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# Forage Accumulation and Nutritional Characteristics of *Brachiaria* Cultivars Grown in a Semi-arid Environment

**J. G. Rodrigues, G. dos S. Difante, L. C. V. Ítavo, M. de G. Pereira, A. L. C. Gurgel*, A. B. G. da Costa, E. L. de L. Véras, G. O. de A. Monteiro, A. M. Dias, & C. C. B. F. Ítavo**
Faculty of Veterinary Medicine and Animal Science, Federal University of Mato Grosso do Sul - Avenida Senador Filinto Müller 2.443, Campo Grande, 79074-460, MS, Brazil
*Corresponding author: antonioleandro09@gmail.com
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## ABSTRACT

**The evaluation of the nutritional value and parameters of ruminal fermentation of tropical forages is important, aiming at its efficient use in ruminant feeding. This study was developed to evaluate the forage accumulation, chemical composition, protodioscin content, digestibility, and *in vitro* degradation kinetics of different *Brachiaria* spp. cultivars in different evaluation periods. The research was carried out in the semi-arid region of Brazil, from April 2016 to April 2017. The experiment was laid out as a split-plot arrangement in a randomized block design in which five cultivars (*B. decumbens* cv. Basilisk, and *B. brizantha* cvs. Marandu, Paiaguás, Piatã, and Xaraés) represented the plot and four evaluation periods (establishment, transition, dry, and rainy) the subplot. There was a cultivar × period interaction effect for forage accumulation rate (FAR), for which cv. Xaraés showed the highest mean (54.1 kg/ha.day DM) in the rainy season. The lowest yield was found in cv. Paiaguás in the dry season (24.1 kg/ha.day DM). Cultivars Basilisk, Marandu, and Paiaguás had the highest crude protein content. The highest *in vitro* dry matter digestibility values were found in cv. Basilisk in the dry, transition, and rainy periods; cv. Marandu in the transition period, and cvs. Paiaguás and Piatã in their establishment. A correlation was found between the parameters of *in vitro* degradation kinetics and chemical composition. The protodioscin content was highest in cv. Basilisk, during its establishment (5.5 g/kg DM). Cultivars Paiaguás, Piatã, and Xaraés showed low protodioscin values (<1.0 g/kg DM). Cultivars Basilisk, Marandu, Paiaguás, Piatã, and Xaraés can be used as forage options in the semi-arid environment, as their forage accumulation potential and nutritional value are suitable for animal production even in periods of lower water availability.**

*Keywords: Brachiaria genus; chemical composition; forage yield; in vitro digestibility; semi-arid conditions*

## INTRODUCTION

Making the best use of the produced forage—a nutritional resource that constitutes the basis of most farming systems—entails knowing the plant-environment interactions (Neves *et al.*, 2018). In Brazil, beef cattle husbandry is essentially held on pastures, the majority of which are formed by the genus *Brachiaria*, which occupies an average of 50% of cultivated pasture areas (Berchielli *et al.*, 2012).

A matter of concern for livestock exploitation in tropical regions is producing forage in rainfed conditions. The constant periods of drought, associated with successive years of low rainfall, change forage yield and quality, constituting a serious problem for producers (Lopes *et al.*, 2018). On this basis, research is being conducted to assess the adaptability and production capacity of different forage species and cultivars to identify resistant and productive crops to improve production indices in the semi-arid region (Lima *et al.*, 2017; Fernandes *et al.*, 2020). Physical, chemical, and biological factors, as well as light, temperature, and water, influence tissue flow in a plant (Reis *et al.*, 2012; Gastal & Lemaire, 2015). Therefore, these factors must be considered when choosing the grass to be used in the production system (Magalhães *et al.*, 2012; Ferreira *et al.*, 2013; Tsuzukibashi *et al.*, 2015).

Cultivars Marandu, Basilisk, and Paiaguás are known to produce more forage and have higher nutritional value during the period of water restriction when compared with other *Brachiaria* cultivars (Euclides *et al.*, 2016; Veras *et al.*, 2020). However, outbreaks of intoxication related to the consumption of Brachiaria spp. have been demonstrated in the main production systems with small ruminants (Faccin *et al.*, 2014; Costa *et al.*, 2021), with protodioscin being one of the main bioactive compounds present in forages and considered responsible for this toxicity condition (Costa *et al.,* 2021). It is necessary not only to evaluate the nutritional value of forage plants intended for sheep production but also to quantify these anti-nutritional components, aiming to efficiently use and feed ruminants.

Therefore, it is hypothesized that these cultivars are more resistant to seasonal effects both in terms of forage yield and maintenance of nutritional value. The present study was thus carried out to examine the forage accumulation, chemical composition, protodioscin content, digestibility, and *in vitro* degradation kinetics of different *Brachiaria* spp. cultivars in different evaluation periods.

## MATERIAL AND METHODS

### Location

The field experiment was conducted at the Academic Unit Specialized in Agricultural Sciences (UAECA), Federal University of Rio Grande do Norte (UFRN) (5°53′35.12″ S and 35°21′47.03″ W, 11 m above sea level) from April 10, 2016, to April 1, 2017. The region is characterized by a dry sub-humid climate (Thornthwaite, 1948), with water surpluses from May to August. The average annual precipitation in the region is 1052 mm and the average temperature is 25.5. Mean temperature, monthly precipitation, mean water deficit, and surplus data were obtained from the database of the National Institute of Meteorology (INMET) (Figure 1).

### Soil and Fertilization

The soil of the experimental area is classified as an Arenosol (Santos *et al.,* 2018). Fertilization and soil correction were performed based on the soil analysis result (Table 1). For time-of-planting fertilization, 500 kg/ha limestone, 105 kg/ha single superphosphate, and 164 kg/ha potassium chloride were applied. Nitrogen fertilization was performed using ammonium sulfate, which was applied 42 days after sowing (50 kg/ha N) and again after the establishment period (50 kg/ha N), totaling 100 kg/ha N.

### Experimental Design

The experiment was laid out as a split-plot arrangement in a randomized-block design with four replicates, where cultivars were the main plots and the evaluation periods were the subplots. The treatments consisted of five cultivars, namely, Basilisk, Marandu, Paiaguás, Piatã, and Xaraés, which were evaluated in four periods (establishment, dry, transition, and rainy). The area was divided into plots of 2.0 m × 2.0 m, totaling 4.0 m², with a usable area of 1.3 m². In each plot, the outermost 0.70 m of each side was considered bordering area. Blocks were spaced 2.0 m apart and plots 1.0 m apart, totaling 20 plots in an area of 324 m². The plants were sown by seed broadcasting. The sowing density was calculated considering the recommendation for each of the cultivars and the crop value (CV%) of the seeds.

### Data Collection

Samples were collected to quantify the forage mass and determine the morphological components when the plants intercepted 90% of incident light in the canopy. To this end, a canopy analyzer (AccuPAR Linear PAR/LAI ceptometer, model PAR - 80, Decagon Devices) was used. Readings were taken weekly (eight above the forage canopy and eight at ground level, per plot) in sunny weather conditions. To calculate the percentage of light intercepted by the canopy (%LI), the following formula was used: % LI = 100% – (Soil/Above × 100).

The interception target was reached at 110 days (establishment period), 84 days (dry season), 111 days (transition period), and 51 days (rainy season) after sowing. The forage contained in a 1.0-m² area of each plot was cut at 15 cm above ground level and weighed individually. Two sub-samples were taken—one to determine the dry matter (DM) and another to separate the

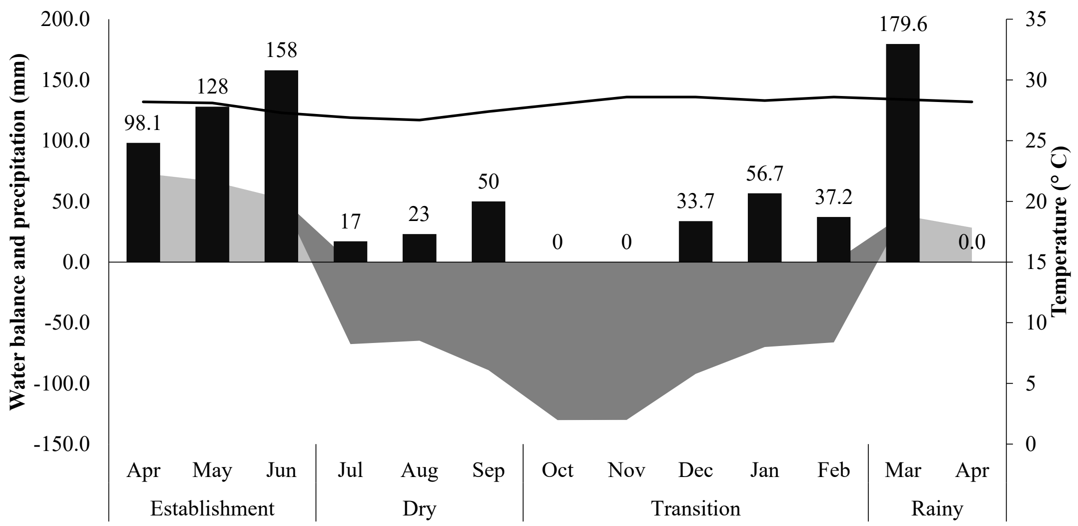


Figure 1. Water balance, precipitation, and average temperature from April 2016 to April 2017. surplus; deficit; precipitation; temperature.

Table 1. Chemical characteristics of the soil in the experimental area in the 0-20 and 20-40 cm deep layers

| Layer (cm) | Chemical characteristics | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | K | Na | pH | Ca | Mg | Al | H+Al | CEC | BS (%) | Particle size (%) | | |
| | --mg/dm³-- | | | | cmol$_c$/dm³ | | | | | | Sand | Silt | Clay |
| 0-20 | 18 | 63 | 20 | 6.6 | 3.1 | 0.2 | 0 | 1.2 | 4.4 | 72.7 | 84.6 | 4.0 | 11.4 |
| 20-40 | 8 | 49 | 13 | 5.6 | 0.9 | 0.1 | 0 | 1.1 | 2.2 | 50.0 | 85.2 | 2.0 | 12.8 |

Note: Source: Soil Laboratory of the Agricultural Research Corporation of Rio Grande do Norte. pH= pH in water (1:2.5); CEC= cation-exchange capacity; BS= base saturation.

morphological components, namely, leaf blade, stem + sheath, and dead material. The samples were then dried in a forced-air oven at 55 °C for 72 h to determine the dry weight.

The daily forage accumulation rate (FAR) was calculated as the ratio between forage yield and the number of days in each period. Leaf:stem ratio (LSR) was determined as the ratio between leaf dry weight by stem dry matter. Finally, the live:dead leaf ratio (L:D) was calculated as the sum of the dry weight of leaves and stems divided by the dead-material weight. The samples of morphological components were ground in a mill with a 1-mm sieve for laboratory analysis.

### Laboratory Analysis

Chemical composition, *in vitro* digestibility, and cumulative *in vitro* gas production were measured at the Laboratory of Applied Animal Nutrition at the Faculty of Veterinary Medicine and Animal Science. The protodioscin content, in turn, was analyzed at the Institute of Chemistry, both at the Federal University of Mato Grosso do Sul.

### Chemical Composition of Morphological Components

The dry matter (DM) content was determined after pre-drying in an oven at 55 °C and definitive drying in an oven at 105 °C. Organic matter (MO = 100 – ash) was determined after burning the sample at 550 °C for 3 h in a muffle furnace (932.05; AOAC, 2000). The concentration of nitrogen (N) was quantified by the Kjeldahl method (976.05; AOAC, 2000), and a constant factor of 6.25 was considered for the conversion of nitrogen values into crude protein (CP). For the quantification of fiber components, the samples were boiled for one hour in neutral detergent to determine the levels of neutral detergent fiber (NDF), and then boiled for another hour in acid detergent to determine the levels of acid detergent fiber (ADF) following the methodology proposed by Van Soest *et al*. (1991).

### Extraction and Quantification of Protodioscin

The leaves of the evaluated cultivars were dried at room temperature and ground in the Wiley mill (3 mm). Analyzes were performed in duplicates. Each sample (1 g) was extracted three times in 50% acetonitrile and water (v/v) using volumes of 8 mL, 5 mL, and 3 mL under ultrasound stirring for 30, 30, and 20 min, respectively. Then, the extracts were transferred to a test tube and centrifuged for 30 min at 5.000 rpm at 13 °C. The extracted solution was completed at 10 mL volume for analysis using HPLC/ELSD (high performance liquid chromatography/light scattering) (Lima *et al.,* 2012; Ganzera *et al.,* 2001).

### *In Vitro* Digestibility

*In vitro* digestibility was determined according to the methodology of Tilley & Terry (1963), which was adapted to the Ankom Daisy system (Ankom Technology Corp., Macedon, NY, USA). The procedure consisted of incubating 0.5-g samples of plant material inside synthetic polypropylene fabric bags (5×5 cm, 100 μm), for 48 h, in bottles equipped with a Bunsen valve, containing 1.600 mL of buffer solution and 400 mL of sheep rumen fluid. After 48 h, 40 mL HCL (6N) and 8 g pepsin were added and the incubation continued until 72 h were completed. The buffer solution was composed of type-A solution (g/L: 10.0 g $KH_2PO_4$, 0.5 g $MgSO_47H_2O$, 0.5 g NaCl, 0.1 g $CaCl_22H_2O$, and 0.5 g urea) and type-B solution (g/100 mL: 15.0 g $Na_2CO_3$; 1.0 g $Na_2S9H_2O$) at a B:A ratio of 1:5, to reach a final pH value of 6.8. At the end of the incubation period, the samples were washed, dried in a forced-air oven, weighed, and sent for laboratory analysis to determine the *in vitro* nutrient digestibility coefficients (DM, OM, NDF, and ADF), which were calculated by the following equation:

IVD (g kg$^{-1}$)= (Weight of incubated nutrient (g) – Weight of residual nutrient (g) – Weight of blank (g)) / (Weight of incubated nutrient (g)) × 1000.

### Cumulative *In Vitro* Gas Production

Cumulative *in vitro* gas production was analyzed using the ANKOM® RF Gas Production System (Ankom Technology Corp., Macedon, NY, USA). Twenty-four bottles equipped with pressure sensors wirelessly connected to a computer were used. The samples were incubated in triplicate for 48 h. Each bottle (310 mL) was filled with 0.5000±0.0005 g of plant material, 100 mL of buffer solution composed of solutions A and B mentioned above. The mixture was preheated to 39 °C to receive 25 mL of inoculum, which was obtained by mixing ruminal fluids collected from three sheep. Before attaching the pressure sensor to the flask, $CO_2$ was purged into them to provide an anaerobic environment. Three bottles containing only solutions A and B and rumen fluid were incubated to correct bacterial growth. Data were recorded every 5 min and, in the end, processed for cumulative gas production in mL of gas/100 mg of incubated DM.

### Statistical Analysis

Data were subjected to analysis of variance, and when the F test found a significant effect, the effects of sources of variation and their interactions were compared by Tukey's test at 5% significance. For FAR, nutritional value and digestibility, the following model was used:

$$Yijk = \mu + Ci + Bj + \alpha ij + Pk + (C^*P)ik + \beta ijk,$$

Yijk was value observed in cultivar i, block j, and period k; μ was overall mean effect; Bj was effect of block J; Ci was effect of cultivar i; αij was effect of random error attributed to the plot; Pk was effect of period k; (C*P)ik was interaction effect between cultivar and period; and βijk was random error attributed to the subplot.

The cumulative *in vitro* gas production parameters were estimated using the two-compartment logistic model proposed by Schofield *et al.* (1994).

$$Vtotal(Y)= V_{fr}/(1+\exp(2–4Kd_{ff}(t–L))) + V_{fl}/(1+\exp(2–4Kd_{fl}(t–L))),$$

where Vtotal (mL/100 mg DM) was the total volume of gas produced *in vitro* at time t (h); $V_{fr}$ (mL) was the total volume of gas produced *in vitro* by the degradation of the rapidly degradable fraction of the Cornell System (A + B1); $Kd_{ff}$ (/h) was the fractional rate of gas produced *in vitro* by the degradation of the rapidly fermentable fraction of the feed; $V_{fl}$ (mL) was the total volume of gas produced *in vitro* by the degradation of the slowly degradable fraction of the Cornell System (B2); $K_{sf}$ (/h) was the fractional rate of gas produced *in vitro* by the degradation of the slowly fermentable fraction of the feed; and L (h) was the time elapsed until fermentation begins (lag time).

Correlations were obtained by Pearson correlation analysis and the t-test, considering significance at p≤0.05.

## RESULTS

### Pasture Structure

There was a cultivar × period interaction effect (p<0.05) for forage accumulation rate (FAR), live:dead leaf ratio (LDR), and leaf:stem ratio (LSR). The highest FAR was observed in cv. Xaraés in the rainy season (91.0 kg/ha.day DM), and the lowest in cv. Paiaguás in the dry and rainy seasons (24.1 and 49.8 kg/ha.day DM, respectively) (Table 2). Between the periods, forage accumulation was greater in the rainy season, for all cultivars. The highest LSR was observed in cv. Xaraés (3.00) and the lowest in cv. Basilisk (1.00), both in the transition period. Only cv. Xaraés showed changes in LSR between the periods, with a sharp decrease in the rainy season.

Live:dead leaf ratio was highest in cv. Piatã in the dry and rainy seasons (11.5 and 10.4, respectively) and in cv. Xaraés in the rainy season (9.4). Cultivars Basilisk, Marandu, Paiaguás, and Piatã showed a decline in LDR in the dry and transition periods, in comparison to the rainy season.

Table 2. Forage accumulation rate and structural composition of *Brachiaria* spp. cultivars grown

| Period | Cultivar | | | | | |
|---|---|---|---|---|---|---|
| | Basilisk | Marandu | Paiaguás | Piatã | Xaraés | P |
| | | | Forage accumulation rate (kg/ha.day DM) | | | |
| Establishment | 33.2$^{aB}$ | 34.4 $^{aAB}$ | 35.8 $^{aAB}$ | 36.5 $^{aB}$ | 36.9 $^{aB}$ | 0.973 |
| Dry | 45.7 $^{aB}$ | 39.8 $^{abAB}$ | 24.1$^{bB}$ | 45.9 $_{aAB}$ | 49.3 $^{aB}$ | 0.004 |
| Transition | 31.7 $^{aB}$ | 31.5 $^{aB}$ | 25.4 $^{aB}$ | 29.9 $^{aB}$ | 39.1 $^{aB}$ | 0.385 |
| Rainy | 67.7$^{bA}$ | 51.4$^{bA}$ | 49.8$^{aB}$ | 56.7 $^{aB}$ | 91.0 $^{aA}$ | <0.001 |
| P-value | <0.001 | 0.034 | 0.001 | <0.001 | <0.001 | |
| | | | Leaf:stem ratio | | | |
| Establishment | 1.1 $^{bA}$ | 1.9 $^{bA}$ | 1.5 $^{bA}$ | 2.2 $^{bA}$ | 3.5 $^{aA}$ | <0.001 |
| Dry | 1.2 $^{bA}$ | 2.2 $^{abA}$ | 1.6 $^{abA}$ | 1.7 $^{abA}$ | 2.5 $^{aAB}$ | 0.028 |
| Transition | 1.0$^{bA}$ | 2.0 $^{abA}$ | 1.7 $^{bA}$ | 1.7 $^{bA}$ | 3.0$^{aA}$ | <0.001 |
| Rainy | 1.2 $^{aA}$ | 1.7 $^{aA}$ | 1.3 $^{aA}$ | 1.7 $^{aA}$ | 1.5 $^{aB}$ | 0.668 |
| P-value | 0.945 | 0.726 | 0.831 | 0.571 | <0.001 | |
| | | | Live:dead leaf ratio | | | |
| Establishment | 5.4$^{aB}$ | 3.9 $^{aA}$ | 2.3 $^{aB}$ | 5.3 $^{aB}$ | 5.1 $^{aC}$ | 0.281 |
| Dry | 10.5 $^{abA}$ | 6.0$^{bcA}$ | 3.5$^{cAB}$ | 11.7 $^{aA}$ | 6.3 $^{bcBC}$ | <0.001 |
| Transition | 5.2 $^{bB}$ | 5.3 $^{bA}$ | 6.7 $^{bA}$ | 7.9 $^{aAB}$ | 8.5 $^{aA}$ | <0.001 |
| Rainy | 6.4$^{bAB}$ | 8.0 $^{abA}$ | 6.6 $^{bAB}$ | 10.4 $^{aA}$ | 9.4 $^{aB}$ | 0.017 |
| P-value | 0.006 | 0.095 | 0.016 | <0.001 | <0.001 | |

Note: Different lowercase letters indicate differences between cultivars by Tukey's test at 5% significance and different capital letters indicate differences between periods by Tukey's test at 5% significance. Establishment period (110 days), dry season (84 days), transition period (111 days), and rainy season (51 days).

### Chemical Composition

There was no significant cultivar × period interaction effect for the DM (p=0.2770) or CP (p=0.8976) contents. A cultivar effect, however, occurred for the DM content (p=0.0014), whose highest values were observed in cv. Paiaguás and the lowest in cvs. Basilisk and Xaraés, while the other cultivars showed intermediate values (Figure 2a). As regards the effect of evaluation periods (p<0.0001), the establishment, dry, and transition periods did not differ from each other, whereas the rainy season was the period when the lowest DM values were recorded (Figure 2b).

The cultivars differed in their CP contents (p<0.0082), with the highest values occurring in cv. Basilisk and the lowest in cvs. Piatã and Xaraés (Figure 3a). A period effect (p<0.000) was also detected for CP content, which was highest in the dry and transition periods and lowest in the establishment and rainy periods (Figure 3b).

There was a cultivar × period interaction effect for the percentages of OM (p<0.0001), NDF (p<0.0000), ADF (p<0.0001), and protodioscin content (p<0.0001). The highest concentrations of OM were observed in cvs. Piatã and Xaraés in the establishment period; cv. Marandu in the dry season; cv. Paiaguás in the transition period; and cv. Basilisk in the rainy season. The lowest OM content was detected in cv. Basilisk in the dry season (Table 3).

The highest NDF contents were observed in cv. Marandu in the establishment and dry periods (737.7 and 722.0 g/kg DM, respectively); cv. Piatã in its establishment (728.7 g/kg DM); and cv. Xaraés in the rainy season (734.7 g/kg DM). The lowest NDF content was found in cv. Piatã in the transition period (633.0 g/kg DM). As for ADF, the highest contents were observed in cvs. Basilisk, Piatã, and Xaraés in the establishment period (408.0, 408.2, and 443.7 g/kg DM, respectively); cvs. Marandu, Piatã, and Xaraés in the dry season; cvs. Piatã and Xaraés in the transition period; and cvs. Paiaguás and Xaraés in the rainy season. On the other hand, the lowest ADF content was detected in cv. Basilisk (329.0 g/kg DM) in the transition period (Table 3).

In terms of protodioscin, the highest contents were observed in cv. Basilisk (5.5 g/kg DM) in its establishment period, and the lowest in cv. Piatã (1.0 g/kg DM) in the dry season.

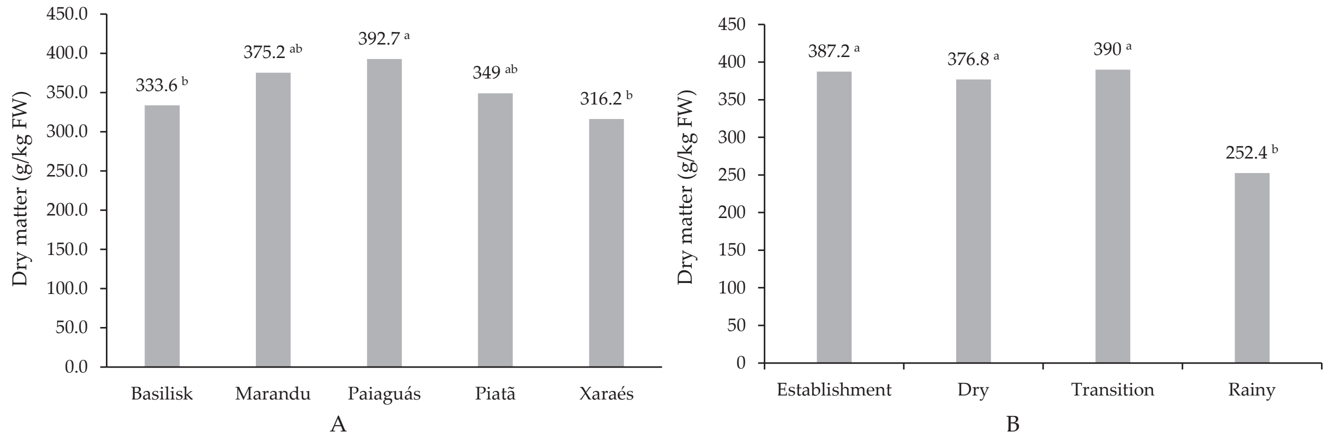


Figure 2. Dry matter contents of *Brachiaria* spp. in different cultivars (a) and periods (b). Values followed by different lowercase letters differ (p<0.05) according to Tukey's test.

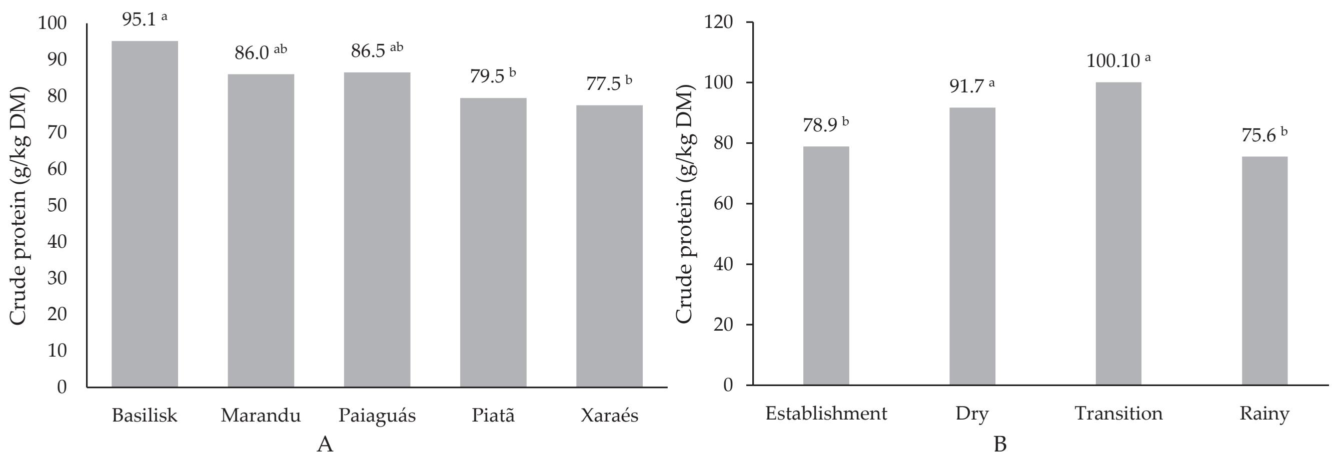


Figure 3. Crude protein contents of the leaf blade of *Brachiaria* spp. in different cultivars (a) and periods (b). Values followed by different lowercase letters differ (p<0.05) according to Tukey's test.

Table 3. Chemical composition of leaf blades of *Brachiaria* cultivars evaluated in different periods

| Period | Cultivar | | | | | |
|---|---|---|---|---|---|---|
| | Basilisk | Marandu | Paiaguás | Piatã | Xaraés | P |
| | | | Organic matter (g/kg DM) | | | |
| Establishment | 937.2 $^{aB}$ | 945.5 $^{aB}$ | 915.2 $^{bA}$ | 947.7 $^{aA}$ | 946.2 $^{aA}$ | <0.001 |
| Dry | 913.5 $^{cC}$ | 981.2 $^{aA}$ | 929.2 $^{bcA}$ | 931.0 $^{bB}$ | 918.5 $^{bcB}$ | <0.001 |
| Transition | 921.2 $^{aC}$ | 926.0 $^{aC}$ | 924.7 $^{aA}$ | 934.0 $^{aAB}$ | 930.7 $^{aAB}$ | 0.234 |
| Rainy | 982.7 $^{aA}$ | 928.2 $^{bC}$ | 921.0 $^{bA}$ | 925.5 $^{bB}$ | 930.5 $^{bAB}$ | <0.001 |
| P-value | <0.001 | <0.001 | 0.129 | 0.004 | <0.001 | |
| | | | Neutral detergent fiber (g/kg DM) | | | |
| Establishment | 675.7 $^{bAB}$ | 737.7 $^{aA}$ | 714.0 $^{abA}$ | 728.7 $^{aA}$ | 721.0 $^{abAB}$ | 0.009 |
| Dry | 654.0 $^{bcB}$ | 722.0 $^{aA}$ | 633.0 $^{cB}$ | 667.5 $^{bcB}$ | 696.0 $^{abAB}$ | <0.001 |
| Transition | 610.2 $^{bC}$ | 616.0 $^{bB}$ | 622.7 $^{bB}$ | 559.2 $^{cC}$ | 683.0 $^{aB}$ | <0.001 |
| Rainy | 706.2 $^{abA}$ | 704.0 $^{abA}$ | 713.2 $^{abA}$ | 679.7 $^{bB}$ | 734.7 $^{aA}$ | 0.048 |
| P-value | <0.001 | <0.001 | <0.001 | <0.001 | 0.013 | |
| | | | Acid detergent fiber (g/kg DM) | | | |
| Establishment | 408.0 $^{aA}$ | 396.5 $^{aAB}$ | 389.0 $^{aAB}$ | 408.2 $^{aA}$ | 443.7 $^{aA}$ | 0.069 |
| Dry | 354.2 $^{bBC}$ | 420.2 $^{aA}$ | 357.5 $^{bB}$ | 427.0 $^{aA}$ | 436.5 $^{aA}$ | <0.001 |
| Transition | 329.0 $^{bC}$ | 358.2 $^{abB}$ | 374.0 $^{abB}$ | 383.5 $^{aA}$ | 407.0 $^{aA}$ | 0.001 |
| Rainy | 379.7 $^{bAB}$ | 390.0 $^{abAB}$ | 437.5 $^{aA}$ | 430.7 $^{abA}$ | 439.5 $^{aA}$ | 0.004 |
| P-value | 0.005 | 0.016 | 0.001 | 0.068 | 0.245 | |
| | | | Protodioscin content (g/kg DM) | | | |
| Establishment | 5.5 $^{Aa}$ | 3.0 $^{bA}$ | 2.9 $^{bA}$ | 1.9 $^{cB}$ | 1.9 $^{Ca}$ | <0.001 |
| Dry | 1.5 $^{Bc}$ | 2.3 $^{aC}$ | 2.2 $^{aB}$ | 1.2 $^{cC}$ | 1.3 $^{Cb}$ | <0.001 |
| Transition | 5.1 $^{aB}$ | 2.7 $^{cB}$ | 2.9 $^{bA}$ | 2.0 $^{dA}$ | 2.0 $^{dA}$ | <0.001 |
| Rainy | 1.2 $^{Ad}$ | 0.9 $^{bD}$ | 0.9 $^{bC}$ | 1.0 $^{bD}$ | 0.9 $^{Bc}$ | <0.001 |
| P-value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | |

Note: Different lowercase letters indicate differences between cultivars by Tukey's test at 5% significance and different capital letters indicate differences between periods by Tukey's test at 5% significance. Establishment period (110 days), dry season (84 days), transition period (111 days), and rainy season (51 days).

## *In Vitro* Digestibility

There were significant interaction effects between cultivars and periods ($p<0.05$) for the *in vitro* digestibility of dry matter ($IVD_{DM}$), crude protein ($IVD_{CP}$), neutral detergent fiber ($IVD_{NDF}$), and acid detergent fiber ($IVD_{ADF}$) of the leaf blade (Table 4).

The highest $IVD_{DM}$ means of the leaf blade were observed in cv. Basilisk in the dry, transition, and rainy periods; cv. Marandu in the transition period; and cvs. Paiaguás and Piatã in their establishment. The lowest $IVD_{DM}$ mean, in turn, was detected in cv. Marandu in its establishment period (Table 4).

For $IVD_{CP}$, the highest values were obtained by cv. Basilisk in the rainy season; cvs. Basilisk, Marandu, and Xaraés in the transition period; and cv. Xaraés in the rainy season. The lowest $IVD_{CP}$ was detected in cvs. Paiaguás and Piatã in the rainy season (Table 4).

The highest $IVD_{NDF}$ was observed in cvs. Piatã and Xaraés in the establishment period; cv. Xaraés in the dry season; cv. Marandu in the transition period; and cv. Paiaguás in the rainy season. The lowest $IVD_{NDF}$, in turn, was found in cv. Piatã in the transition period. In the period of establishment, the lowest $IVD_{ADF}$ occurred in cv. Marandu (387.0 g/kg of DM) and the highest in cv. Piatã (721.0 g/kg DM). Cultivar Xaraés in the dry season and cv. Paiaguás in the rainy season exhibited the highest $IVD_{ADF}$ means (668.5 and 715.0 g/kg DM, respectively). The $IVD_{ADF}$ of cv. Basilisk did not vary between the periods. Cultivars Marandu and Paiaguás showed lower digestibility in the establishment and dry periods. These results increased with the increasing rainfall in the transition and rainy periods. Cultivars Piatã and Xaraés, in turn, showed the opposite behavior, with higher $IVD_{ADF}$ means in the establishment and dry periods and lower values in the transition and rainy periods.

## Cumulative *In Vitro* Gas Production

There was a cultivar × period interaction effect for cumulative gas production (Y) ($p<0.0001$), volume of gas produced by the degradation of NFC and soluble compounds ($V_{fr}$) ($p<0.0001$), rate of degradation of the rapid fraction ($Kd_{ff}$) ($p<0.0001$), time of particle colonization by the bacteria (Lag time) ($p<0.0001$), the volume of gas produced by the degradation of fibrous carbohydrates ($V_{fl}$) ($p<0.0001$), and rate of degradation of fibrous carbohydrates ($K_{sf}$) ($p<0.0001$) (Table 5).

Cumulative gas production was highest in cv. Basilisk in the establishment period and dry season and in cv. Piatã in the rainy season, whereas the lowest average total production was found in cv. Xaraés in the dry season (Table 6). Regarding the $V_{fr}$ fraction, the largest amounts were produced by cv. Marandu in the transition period; cv. Paiaguás in the transition period and rainy season; cv. Piatã in the establishment and dry

Table 4. *In vitro* digestibility of the leaf blade of *Brachiaria* spp. cultivars in different periods

| Period | Cultivar | | | | | |
|---|---|---|---|---|---|---|
| | Basilisk | Marandu | Paiaguás | Piatã | Xaraés | P |
| | | | $IVD_{DM}$ (g/kg DM) | | | |
| Establishment | 761.5$^{aB}$ | 641.0$^{cC}$ | 761.0$^{aA}$ | 763.5$^{aA}$ | 699.0$^{bA}$ | <0.001 |
| Dry | 820.0$^{aA}$ | 752.5$^{bcB}$ | 765.5$^{bA}$ | 737.5$^{bcA}$ | 725.0$^{cA}$ | <0.001 |
| Transition | 825.5$^{aA}$ | 796.0$^{aA}$ | 752.0$^{bA}$ | 747.5$^{bA}$ | 714.0$^{cA}$ | <0.001 |
| Rainy | 804.0$^{aA}$ | 762.0$^{bB}$ | 759.5$^{bA}$ | 738.5$^{abA}$ | 715.5$^{bA}$ | <0.001 |
| P-value | <0.001 | <0.001 | 0.617 | 0.066 | 0.099 | |
| | | | $IVD_{CP}$ (g/kg DM) | | | |
| Establishment | 884.5 $^{aAB}$ | 792.5$^{cdB}$ | 834.0$^{bcA}$ | 786.5$^{dB}$ | 875.0$^{abA}$ | <0.001 |
| Dry | 919.5$^{aA}$ | 878.5$^{abA}$ | 828.5$^{cA}$ | 856.5$^{bcA}$ | 867.0$^{bcA}$ | <0.001 |
| Transition | 909.5$^{aA}$ | 891.5$^{aA}$ | 819.5$^{bA}$ | 863.0$^{abA}$ | 885.5 $^{aA}$ | <0.001 |
| Rainy | 839.5$^{aB}$ | 824.0$^{aB}$ | 767.0$^{bB}$ | 776.0$^{bB}$ | 849.0$^{aA}$ | <0.001 |
| P-value | <0.001 | <0.001 | 0.001 | <0.001 | 0.172 | |
| | | | $IVD_{NDF}$ (g/kg DM) | | | |
| Establishment | 614.5$^{cB}$ | 550.5$^{dB}$ | 707.0$^{bB}$ | 747.0$^{aA}$ | 771.5$^{aA}$ | <0.001 |
| Dry | 691.0$^{bA}$ | 707.0$^{bA}$ | 714.0$^{bB}$ | 684.0$^{bB}$ | 795.5$^{aA}$ | <0.001 |
| Transition | 679.5$^{bA}$ | 714.0$^{aA}$ | 703.0$^{abB}$ | 642.5$^{cC}$ | 639.5$^{cB}$ | <0.001 |
| Rainy | 696.0$^{bA}$ | 711.0$_{bA}$ | 754.5 $^{aA}$ | 718.0$^{bA}$ | 655.5$^{cB}$ | <0.001 |
| P-value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | |
| | | | $IVD_{ADF}$ (g/kg DM) | | | |
| Establishment | 520.5$^{cA}$ | 387.0$^{dB}$ | 610.0$^{bB}$ | 721.0$^{aA}$ | 622.0$^{bA}$ | <0.001 |
| Dry | 564.5$^{bA}$ | 608.0$^{abA}$ | 653.0$^{aAB}$ | 656.5$^{aAB}$ | 668.5$^{aA}$ | 0.009 |
| Transition | 538.5$^{bA}$ | 614.0$^{abA}$ | 660.5$^{aAB}$ | 630.5$^{aB}$ | 535.5$^{bB}$ | <0.001 |
| Rainy | 570.0$^{cA}$ | 598.0$^{bcA}$ | 715.0$^{aA}$ | 683.0$^{abAB}$ | 525.0$^{cB}$ | <0.001 |
| P-value | 0.248 | <0.001 | 0.018 | 0.044 | <0.001 | |

Note: Different lowercase letters indicate differences between cultivars by Tukey's test at 5% significance and different capital letters indicate differences between periods by Tukey's test at 5% significance. Establishment period (110 days), dry season (84 days), transition period (111 days), and rainy season (51 days).

periods; and cv. Xaraés in the establishment period. The lowest production of fraction A was found in cv. Paiaguás during the establishment period.

The $K_{dff}$ fraction value was highest in cv. Xaraés in the dry season and lowest in cvs. Xaraés and Basilisk in the establishment and transition periods, respectively. Lag time was the longest in cvs. Paiaguás, Piatã, and Xaraés and shortest in cvs. Basilisk and Marandu in the dry season. As for the $V_{fl}$ fraction, the largest volumes were produced by cv. Marandu in the establishment period and cv. Piatã in the rainy season, and the smallest by cv. Paiaguás in the rainy season. With respect to the $K_{sf}$ fraction, the highest production was achieved by cv. Xaraés in the establishment period and the lowest by the same cultivar in the dry, transition, and rainy periods.

### Pearson Correlation Analysis

Dry matter was positively correlated with CP and $IVD_{CP}$. Crude protein correlated positively with $IVD_{DM}$, $IVD_{CP,}$ and protodioscin. Neutral detergent fiber showed a negative correlation with $IVD_{DM}$, $IVD_{OM}$, $IVD_{CP,}$ and protodioscin. Finally, ADF fiber correlated negatively with $IVD_{CP}$ and protodioscin and positively with L:S (Table 6).

The $IVD_{DM}$ correlated positively with the *in vitro* digestibility of CP, NDF, and ADF. The $IVD_{CP}$, in turn, was negatively correlated with $IVD_{ADF}$ and positively correlated with the protodioscin content. There was a negative correlation between $IVD_{NDF}$ and protodioscin and a positive correlation between $IVD_{NDF}$ and L:S. The $IVD_{ADF}$ correlated negatively with protodioscin.

## DISCUSSION

### Pasture Structure

The observed interactions between the *Brachiaria* cultivars and evaluation periods (Table 2) demonstrate the influence of climatic variability in the semi-arid environment on the development of these grasses. During their establishment, plants naturally require a longer time interval to reach the harvest target than expected in already established pastures, as the processes of emergence and initial tissue growth occur in this interval (Mariani *et al.,* 2012). Due to the initial plant development processes, the evaluated *Brachiaria* cultivars took 110 days to reach the harvest target in the establishment period (Rodrigues *et al.*, 2021); however, forage accumulation was lower than in the transition and rainy periods (84 and 51 days, respectively).

Although the establishment period was the longest in the number of days, forage accumulation in this period was lower than in the transition and rainy seasons. In the rainy season, the plants reached the 90% LI target in 51 days, corresponding to approximately 50% of the time they took to reach the same goal in the other periods. Water availability was probably the limiting factor

Table 5. Gas production kinetics of *Brachiaria* spp. cultivars in different periods

| Variables | Cultivar | | | | | |
|---|---|---|---|---|---|---|
| | Basilisk | Marandu | Paiaguás | Piatã | Xaraés | P |
| | | | Establishment | | | |
| Y (mL/100 mg DM) | $19.10^{aA}$ | $17.32^{bcA}$ | $14.91^{dB}$ | $16.73^{cB}$ | $18.60^{abA}$ | <0.001 |
| $V_{fr}$ (mL/100 mg DM) | $5.57^{aAB}$ | $3.26^{bB}$ | $3.22^{bC}$ | $6.59^{aA}$ | $5.89^{aA}$ | 0.002 |
| $K_{dff}$ (/h) | $0.82^{abAB}$ | $0.98^{aA}$ | $0.98^{aA}$ | $0.85^{abA}$ | $0.60^{bB}$ | <0.001 |
| *Lag* (h) | $0.49^{bcA}$ | $0.35^{cAB}$ | $0.61^{bB}$ | $1.00^{aA}$ | $1.00^{aA}$ | <0.001 |
| $V_{fl}$ (mL/100 mg DM) | $13.53^{abA}$ | $14.05^{aA}$ | $11.69^{bcA}$ | $10.13^{cB}$ | $12.71^{abA}$ | <0.001 |
| $K_{sf}$ (/h) | $0.03^{bA}$ | $0.03^{bA}$ | $0.03^{bA}$ | $0.03^{bA}$ | $0.51^{Aa}$ | <0.001 |
| $R^2$ | 0.99 | 0.99 | 0.97 | 0.99 | 0.98 | |
| | | | Dry | | | |
| Y (mL/100 mg DM) | $19.00^{aA}$ | $15.84^{cB}$ | $17.03^{bcA}$ | $17.99^{abB}$ | $13.97^{dC}$ | <0.001 |
| $V_{fr}$ (mL/100 mg DM) | $7.17^{abA}$ | $3.49^{cB}$ | $5.31^{bcB}$ | $7.79^{aA}$ | $4.59^{cA}$ | <0.001 |
| $K_{dff}$ (/h) | $0.61^{bB}$ | $0.98^{aA}$ | $0.84^{abA}$ | $0.78^{abA}$ | $0.98^{aA}$ | <0.001 |
| *Lag* (h) | $0.12^{bB}$ | $0.26^{bB}$ | $1.00^{aA}$ | $1.00^{aA}$ | $1.00^{aA}$ | <0.001 |
| $V_{fl}$ (mL/100 mg DM) | $11.82^{abA}$ | $12.34^{aAB}$ | $11.72^{abA}$ | $10.20^{bcB}$ | $9.38^{cB}$ | <0.001 |
| $K_{sf}$ (/h) | $0.03^{aA}$ | $0.03^{aA}$ | $0.03^{aA}$ | $0.02^{aA}$ | $0.04^{aB}$ | 0.998 |
| $R^2$ | 0.97 | 0.98 | 0.99 | 0.99 | 0.99 | |
| | | | Transition | | | |
| Y (mL/100 mg DM) | $14.60^{cC}$ | $15.93^{bcB}$ | $18.12^{aA}$ | $14.89^{cC}$ | $16.80^{abB}$ | <0.001 |
| $V_{fr}$ (mL/100 mg DM) | $5.30^{bAB}$ | $6.02^{aA}$ | $7.55^{aA}$ | $6.27^{abAB}$ | $6.02^{abA}$ | 0.04 |
| $K_{dff}$ (/h) | $0.59^{bB}$ | $0.74^{abA}$ | $0.85^{abA}$ | $0.88^{aA}$ | $0.69^{abB}$ | 0.021 |
| *Lag* (h) | $0.44^{bA}$ | $0.47^{bA}$ | $1.00^{aA}$ | $1.00^{aA}$ | $1.00^{aA}$ | <0.001 |
| $V_{fl}$ (mL/100 mg DM) | $9.30^{abB}$ | $9.91^{abC}$ | $10.56^{aA}$ | $8.60^{bB}$ | $10.77^{aB}$ | 0.014 |
| $K_{sf}$ (/h) | $0.03^{aA}$ | $0.03^{aA}$ | $0.02^{aA}$ | $0.03^{aA}$ | $0.03^{aB}$ | 10.000 |
| $R^2$ | 0.98 | 0.98 | 0.99 | 0.98 | 0.99 | |
| | | | Rainy | | | |
| Y (mL/100 mg DM) | $16.59^{bB}$ | $15.27^{bcB}$ | $14.92^{cB}$ | $20.31^{aA}$ | $14.69^{cC}$ | <0.001 |
| $V_{fr}$ (mL/100 mg DM) | $4.53^{bB}$ | $3.47^{bB}$ | $7.41^{aA}$ | $4.45^{bB}$ | $4.26^{bA}$ | <0.001 |
| $K_{dff}$ (/h) | $0.88^{aA}$ | $0.99^{aA}$ | $0.85^{aA}$ | $0.98^{aA}$ | $0.98^{aA}$ | 0.445 |
| *Lag* (h) | $0.64^{bA}$ | $0.27^{cAB}$ | $1.00^{aA}$ | $1.00^{aA}$ | $1.00^{aA}$ | <0.001 |
| $V_{fl}$ (mL/100 mg DM) | $12.06^{bA}$ | $11.80^{bB}$ | $7.50^{cB}$ | $15.86^{aA}$ | $10.70^{bB}$ | <0.001 |
| $K_{sf}$ (/h) | $0.03^{aA}$ | $0.02^{aA}$ | $0.04^{aA}$ | $0.04^{aA}$ | $0.04^{aB}$ | 0.100 |
| $R^2$ | 0.99 | 0.97 | 0.97 | 0.99 | 0.98 | |

Note: Different lowercase letters indicate differences between cultivars by Tukey's test (p<0.05) and different capital letters indicate differences between periods by Tukey's test (p<0.0001). Y= cumulative gas production; $V_{fr}$= volume of gas produced by the degradation of non-fibrous carbohydrates and soluble compounds; $K_{dff}$= rate of degradation of non-fibrous carbohydrates and soluble compounds; L= time of particle colonization by the bacteria; $V_{fl}$= volume of gas produced by the degradation of fibrous carbohydrates; $K_{sf}$= fibrous carbohydrate degradation rate. Establishment period (110 days), dry season (84 days), transition period (111 days), and rainy season (51 days).

Table 6. Pearson correlations between chemical composition, digestibility, and *in vitro* degradation kinetic variables and structural components of *Brachiaria* spp.

| Variables | DM | OM | CP | NDF | ADF | $IVD_{DM}$ | $IVD_{CP}$ | $IVD_{NDF}$ | $IVD_{ADF}$ | PROTO | L:S | $V_{fr}$ | $V_{fl}$ | Y |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DM | - | $-0.12^{ns}$ | $0.28^{*}$ | $-0.34^{*}$ | $-0.39^{*}$ | $0.01^{ns}$ | $0.28^{*}$ | $-0.10^{ns}$ | $0.04^{ns}$ | $0.42^{ns}$ | $0.14^{ns}$ | $0.09^{ns}$ | $-0.05^{ns}$ | $0.02^{ns}$ |
| $OM_{DM}$ | | - | $-0.17^{ns}$ | $0.34^{*}$ | $0.15^{ns}$ | $-0.08^{ns}$ | $0.02^{ns}$ | $-0.10^{ns}$ | $-0.15^{ns}$ | $0.04^{ns}$ | $0.09^{ns}$ | $-0.21^{*}$ | $0.23^{*}$ | $0.07^{*}$ |
| CP | | | - | $-0.58^{ns}$ | $-0.46^{ns}$ | $0.41^{*}$ | $0.39^{*}$ | $-0.09^{*}$ | $0.02^{ns}$ | $0.26^{*}$ | $-0.05^{ns}$ | $0.24^{*}$ | $-0.16^{ns}$ | $0.01^{ns}$ |
| NDF | | | | - | $0.58^{ns}$ | $-0.40^{*}$ | $-0.34^{*}$ | $0.10^{ns}$ | $-0.13^{ns}$ | $-0.28^{*}$ | $0.14^{ns}$ | $-0.37^{*}$ | $0.29^{*}$ | $0.02^{ns}$ |
| ADF | | | | | - | $-0.50^{ns}$ | $-0.29^{*}$ | $0.18^{ns}$ | $0.16^{ns}$ | $-0.35^{*}$ | $0.21^{*}$ | $0.03^{ns}$ | $0.09^{ns}$ | $0.06^{ns}$ |
| $IVD_{DM}$ | | | | | | - | $0.35^{*}$ | $0.28^{*}$ | $0.25^{*}$ | $0.17^{ns}$ | $-0.44^{ns}$ | 0.22* | $-0.28^{*}$ | $-0.12^{ns}$ |
| $IVD_{CP}$ | | | | | | | - | $-0.11^{ns}$ | $-0.34^{*}$ | 0.36* | $0.03^{ns}$ | $0.14^{ns}$ | $-0.12^{ns}$ | $0.02^{ns}$ |
| $IVD_{NDF}$ | | | | | | | | - | $0.81^{ns}$ | $-0.41^{*}$ | $0.24^{*}$ | $0.18^{ns}$ | -0.29* | $-0.17^{ns}$ |
| $IVD_{ADF}$ | | | | | | | | | - | $-0.40^{*}$ | $0.14^{ns}$ | $0.39^{*}$ | $-0.35^{*}$ | $-0.07^{ns}$ |
| PROTO | | | | | | | | | | - | $-0.21^{ns}$ | $-0.05^{ns}$ | $0.09^{ns}$ | $0.11^{ns}$ |
| L:S | | | | | | | | | | | - | $0.45^{ns}$ | $0.02^{ns}$ | $0.06^{ns}$ |
| $V_{fr}$ | | | | | | | | | | | | - | $-0.46^{ns}$ | $0.29^{*}$ |
| $V_{fl}$ | | | | | | | | | | | | | - | $0.70^{ns}$ |
| Y | | | | | | | | | | | | | | - |

Note: *p<0.05; DM = dry matter; OM= organic matter; NDF= neutral detergent fiber; ADF= acid detergent fiber; IVDDM= *in vitro* digestibility of dry matter; IVDCP= *in vitro* digestibility of crude protein; IVDNDF= *in vitro* digestibility of neutral detergent fiber; IVDADF= *in vitro* digestibility of acid detergent fiber; PROTO= protodioscin content (g/kg DM); L:S= leaf:stem ratio; $V_{fr}$= gas volume resulting from the fermentation of the rapidly degradable fraction (mL gas/100 g DM); $V_{fl}$= gas volume resulting from the fermentation of the slowly degradable fraction (mL gas/100 g DM) and Y= cumulative gas production.

for forage production in the other periods (Figure 1) since the soil was corrected and the nutritional requirements of the plants were properly met (Oliveira *et al.,* 2016).

Increases in forage mass lead to changes in the structural composition of the pasture, with increasing percentages of the stem relative to leaf blades, and, at critical times, increasing senescent material (Table 2). However, in cv. Xaraés, the increase in accumulation rate being concomitant with increases in the live:dead leaf and leaf:stem ratios during the rainy season suggest better pasture quality since the leaf blade is the plant component with the highest nutritional value (Emerenciano *et al.,* 2018 ).

### Chemical Composition

The DM content of a forage plant is a consequence of its momentary physiological stage in response to environmental conditions (Campos *et al.,* 2016). Accordingly, low water availability in the soil reduces the amount of water in the plant tissues (Oliveira *et al.,* 2017). This was evidenced by the higher DM contents recorded in the dry and transition periods and lower values found in the rainy season for all forage cultivars evaluated (Figure 2b).

Under the same climatic and management conditions, variability between plants tends to be the result of genetic expression. In dry environments, mainly, the quality of forage species depends on the ability of plants to maintain a favorable water status in their tissues (Mwendia *et al.,* 2013). In this respect, Euclides *et al.* (2016) observed that the leaf blades of *Brachiaria decumbens* had higher CP contents than the leaves of *Brachiaria brizantha* (Figure 3a). Cultivar Paiaguás, in turn, has the characteristic of maintaining its nutritional value even during the dry season (Valle *et al.,* 2013).

The high concentrations of CP in the transition period may be related to a compensation mechanism employed by plants, whereby they use the accumulated and unused solutes for biosynthesis during the period of water scarcity to enable a rapid increase in leaf tissue when the ideal conditions for plant growth are resumed (Guenni *et al.,* 2002).

The lower ADF content found in cv. Basilisk in the transition period is a favorable condition, as it increases the digestibility potential of the forage (Table 3). In forages, the ADF concentration refers to the proportion of cell walls made up of cellulose and lignin (Sriagtula *et al.,* 2021), constrictors representing the main digestibility limit (Pariz *et al.,* 2011). According to Van Soest (1994), ADF starts to have a negative correlation with digestibility when it exceeds 40%, a percentage that was not found in the leaf blade of these cultivars.

There was a high correlation between the protodioscin content in the leaf blade and the different regrowth ages obtained in the different periods (Table 6). The highest protodioscin levels were associated with the periods in which the plants took the longest to reach the harvest target, regardless of rainfall. A different response was described by Moreira *et al.* (2009), who found a higher protodioscin content in the rainy season.

The shorter periods (dry and rainy), with younger plants, correspond to the times when this protodioscin is found at lower concentrations. These results differ from those presented by Riet-Correa *et al.* (2011), who found higher saponin contents in *Brachiaria* plants at budding. However, photosensitization outbreaks in cattle and sheep at all times of the year (Souza *et al.,* 2010; Mustafa *et al.,* 2012) suggest that other unknown factors influence the protodioscin content of pastures. This shows that the levels of saponins in plants are not constant, varying between cultivars and times of year (Melo *et al.,* 2019; Leal *et al.,* 2020).

### *In Vitro* Digestibility and Degradation Kinetics

*In vitro* dry matter digestibility was negatively correlated with the NDF content. As seen in the present study, cv. Basilisk had the lowest NDF contents and the highest $IVD_{DM}$. Leal *et al.* (2016) worked with different *Brachiaria* cultivars and found similar results, where increasing fiber percentages led to lower $IVD_{DM}$ values.

The higher $IVD_{DM}$, $IVD_{CP,}$ and $IVD_{NDF}$ percentages of the cultivars seen in the dry season may be a response to the nitrogen fertilization applied after the establishment period. Dupas *et al.* (2016) described results showing that the application of nitrogen affected the CP of the forage, increasing its quality due to digestion rates, including fibrous components. Likewise, Peyraud *et al.* (1998) explained that the concentration of fibrous components could decrease when there is an increase in CP and other soluble substances in the cell content, which accumulate inside the cell, diluting the cell wall.

The lower digestibility of cvs. Basilisk, Marandu, Paiaguás, and Piatã in the rainy season can be explained by the acceleration of forage growth processes that occurs under optimal rainfall conditions, which favor an increase in structural components. Quintino *et al.* (2013) examined the production and nutritional value of *Brachiaria* cultivars at different harvest ages and observed that $IVD_{DM}$ was lowest in the rainy season when climatic conditions favored pasture growth. This result, coupled with the yield data, demonstrates that water availability can indicate the yield potential. However, in qualitative terms, this statement may depend on other factors, such as temperature or genetic mechanisms. Cultivars Paiaguás, Piatã, and Xaraés showed no variation in $IVD_{DM}$ between the periods. This finding indicates that these cultivars may have stability mechanisms in their fibrous components even with climatic variations. Additionally, it suggests that they would exhibit good adaptation and productivity in a semi-arid region.

In this study, the degradation of fibrous fractions in the rumen fluid varied across cultivars and periods. This variable is linked to the amount of fiber present in each grass, and different types of tissue influence forage digestibility. The higher percentages of NDF and ADF found to explain the lower $IVD_{DM}$ detected in cvs. Piatã and Xaraés as well as the lower cumulative gas production over the periods in cv. Xaraés. Thus, the fibrous components present in the cell wall—indigestible compounds, mainly—form a barrier that prevents microbial adhesion and enzymatic hydrolysis of cellulose and hemicellulose. As a result, the potentially degradable structural carbohydrates become unavailable, reducing fiber digestibility and the quality, and utilization of the forage plant (Rodrigues *et al.*, 2014).

The negative correlation observed between the volume of gas produced by the fermentation of the rapidly degradable fraction ($V_{fr}$) and the NDF content (Table 6) is expected since larger volumes of gas at this stage of degradation are associated with cell content. High fiber levels translate into low amounts of cell content (Reis *et al.*, 2012; Rodrigues *et al.*, 2015). The positive correlation between the $V_{fr}$ fraction and CP, $IVD_{DM,}$ and $IVD_{OM}$ implies that the higher the CP content and the more digestible the dry matter and organic matter, the larger the amount of gas produced by the $V_{fr}$ fraction.

Fraction D corresponds to the degradation of fibrous carbohydrates (Oliveira *et al.*, 2017). Thus, larger volumes of gas produced from the $V_{fl}$ fraction positively correlate with NDF and fiber quality in a forage plant, which can strongly indicate pasture quality.

The results presented here regarding production, digestibility, and kinetics of *in vitro* rumen degradation disprove the hypothesis that cvs. Marandu, Basilisk, and Paiaguás are more resistant to the seasonal effects of forage production and have fewer oscillations in nutritional value than other *Brachiaria* cultivars (Euclides *et al.*, 2016; Veras *et al.*, 2020) since all cultivars responded similarly during the evaluated periods. Therefore, the five cultivars evaluated in this study are potential alternatives for use in the semi-arid region of Brazil.

## CONCLUSION

The *Brachiaria* spp. cultivars Basilisk, Marandu, Paiaguás, Piatã, and Xaraés are potential options for forage production in semi-arid environments, given their ability to maintain increases in forage accumulation rate and low variability of nutritional value even in periods of lower water availability. This is an important characteristic of production systems in the Brazilian semi-arid environment, where forage restriction is the major limiting factor for animal production. However, due to the high content of protodioscin, the Basilisk cultivar should not be used in small ruminant production systems.

## CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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