)	Supplement* (kg/d)	Total (kg/d)	DMD (%)	pH Mean	Liquid (kg)	Solid (kg)	Total mass (kg)	Kt (%/h)	Kt (kg/h)
DEG		619.8	9.1	0.375 [A]	9.5	52.4 [B]	6.85	51.6	7.15	58.7	6.24	0.422
REC		610.8	10.8	0.249 [B]	11.1	55.2 [B]	6.92	50.4	6.87	57.3	7.03	0.466
MIX		625.6	10.0	0.184 [C]	10.2	59.1 [A]	6.91	52.1	7.13	59.2	6.22	0.437
	Rainy	613.9	13.2	0.161	13.3	67.1	6.76	49.0	6.83	55.8	8.26	0.557
	Dry	623.6	6.8	0.377	7.3	44.1	7.03	53.7	7.28	61.0	4.73	0.326
Average		618.8	10.0	0.270	10.3	55.6	6.89	51.4	7.06	58.4	6.50	0.440
SEM		15.05	0.72	0.032	0.71	2.35	0.06	2.08	0.33	2.40	0.36	0.024
Statistical Probabilities (*P* value)												
Trat.		NS	NS	< .0001	NS	0.0038	NS	NS	NS	NS	NS	NS
Seasons		NS	< .0001	< .0001	< .0001	< .0001	0.0146	0.0102	NS	0.0114	< .0001	< .0001
Treat. × Season		NS	0.0212	0.0221	0.0228	NS	NS	NS	NS	NS	0.0022	NS

* Average annual supplement consumption, in the dry season MIX animals received the mineral formulation, while REC and DEG animals received a mineral-energetic-protein supplementation. [A, B, C] Different capital letters in the same column represent treatments that differ from each other ($P < 0.05$) by Fisher's test. DMD, Dry Matter Digestibility; DMI, dry matter intake; ABW, average body weight; pH Mean, mean of ruminal pH day; Kt, Total disappearance rate; SEM, Standard error of the mean. DEG, degraded pasture of *Urochloa decumbens* cv. Basilisk, REC, mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu fertilized with 200 kg of N-urea ha^{-1} year, MIX, mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu intercropped with *Cajanus cajan* (L. Millsp.) cv. BRS Mandarim.

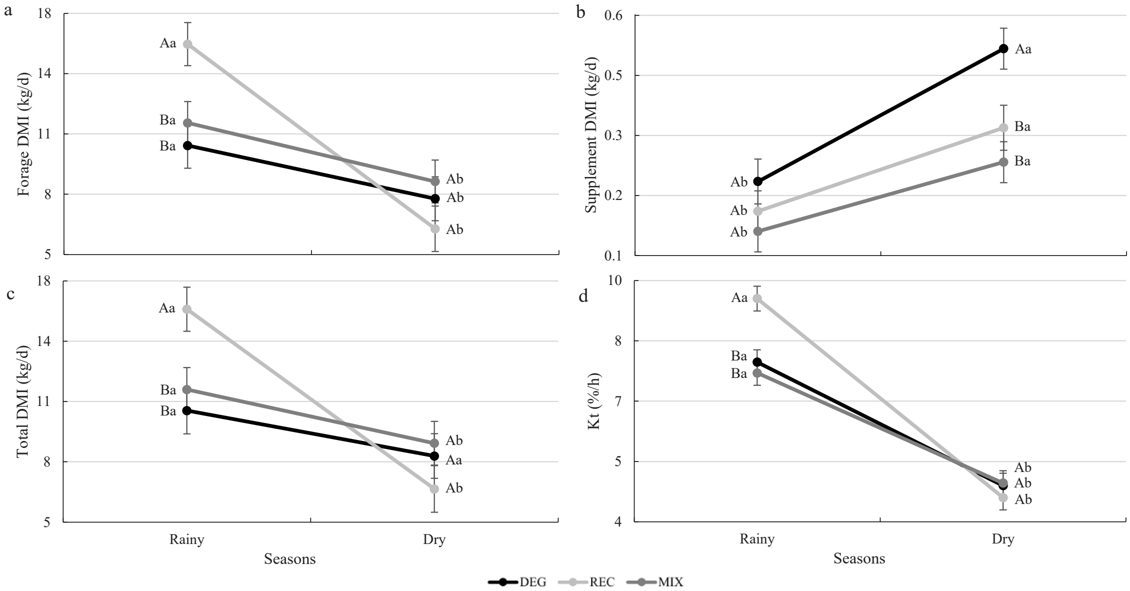

Fig. 3. Decomposition of the treatment×season interaction for the forage, supplement, and total Dry Matter Intake (DMI), DM Disappearance rate (Kt). Different capital letters indicate statistical differences between treatments in the same season, while different lowercase letters indicate statistical differences between seasons for each treatment by Fisher's test ($P < 0.05$). Vertical bars are the standard error of the mean.

season forage DMI, total DMI and disappearance rate (Kt) did not differ, while the DEG pasture presented the highest supplemental DMI.

In Table 5 we observed that REC and MIX pastures presented the lowest REL values, while REC pasture presented the highest NH_3-N values (0 min and 30 min) and was the only one to present a positive balance. All the variables related to the production of SCFA and CH_4 were affected by season (Table 5). The highest value of propionate was observed in the REC pasture during the rainy season, without differences in the dry season (Fig. 4a). Although the highest values of NH_3-N 0 min and 30 min were observed in REC pasture during the dry season, in the rainy season there were no differences among treatments (Fig. 4b and c).

Treatments in the DEG and REC pastures presented higher ruminal degradation rate of DM and NDF (Tables 6 and 7). Seasons affected all parameters presented in Table 6. In the rainy season, DEG and REC pastures presented the highest ruminal degradability rate of DM and NDF (Fig. 5a and f); REC pasture presented the highest values for effective degradability for ruminal passage rates of 2, 5 and 8 % and ruminal degradability rate of CP (Fig. 5b, c, d and g). The fraction b of NDF did not differ among treatments (Fig. 5e). In the dry season, the ruminal degradability rate of DM and NDF, and effective degradability for ruminal passage rates of 2 % were similar among treatments (Fig. 5a, f and b); the MIX pasture presented the highest values of effective degradability for ruminal passage rates of 5 and 8 % and ruminal degradability rate of CP (Fig. 5c, d and g), and it presented the lowest values of fraction b of NDF (Fig. 5e).

4. Discussion

4.1. Carbon isotopic composition and nutrient consumption

The estimation of the proportion legume and grass consumption is a difficult task in pasture systems. For this purpose, the isotopic composition of $\delta^{13}C$ from C3 (legume) and C4 (tropical grass) plants and animal feces was used to estimate the proportion of the consumption of legumes and the nutritional quality of the diet in the MIX pasture diet consumed by the animals (Norman et al., 2009; Ovani et al., 2022). The MIX system presented legume consumption ranging from 4.09 % to 40.75 %; however, large amounts of tanniniferous legumes can cause metabolic disorders or even limit the digestive process, decreasing the production of SCFA, CH_4 and *in vitro* digestibility (Furtado et al., 2024).

Both MIX and REC pastures presented higher CP content compared to the DEG. For MIX pasture, this increase can be attributed to the presence of pigeon pea in the pasture. While higher CP forage content in REC pasture can be attributed through N fertilization, which improves the production and quality of tropical grasses (Astigarraga et al., 2002; Pasquini Neto et al., 2024). Pigeon pea CP content usually varies from 16 % to 20 % (Paludo et al., 2012), supporting its potential for feeding grazing ruminants due to its high nutritional value (Onim et al., 1985). Given this, it was possible to verify that CP content in the MIX pasture was higher during the dry season, which is when pigeon pea consumption by animals is at its highest. Conversely, DEG pastures had low forage availability during the experimental period, leading animals to ingest older leaves and thus, with higher NDF content (Pezzopane et al., 2024). In addition, the lower NDF of pigeon pea compared to *Urochloa* spp. justifies the lower NDF in MIX pasture (Neres et al., 2012). It is well known that during dry season and in periods of water deficit, the nutritional quality of tropical grasses is worse than in rainy seasons,

Table 5
Production of SCFA, CH_4 and NH_3-N in the different pasture-based systems and seasons.

Effects		Variables									
Treatment	Seasons	Production of SCFA/day					CH_4/day	REL	NH_3-N		
		Acetate (g/kgDM)	Propionate (g/kgDM)	Butyrate (g/kgDM)	tSCFA (g/kgDM)	A:P	(g/kgDM)	(%)	0 min (mg/dL)	30 min (mg/dL)	Balanced/h (mg/dL)
DEG		101.5	34.2	29.3	164.6	2.91	18.9	29.7 ^{A}	8.0^{B}	7.9^{B}	-0.126^{B}
REC		112.3	36.0	32.3	180.2	3.08	17.7	22.9^{B}	11.2 ^{A}	11.4 ^{A}	0.313 ^{A}
MIX		103.3	33.4	29.9	166.7	3.25	17.4	24.3^{B}	8.8^{B}	8.7^{B}	-0.133^{B}
	Rainy	131.6	38.1	36.2	204.5	3.52	21.1	25.6	9.0	8.8	-0.113
	Dry	79.5	30.9	24.8	135.9	2.63	14.9	25.7	9.7	9.8	0.149
Average		105.6	34.5	30.5	170.2	3.08	18.0	25.7	9.3	9.3	0.018
SEM		7.69	1.48	2.08	9.60	0.20	0.79	1.24	0.53	0.55	0.010
Statistical Probabilities (*P* value)											
Trat.		NS	NS	NS	NS	NS	NS	0.0367	0.0007	0.0001	0.0454
Seasons		0.0005	0.0137	0.0058	< .0001	0.0160	< .0001	NS	NS	NS	NS
Treat. × Season		NS	0.0415	NS	NS	NS	NS	NS	0.0020	0.0052	NS

[A, B, C] Different capital letters in the same column represent treatments that differ from each other ($P < 0.05$) by Fisher's test. kgDM, kilogram ruminal dry matter; SCFA, short-chain fatty acid; tSCFA, total short-chain fatty acid production; CH_4, methane; REL, relative energy loss; A:P, acetate to propionate ruminal ratio; NH_3-N, ammonia nitrogen; SEM, Standard error of the mean. DEG, degraded pasture of *Urochloa decumbens* cv. Basilisk, REC, mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu fertilized with 200 kg of N-urea ha^{-1} year, MIX, mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu intercropped with *Cajanus cajan* (L. Millsp.) cv. BRS Mandarim.

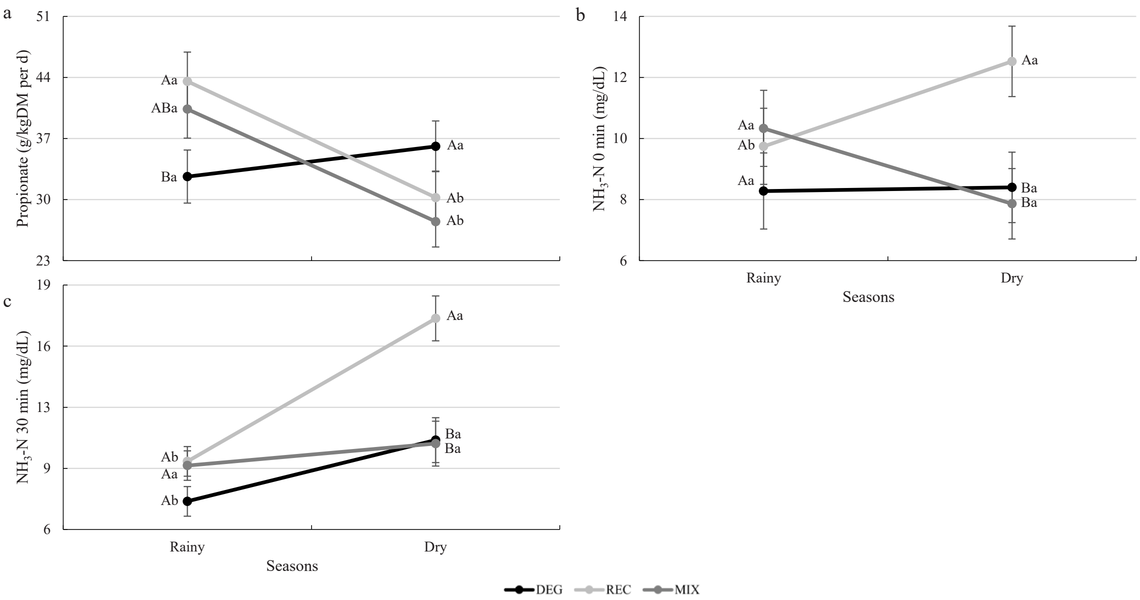

Fig. 4. Decomposition of the treatment×season interaction for the propionate, ammonia nitrogen in time zero (NH_3-N 0 min), ammonia nitrogen in thirty minutes of incubation (NH_3-N 30 min). Different capital letters indicate statistical differences between treatments in the same season, while different lowercase letters indicate statistical differences between seasons for each treatment by Fisher's test ($P < 0.05$). Vertical bars are the standard error of the mean.

Table 6
Dry matter ruminal degradability and ruminal parameters estimated using the Ørskov and McDonald (1979) model in *Urochloa* spp. and *Cajanus cajan* forages under different pasture-based systems and seasons.

Effects		Variables							
Treatment	Seasons	Ruminal Parameters of DM							
		a (%)	b (%)	c (%/h)	ED2 (%)	ED5 (%)	ED8 (%)	PD (%)	IND (%)
DEG		27.6	44.8	0.042 [A]	57.0	47.3	42.4	72.4	27.7
REC		28.4	46.1	0.042 [A]	58.5	47.8	42.8	73.9	26.1
MIX		32.3	41.2	0.031 [B]	57.6	48.4	44.1	73.5	26.5
	Rainy	27.6	48.7	0.046	61.4	50.4	45.0	76.3	23.7
	Dry	31.3	39.3	0.030	54.0	45.2	41.2	70.2	29.8
Average		29.5	44.0	0.038	57.7	47.8	43.1	73.3	26.7
SEM		1.02	1.85	0.002	1.03	0.89	0.84	1.35	1.35
Statistical Probabilities (*P* value)									
Trat.		NS	NS	< .0001	NS	NS	NS	NS	NS
Seasons		0.0331	< .0001	< .0001	< .0001	< .0001	0.0004	< .0001	< .0001
Treat. × Season		NS	NS	0.0070	< .0001	0.0004	0.0008	NS	NS

[A, B, C] Different capital letters in the same column represent treatments that differ from each other ($P < 0.05$) by Fisher's test. DMI, dry matter intake; a, rapidly soluble fraction at time zero; b, potentially degradable fraction; c, degradation rate of the potentially degradable fraction; ED2, ED5 and ED8, effective degradability for ruminal passage rates of 2, 5 and 8 %, respectively; PD, potential degradability; IND, non-degraded portion (100-PD); SEM, Standard error of the mean. DEG, degraded pasture of *Urochloa decumbens* cv. Basilisk, REC, mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu fertilized with 200 kg of N-urea ha^{-1} year, MIX, mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu intercropped with *Cajanus cajan* (L. Millsp.) cv. BRS Mandarim.

and pastures that underwent recovery processes usually present greater biomass production with better nutritional quality than extensively managed pastures not receiving fertilization or soil corrections (Souza et al., 2023; Pasquini Neto et al., 2024). This is consistent with our findings for CP and NDF values. Higher GE concentration during dry season and in MIX pasture align with those observed by Furtado et al. (2023) and justified the nutritional value of pigeon pea green forage (CQBAL 4.0, 2024). In addition, MIX pasture provided, on average, a CT content of 12.5 $g.kg^{-1}$ in their available biomass, which is below 18 $g.kg^{-1}$ and the critical concentrations above which some authors observed reductions in feed intake and digestibility (Grainger et al., 2009; Perna Junior et al., 2022). CT concentrations between 20 and 45 $g.kg^{-1}$ can interfere with the digestive process of ruminants (Perna Junior et al., 2022); however, it is important to consider that CT may have beneficial or adverse effects depending on concentration and other factors such

Table 7
Neutral detergent fiber and crude protein ruminal degradability using the Ørskov and McDonald (1979) model in *Urochloa* spp. and *Cajanus cajan* forages under different pasture-based systems and seasons.

Effects		Variables					
Treatment	Seasons	Ruminal Degradation of NDF			Ruminal Degradation of CP		
		a (%)	b (%)	c (%/h)	a (%)	b (%)	c (%/h)
DEG		18.0	55.9	0.037 [A]	36.5	38.3	0.043
REC		16.2	57.2	0.039 [A]	40.5	38.0	0.042
MIX		17.0	51.9	0.029[B]	34.0	41.8	0.040
	Rainy	15.8	60.5	0.046	29.5	51.2	0.045
	Dry	18.4	49.5	0.024	44.5	27.6	0.039
Average		17.1	55.0	0.035	37.0	39.4	0.042
SEM		1.12	2.46	0.002	3.03	2.93	0.003
		Statistical Probabilities (*P* value)					
Trat.		NS	NS	0.0120	NS	NS	NS
Seasons		NS	0.0001	< .0001	0.0014	< .0001	NS
Treat. × Season		NS	0.0042	0.0494	NS	NS	0.0238

[A, B, C] Different capital letters in the same column represent treatments that differ from each other ($P < 0.05$) by Fisher's test. NDF, neutral detergent fiber; CP, crude protein; a, rapidly soluble fraction at time zero; b, potentially degradable fraction; c, degradation rate of the potentially degradable fraction; SEM, standard error of the mean. DEG, degraded pasture of *Urochloa decumbens* cv. Basilisk, REC, mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu fertilized with 200 kg of N-urea ha^{-1} year, MIX, mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu intercropped with *Cajanus cajan* (L. Millsp.) cv. BRS Mandarim.

as animal species, plant phenological and physiological stage, as well as diet composition (Dhanasekaran et al., 2020).

4.2. DMI, DMD and Rumen pH

The higher forage and total DMI intakes observed in REC pasture during the rainy season may be related to the greater forage availability of *Urochloa* spp. in this treatment, justifying the highest disappearance rate (Fig. 3d) due a pasture with better nutritional quality, directly influenced by N fertilization (Perna Junior et al., 2017; Tseu et al., 2020; Pasquini Neto et al., 2024; Pezzopane et al., 2024). The beneficial effects of fertilization are evident when comparing REC pasture with DEG pasture. On the other hand, during the dry season, animals in DEG pasture presented higher supplement DMI due to the poorer nutritional quality of its forage (Silva et al., 2009). Ruminant diets on tropical pastures usually require nutrient supplementation to improve fiber digestion, especially during the dry seasons when digestibility is low (Almeida et al., 2022; Arco et al., 2024). By including pigeon pea, animals in MIX pasture consumed less supplements, not needing use of energetic-protein mineral supplements. This is especially relevant during periods in times of high prices, making the introduction of this legume even more beneficial, but extra benefits are less operational costs and a richer protein profile in relation to the supplement. Furthermore, with the inclusion of pigeon pea, animals in MIX pasture presented higher DMD (Table 4). This effect could be attributed to the improved N supply to ruminal microorganisms, resulting in increased efficiency of fiber and protein digestion (Niderkorn and Baumont, 2009; Boddey et al., 2020; Furtado et al., 2023).

According to Peters et al. (2025), it is important to highlight that internal markers (iNDF) may not have 100 % recovery, presenting relatively low accuracy when used in isolation. To improve data accuracy, a TiO_2 recovery correction of 93 % (Titgemeyer et al., 2001) and 87.5 % for iNDF (Peters et al., 2025) was applied. Given the practical constraints of total fecal collection in grazing trials and the widespread use of the dual-marker approach (TiO_2 and iNDF) in the literature, we consider the estimates obtained to be acceptable for comparative and interpretive purposes, especially when treatment effects are of interest (Velásquez et al., 2021; Andrade et al., 2025; Pasquini Neto et al., 2025).

The negative relationship between lignin concentration and cell wall digestibility is an important fact to be considered in MIX pasture, which presented a higher lignin content; lignin is more inhibitory for digestion of grasses than legumes (Moore and Jung, 2001), which may justify the higher DMD in the MIX pasture.

The ruminal pH did not vary between treatments, being close to 7, which is expected for grazing cattle (Berchielli et al., 2011). Ruminal pH is an indicator of rumen fermentation intensity and the rumen buffer capacity to resist to its change and different feeding regimes directly influence rumen pH, which can alter the final products of fermentation (Wanapat et al., 2021). Nevertheless, even the highest pH values observed during the dry season, possibly due to a greater amount of indigestible fiber and lower amounts of fibrous carbohydrates being degraded (Franco et al., 2002; Berchielli et al., 2011), would not be able to have any major influence in the microbiome. The lower fiber quality in the dry season also justifies a lower Kt and greater ruminal filling [increase in liquid mass and total ruminal mass] (Oliveira et al., 2017; Perna Junior et al., 2017).

4.3. SCFA, NH_3-N and CH_4

The ideal ruminal NH_3-N concentration for maximizing DMI and ruminal fermentation in tropical conditions is at least 10 mg/dL (Leng, 1996), a value not achieved by DEG and MIX pastures, indicating NH_3-N consumption in the rumen. Conversely, REC pasture exceeded the minimum values stipulated by Leng (1996), showing a positive NH_3-N balance (0.3125 mg/dL per h). Drought-stressed

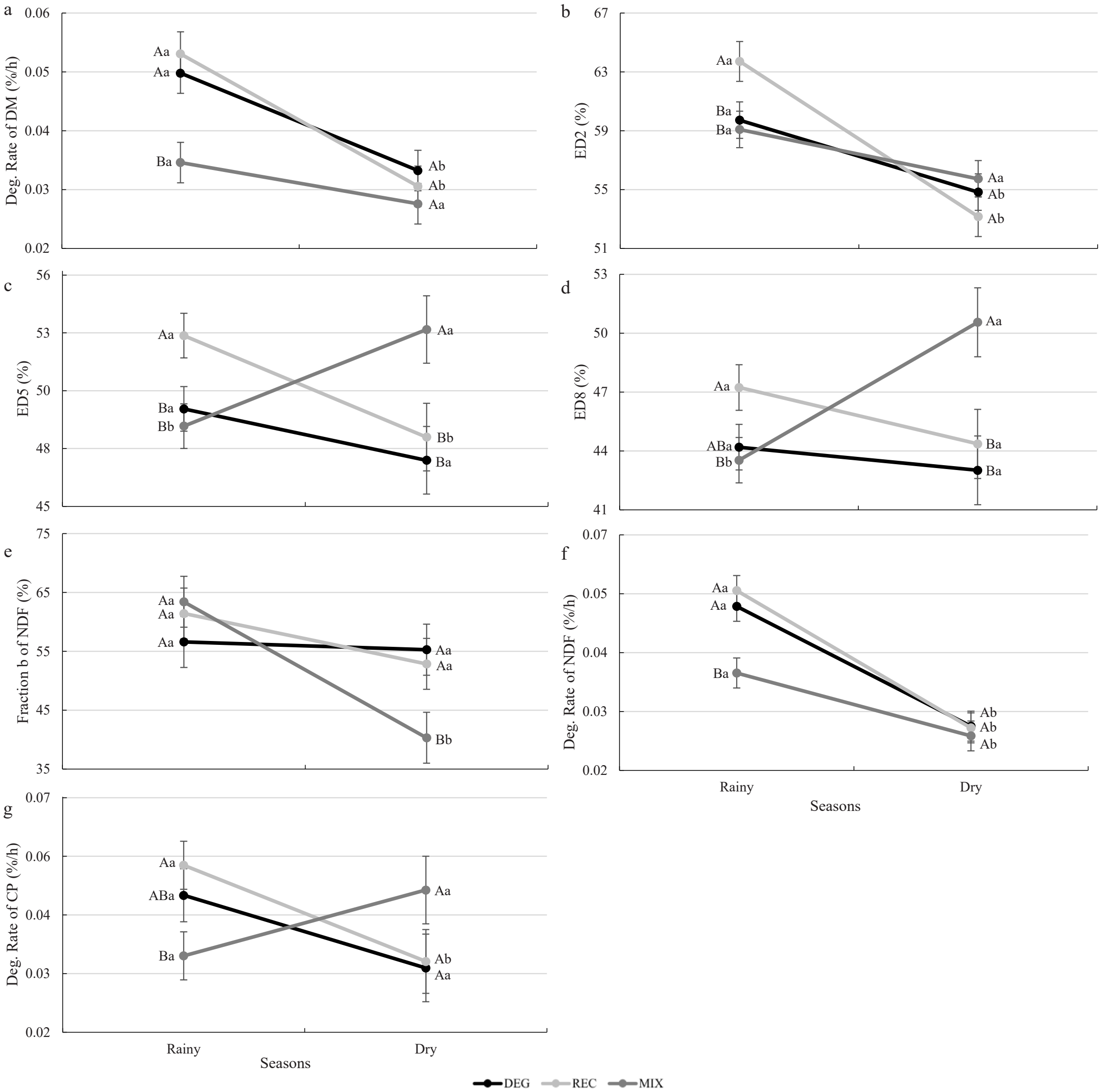

Fig. 5. Decomposition of the treatment×season interaction for the degradation rate of the potentially degradable fraction of DM (Deg. Rate of DM), effective degradability for ruminal passage rates of 2 % (ED2), effective degradability for ruminal passage rates of 5 % (ED5), effective degradability for ruminal passage rates of 8 % (ED8), fraction b of neutral detergent fiber, degradation rate of the potentially degradable fraction of neutral detergent fiber (Deg. Rate of NDF), degradation rate of the potentially degradable fraction of crude protein (Deg. Rate of CP). Different capital letters indicate statistical differences between treatments in the same season, while different lowercase letters indicate statistical differences between seasons for each treatment by Fisher's test ($P < 0.05$). Vertical bars are the standard error of the mean.

legumes may enhance the synthesis of ruminal proteins with efficient N use (Pisulewski et al., 1981; Riede et al., 2019), but CT can reduce soluble protein and NH_3-N levels in the rumen (McMahon et al., 2000; Riede et al., 2019; Dhanasekaran et al., 2020), potentially explaining the results observed in MIX pasture compared to those of the REC pasture for which mineral supplementation and pasture N fertilization probably contributed to maintaining NH_3-N levels in the ideal range (Leng, 1996). Additionally, pasture N fertilization may account for higher propionate production, as increased SCFA productions are associated with fertilized pastures (Noviandi et al., 2012). It's important to consider that the metabolism of rumen microorganisms can be affected by the type of legume and vary throughout the seasons (Riede et al., 2019).

The improved forage quality in the rainy season, as demonstrated by Pezzopane et al. (2024), modified ruminal fermentation, resulted in higher production of acetate, propionate, butyrate, CH_4, and total SCFA, as well as changing the A:P ratio (Table 5). The increase in SCFA production was related to better forage quality, and the higher CH_4 emission was related to increased dry matter intake during the rainy period (Tables 4 and 5).

Lower proportions of energy lost as CH_4 (REL) were observed in REC and MIX pastures, which could be justified by the lower nutrient contents and worse DMD of the DEG pasture, highlighting the potential of adopting more sustainable practices for intensifying pasture-based systems. Previous studies found lower CH_4 production when including pigeon pea (Ligoski et al., 2020; Furtado et al., 2023, 2024); however, in this study we observed just a numerical difference between MIX and DEG pastures when expressed by kg of DMI.

4.4. Ruminal kinetics and degradability

Lower ruminal degradability rate of DM in MIX pasture during the rainy season may be due to the biding proprieties of CT, which reduces the nutrient release rate that may increase microbial synthesis efficiency as in the case of Getachew et al. (2000). Additionally, this could result from some modulation of ruminal passage, making the feed stay longer in the rumen and compensating the lower c value in Table 6 (Perna Junior et al., 2022), as there was no reduction in DMD for this treatment. On the other hand, higher effective degradability at 2 %, 5 % and 8 % were found for the MIX pasture during the dry season, coinciding with higher pigeon pea consumption.

Considering the lower ruminal degradation rate of NDF in MIX pasture during the rainy season, a possible explanation would be a reduction in the number of cellulolytic bacteria due to the direct effect of CT on the ruminal microbiota (McSweeney et al., 2001). As a matter of fact, analysis to determine possible changes on ruminal microorganisms are still being conducted. Another concomitant possibility would be the formation of tannin-cellulose complexes that resist the enzymatic digestion process, with or without difficulty in the adhesion of fibrolytics microorganisms to legumes in the ruminal environment (Bento et al., 2005).

Higher CT concentrations can lead to the formation of tannin-protein complexes interfering in the ruminal degradation (Perna Junior et al., 2022). This may explain the lower degradation rate of the CP that is potentially degradable in the MIX pasture compared to REC pasture during the rainy season.

The botanical composition, season, and cutting height affect the nutritional value and nutrient degradation kinetics of pastures, but there is limited information on pastures composed of different species (Keim et al., 2013). As seen in Table 6, the effective degradability is directly related to the nutritional quality of diet (Tables 2 and 3), considering mainly the dry and rainy seasons. Additionally, the proportion of legumes and grasses consumption also interferes with the rumen degradation process (Tables 6 and 7).

Greater amount of CP in pastures is related to faster degradation in the rumen, in addition to providing an adequate substrate for microbial multiplication that degrades the fiber (Keim et al., 2013). This is consistent with the results observed in Fig. 5g. In MIX pasture, the lower NDF and higher LIG contents (Tables 2 and 3, Fig. 2b and d) justify the lower fraction b of NDF (Fig. 5e). When carrying out an economic feasibility analysis, Tupy et al. (2023) found that pasture systems containing pigeon pea consortium are an economically viable alternative for livestock farmers, increasing forage availability and quality, stocking rate and animal weight gain.

Here we found that intercropping and pasture fertilization directly affected the forage nutritional value and the ruminal fermentation and kinetics processes; however, more research should be carried out including pigeon pea in ruminant diet to better understand its potential for sustainable intensification of tropical pastures by evaluating effects in ruminal microbiome and community and the entire digestion processes of grazing ruminants.

5. Conclusions

The use of pigeon peas provided a greater supply of nutrients compared to single grass pastures. In addition, animals grazing on pigeon pea intercropped with tropical grass presented lower supplement intake and higher DM digestibility. Some lower degradation rates found in the intercropped treatment may have occurred due to the modulation of the microbiota by tannins and the gradual release of the legume nutrients. The energy loss in form of CH_4 was higher for DEG pasture, demonstrating that recovered pastures and the use of pigeon pea intercropped with tropical grass can be valid strategies for reducing the intensity of CH_4 emissions. These facts demonstrate that intercropping with legumes in tropical pasture is an excellent strategy for mitigating GHG emissions in livestock production systems, increasing digestive ruminal efficiency and reducing the need for N-based supplements or fertilizers.

CRediT authorship contribution statement

A. J. Furtado: Conceptualization, Data Curation, Supervision, Investigation, Writing - review & editing. **A. L. Abdalla Filho:** Investigation, Writing - review & editing. **F. Perna Junior:** Methodology, Writing - review & editing, Supervision. **R. Pasquini Neto:** Data curation, Investigation. **G. V. Silva:** Investigation, Conceptualization, Validation. **A. A. G. Lobo:** Conceptualization, Validation. **L. M. Coelho:** Writing - review & editing. **J. F. Bruno:** Writing - review & editing. **A. Berndt:** Investigation, Supervision. **A. F. Pedroso:** Investigation. **S. R. Medeiros:** Investigation, Validation. **P. P. A. Oliveira:** Funding Acquisition, Investigation, Supervision. **P. H. M. Rodrigues:** Funding Acquisition, Investigation, Supervision.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Paulo Henrique Mazza Rodrigues reports financial support and equipment, drugs, or supplies were provided by Fundação de Amparo à Pesquisa do Estado de São Paulo. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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