Research, Society and Development, v. 13, n. 2, e8613244974, 2024
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v13i2.44974

# The tissue composition of Mulato II grass under different canopy structures and its impact on digestibility

**Composição tecidual de capim-mulato II sob diferentes estruturas de dossel e seu impacto sob a digestibilidade**

**Composición tisular de pasto Mulato II bajo diferentes estructuras de dosel y su impacto en la digestibilidad**

Received: 01/29/2024 | Revised: 02/15/2024 | Accepted: 02/16/2024 | Published: 02/20/2024

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**Abstract**
Different heights of forage management modify the plant architecture, changing the proportion of young and mature tissues and its nutritional value. Thus, the objective of this study was to evaluate the tissue composition of leaves of hybrid *Urochloa* cv. Mulato II (syn. *Brachiaria*) kept under four management conditions simulating continuous grazing in the rainy and dry seasons. In addition, the effect of the variation of the percentage of tissues in the leaf on its digestibility was carried out based on the contents of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and *in vitro* dry matter digestibility (IVDMD). Four stocking levels were evaluated simulating continuous grazing, with a canopy height of 10, 20, 30, and 40 cm. It was found that the pastures with 10 and 20 cm had the lowest percentage of lignified tissues in the rainy and dry seasons, which consequently resulted in greater tissue disappearance, mainly of the mesophyll. Pastures with 10 cm had higher CP contents, lower NDF and ADF contents, and consequently higher IVDMD. Therefore, the Mulato II grassland managed with 10 cm had greater degradability of the leaf tissues, due to the higher percentage of rapidly digestible tissues.
**Keywords:** Continuous grazing; Defoliation severity; Leaf anatomy; *Urochloa* hybrid.

**Resumo**
Diferentes alturas de manejo da forragem modificam a arquitetura da planta, alterando a proporção dos tecidos jovens e maduros e o seu valor nutricional. Desta forma, objetivou-se avaliar a composição tecidual de folhas de *Urochloa* híbrida cv. Mulato II (syn. *Brachiaria*) mantida sob quatro condições de manejo simulando pastejo contínuo na época das águas e da seca. Além disso, avaliou-se o efeito da variação da porcentagem de tecidos na folha sob sua digestibilidade com base nos teores de proteína bruta (PB), de fibra em detergente neutro (FDN), de fibra em

detergente ácido (FDA) e na digestibilidade *in vitro* da matéria seca (DIVMS). Foram avaliados quatro níveis de lotação simulando pastejo contínuo, com manutenção da altura do dossel 10, 20, 30 e 40 cm. Foi verificado que os pastos com 10 e 20 cm apresentaram a menor porcentagem de tecidos lignificados nas águas e secas, o que consequentemente resultou em maior desaparecimento de tecidos, principalmente do mesófilo. Pastos com 10 cm apresentaram maiores teores de PB, menores teores de FDN e FDA, e consequentemente maior DIVMS. Portanto, conclui-se que o capim-mulato II manejado com 10 cm apresentou maior degradabilidade dos tecidos foliares, em virtude da maior porcentagem de tecidos de rápida digestão.
**Palavras-chave:** Anatomia foliar; Lotação contínua; Porcentagem de tecidos; *Urochloa* híbrida.

**Resumen**
Las diferentes alturas de manejo del forraje modifican la arquitectura de la planta, alterando la proporción de tejidos jóvenes y maduros y su valor nutricional. Por lo tanto, buscamos evaluar la composición tisular de hojas de *Urochloa* híbrido cv. Mulato II (sin. *Brachiaria*) mantenido bajo cuatro condiciones de manejo simulando pastoreo continuo en las estaciones lluviosa y seca. Además, se determinó el efecto de variar el porcentaje de tejidos de la hoja sobre su digestibilidad con base en los niveles de proteína cruda (PB), fibra insoluble en detergente neutro (FDN), fibra insoluble en detergente ácido (FDA) y digestibilidad *in vitro* de la materia seca. (IVDMS). Se evaluaron cuatro niveles de carga simulando pastoreo continuo, manteniendo alturas de dosel de 10, 20, 30 y 40 cm. Se encontró que los pastos de 10 y 20 cm tuvieron el menor porcentaje de tejidos lignificados en condiciones húmedas y secas, lo que en consecuencia resultó en una mayor desaparición de tejidos, principalmente del mesofilo. Los pastos con 10 cm tuvieron mayores contenidos de PB, menores contenidos de FDN y FDA y, en consecuencia, mayores IVDMS. Por lo tanto, se concluye que el pasto mulato II manejado a 10 cm presentó mayor degradabilidad de los tejidos foliares, debido al mayor porcentaje de tejidos de rápida digestión.
**Palabras clave:** Anatomía de la hoja; Pastoreo continuo; Porcentaje de tejidos; *Urochloa* híbrida.

## 1. Introduction

Studies of forage digestibility associate the digestion of grasses with the arrangement of cells and tissues in plant organs, as well as with the proportion of each tissue and with the thickness of the cell wall (Wilson & Mertens, 1995). Evaluations based on the internal structure of the leaves, associated with digestibility, are a suitable tool in the understanding of the nutritional quality of forage (Basso & Barbero, 2015). In this way, nutritional quality can be better understood through the composition of the cell wall, the contents of crude protein and the *in vitro* digestibility of dry matter (Mott, 1970; Nunes et al., 1985; Paciullo et al., 2001).

The nutritional quality of forage, as well as some anatomical characteristics, vary depending on the plant's age, the organ, and environmental conditions such as season and soil fertility. Structural changes in relation to the plant's development stage, such as values of neutral detergent fiber (NDF) and acid detergent fiber (ADF), for example, usually increase progressively with the maturation of the plant organs, increasing with the thickening of the cell wall (Wilkins, 1969). Habermann et al. (2019) reported that the effects of temperature on the proportion of tissues and leaves of *Panicum maximum* are less obvious than the concentration of atmospheric $CO_2$. The thickening and lignification of cell walls are determining parameters to infer about the quality of forage, since they reduce their digestibility (Paciullo, 2002).

One of the most digestible tissues in forage plants is the chlorophyllous parenchyma, widely distributed throughout the mesophyll leaf. This high digestibility is credited to only the primary cell wall, which has a thickness of 0.1 to 0.2 µm and is not lignified (Cheng et al., 1980). In the case of the parenchyma cells of the vascular sheath, despite the predominance of primary walls in forage plants, the compact arrangement and the positioning in more inner layers of the leaf reduces their digestibility in relation to the chlorophyllous parenchyma. On the epidermis, a cuticle impregnated with hydrophobic compounds makes it difficult for ruminal microorganisms to act and consequently digest it (Wilson et al., 1983). Both the parenchyma and the epidermis have cell walls that can be lignified, which would make them even more resistant to degradation, requiring bacterial physical adhesion (Akin & Rigsby, 1985). The sclerenchyma and the xylem are tissues composed mainly of cells with secondary and lignified walls, being the least digestible in the plant body. In relation to the

phloem, it is a complex tissue composed of different cell types, which can present cells with only the primary wall or also cells with secondary and lignified walls (Evert, 2006).

In this context, establish the relationship of morphological, anatomical and nutritional components of forage with possible management heights and with attributes of plant architecture (Laca & Lemaire, 2000) and physiological parameters (Maranhão et al., 2021) becomes relevant to evaluate the nutritional quality of forage and to obtain the adequate management of pastures. Thus, the present project aimed to evaluate the nutritional value, the percentage of tissues and *in vitro* and *in vivo* digestibility in leaves of *Urochloa* hybrid cv. Mulato II (syn. *Brachiaria*) under different canopy heights, simulating grazing with different levels of continuous grazing in the rainy and dry seasons.

## 2. Methodology

### 2.1 Study area and experimental design

The research was carried out in the Forage Sector of the Experimental Farm Capim Branco of the Federal University of Uberlândia (UFU), Minas Gerais, Brazil, from December 2015 to April 2017. The climate, according to the Köppen classification, is of the "Cwa" humid subtropical mesothermal type with dry winter. The climatic data relating to the experimental period were obtained at the UFU meteorological station. The months from October to March correspond to the rainy season and had an average temperature of 22.9ºC, average precipitation of 198 mm and average humidity of 71%. The months from April to September correspond to the dry season and had an average temperature of 21.4 ºC, average precipitation of 29.9 mm and average humidity of 67%.

The experimental area's soil was classified as dystrophic dark red latosol, of clayey texture (EMBRAPA, 2009). Soil sampling was carried out for chemical characterization before the experiment was implemented (Table 1). According to the interpretation of the soil analysis, there was no need for liming (Cantarutti et al., 1999).

**Table 1 -** Chemical composition analysis of the experimental area's soil from 0 at 20 cm.

| **pH** | **OM** | **P** | **K** | **Ca** | **Mg** | **H+Al** | **SB** | **T** | **V** |
|---|---|---|---|---|---|---|---|---|---|
| H2O | g $dm^{-3}$ | mg $dm^{-3}$ | | | $cmol_c$ $dm^{-3}$ | | | | % |
| 5,3 | 3,1 | 2,6 | 0,27 | 2,2 | 0,8 | 1,8 | 3,27 | 5,07 | 64 |

*OM = organic matter, SB = sum of exchangeable bases; T = cation exchange capacity at pH 7.0; V = base saturation index.
Source: Soil Laboratory (UFU), Uberlândia (2015).

The experiment was designed considering four treatments simulating different levels of grazing stocking, keeping the canopy at heights of 10, 20, 30 and 40 cm. The treatments were allocated in four experimental plots of 4 x 4 m (16 $m^2$) with a completely randomized design and continuous management. The plots with *U.* hybrid cv. Mulato II (Mulato II grass) were established in November 2013, using 9 kg of pure and viable seeds per hectare. The experiment was installed in October 2015, when the standardization cut of the plots was carried out at 10 cm in height. From this moment on, the plots remained in free growth until they reached the desired height. The evaluations were started in the dry season for the stabilization of the stands, when all the plots were at the target height. Monitoring was carried out using transect lines under the plot, at the target height of each treatment. As the tillers exceeded the target height, mechanical cutting was performed with the aid of scissors, simulating a grazing with continuous stocking. The evaluations were carried out until the month of April 2017.

### 2.2 Nutritional value

To determine the nutritional value of the leaves in the different seasons of the year, 50 leaf blades were cut in the region of the insertion of the ligule of the second leaf, completely expanded and intact, in each plot during the rainy and dry

seasons, obtaining the crude protein contents (CP), *in vitro* dry matter digestibility (IVDMD), neutral detergent fiber (NDF) and acid detergent fiber (ADF). The leaves were previously dried in an oven with forced air circulation at 65º C for 72 hours, ground and analyzed using the infrared reflectance spectroscopy (NIRS) system.

To determine the IVDMD, the rumen inoculum was obtained from two rumen cannulated heifers. The animals were fed a diet consisting of hay and concentrate, with a roughage:concentrate ratio of 80:20 based on dry mass (DM). The animals had unrestricted access to water and were adapted to the standard diet for 15 days. All cannulation and handling procedures conducted with the animals were approved by the UFU's animal experimentation ethics committee (CEUA, protocol 099/16). The rumen fluid was collected on a cloth and placed in thermos bottles preheated to a temperature of 39°C. Subsequently, it was filtered through gauze in 1 L beakers. McDougall's buffer solution (1948), added to 5 ml of urea solution (5.5 g 100 $ml^{-1}$) (60:1, v:v), was prepared 24 hours before the start of each incubation battery. The pH of the final solution was reduced to 6.8 by bubbling with $CO_2$, and the solution was stored inside the fermenter at 39°C (Silva & Queiroz, 2002).

The *in vitro* digestibility assays were performed using an artificial MA-443 fermenter (Marconi laboratory equipment, Piracicaba, Brazil), using bags made of non-woven fabric (100 g $m^{-2}$) in the dimensions of 4 x 4.5 cm, heat sealed, washed and weighed for tare quantification, as described by Detmann et al. (2012). In each bag, 500 mg of the dry sample were weighed, using five replicates of each treatment. The bags (24) were inserted into the jugs of the fermenter, including an empty bag representing the blank. Then, 400 ml of ruminal inoculum and 1600 ml of McDougall's buffer solution were added to each jar. The free space of the jars was immediately saturated with $CO_2$, which were closed and placed inside the fermenter at 39°C. After 24 and 36 hours of incubation, the bags were removed from the fermenter and immediately washed with hot distilled water (temperature above 90°C), exerting light manual pressure to remove the gases. After washing, all bags were dried (55°C for 24 hours followed by 105°C for 16 hours) and weighed, yielding an apparently undigested DM residue. The IVDMD coefficient was obtained by the formula IVDMD = [DM – (R – B)]/DM x 100, where DM corresponds to the initial dry mass (g), R corresponds to the residue of the incubation dry mass (g), and B corresponds to the dry mass residue obtained in the "white" bags (g).

### 2.3 Anatomical assessments

**2.3.1 Tissues percentage:** 40 leaf blades were collected, cut in the region of the insertion of the ligule of the second fully expanded leaf and intact, being carried out two collections in the rainy (2016 and 2017) and one in the dry season (2016). Part of these blades was used for the evaluation of *in vitro* tissue degradation, described in the next item. In the evaluation of the percentage of tissues, 10 slides from each plot were used, with cuts of 1 cm fragments in the median third of each slide. The fragments were fixed in FAA (formalin, glacial acetic acid, 50% ethanol, 1:1:18 v/v) for 48 hours and stored in 70% ethanol (Johansen, 1940).

The anatomical evaluations were carried out in the Laboratory of Anatomy, Plant Development and Interactions (LADEVI), belonging to the Institute of Biology at UFU. Leaf blade fragments were subjected to an alcoholic series with tertiary butyl alcohol for about 40 hours (Daykin & Hussey, 1985). The fragments were embedded in Paraplast® and sectioned using a rotating microtome (Kraus & Arduin, 1997). Freehand cuts were also obtained using a razor blade. Subsequently, these sections were clarified with sodium hypochlorite 20% and washed in distilled water (Kraus & Arduin, 1997). All sections were stained with astra blue solution 0.5% and alcoholic safranin 1% (1:9, v/v) (Bukatsch, 1972). Freehand sections were mounted between slide and coverslip, with Kaiser's glycerin gelatin (Johansen, 1940), while sections in a rotary microtome were mounted between slide and coverslip using crystal varnish as mounting medium (Kraus & Arduin, 1997).

Qualitative and quantitative analyzes were performed to characterize the tissue structure of intact leaves. The tissues between the 2nd and 3rd vascular tissues of the intact leaves were measured using the image analysis software (model Image Pro

Plus version 4.5.0.29), being evaluated the area occupied by the epidermis on the adaxial (EpAd) and abaxial (EpAb) faces of the leaf, the areas occupied by the vascular sheath (VS), vascular tissue (VT) and sclerenchyma (Sc). The mesophyll area (Mes) was calculated by the difference between the total area of the cross section and the areas of the other tissues.

**2.3.2 *In situ* tissue degradation:** In the evaluation of *in situ* tissue degradation of leaf blades, fragments of residual DM from digestibility tests (n=10) and fragments of leaf blades from rainy collections (2016 and 2017) and dry (2016) were used, which were cut with the aid of scissors into 1 cm fragments. To evaluate tissue digestion, was followed by the methodology described by Akin (1982) and Wilson et al. (1991).

For a qualitative evaluation of the impact of preserving leaf tissues as a function of time (after 24 and 36 hours of incubation), fragments of incubated leaf blades were included in historesin according to the manufacturer's recommendations (Leica Historesin®), cut transversally at 5 μm thick on a rotating microtome and stained with toluidine blue 0.05% - pH 4.7 (O'BRIEN et al., 1964). All images were obtained using a photomicroscope (Leica® DM500), equipped with an attached digital camera (Leica® ICC50HD).

**2.4 Data analysis**

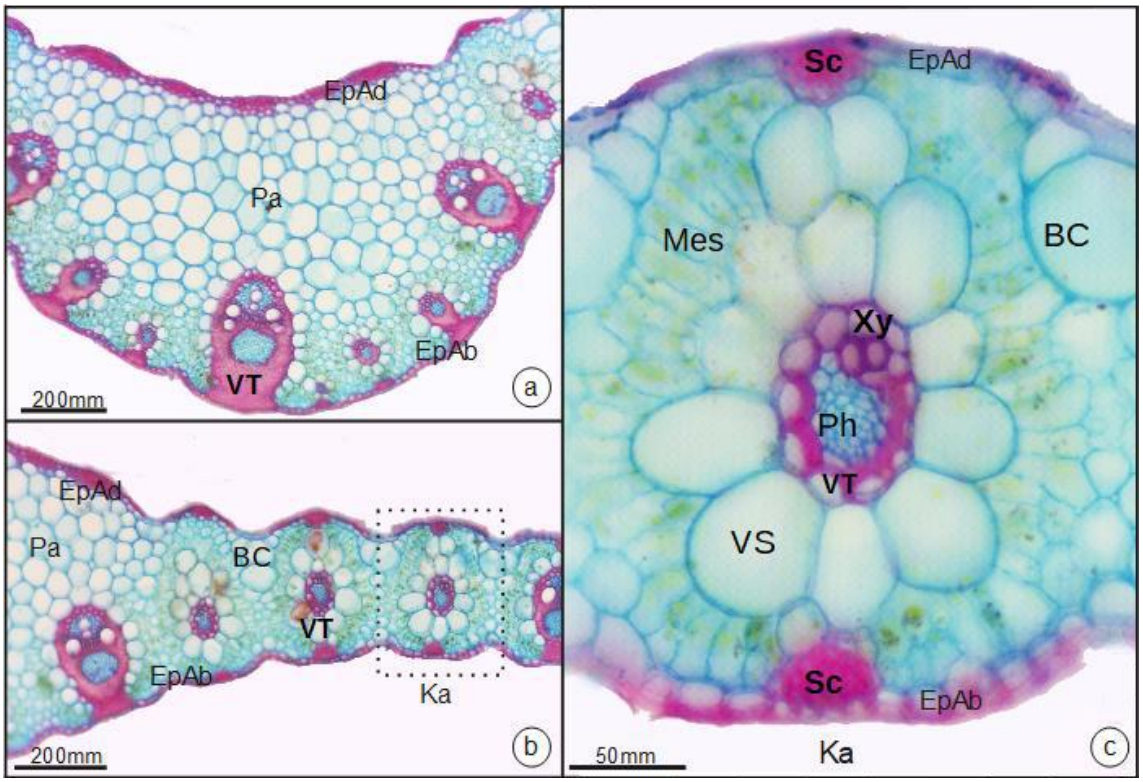
All data sets were tested for normality of error distribution and homogeneity of variances. The effect of treatments on leaf tissues was compared using ANOVA, and when differences were detected, the means were compared by the Tukey test at 5% significance. For all analyses, the SASm-Agri 8.2 software was used.

## 3. Results

The leaves of Mulato II are amphistomatic and have a uniseriate epidermis covered by a thin cuticle (Figure 1a-c). The median region contains a large proportion of fundamental parenchyma, with collateral vascular tissues distributed exclusively on the abaxial surface, and fibers occur internally to the epidermis and associated with vascular tissues (Figure 1a). In the intercostal region, it is possible to observe that the leaf is parallelelinerve, with two types of vascular tissues distinguished by the connection by fibers to the epidermis on the abaxial face of the leaf (Figure 1b), by the caliber and mainly by the structure of the xylem and phloem (Figure 1c). Bulliform epidermal cells occur on both faces, but are more prominent on the adaxial face (Figure 1b). Sclerenchyma fibers are also present on both faces of the leaf blade, always associated with the vascular tissues (Figure 1c). The leaf presents Kranz anatomy, with collateral vascular tissues contoured by a prominent vascular sheath and, in sequence, by 1 to 2 layers of radial mesophyll (Figure 1b and c).

Regarding the percentage of tissues in the leaf blades, only for EpAd between the canopy management heights during the rainy season were not observed differences (Table 2). The percentage of EpAb was lower in the 10 cm pastures in both seasons, however they were similar to the 30 cm in the dry season. In the rainy, sclerenchyma was lower in 10 and 20 cm pastures, which also occurred in the dry season. Pastures of 30 cm showed higher values for vascular sheath, being statistically similar to those of 20 and 40 cm in the rainy and only to those of 40 cm in the dry season. It was observed that the 10 cm pastures had a higher percentage of vascular tissue in the rainy, and the 40 cm in the dry ones. Mesophyll was higher in 10 cm pastures in both seasons.

**Figure 1 -** Cross sections of the intact leaf blade of *Urochloa* hybrid cv. Mulato II (syn. *Brachiaria*), showing general anatomical features: a) Median region; b) Intercostal region indicating one of the tissues used for micromorphometric measurements (dotted square); c) Detail of the vascular tissue.




Abbreviations: BC = bulliform cells, Mes = mesophyll, EpAd = epidermis on the adaxial surface of the leaf, EpAb = epidermis on the abaxial surface of the leaf, Pa = parenchyma, Ph = Phloem, VT = vascular tissues, VS = vascular sheath, Sc = sclerenchyma (fibers), Xy = Xylem, Ka = Kranz anatomy. Source: Authors.

**Table 2 -** Percentage occupied by the different tissues in leaves of *Urochloa* hybrid cv. Mulato II (syn. *Brachiaria*) kept under different canopy heights (10, 20, 30 and 40 cm), simulating grazing with continuous stocking during the rainy and dry season.

| Canopy height (cm) | Tissues (%) on rainy | | | | | |
|---|---|---|---|---|---|---|
| | EpAd | EpAb | Sc | VS | VT | Mes |
| **10** | 16.27 $^{a}$ | 5.96 $^{b}$ | 10.28 $^{b}$ | 25.47 $^{b}$ | 5.93 $^{a}$ | 36.09 $^{a}$ |
| **20** | 15.95 $^{a}$ | 7.87 $^{a}$ | 12.06 $^{b}$ | 26.02 $^{ab}$ | 5.32 $^{ab}$ | 32.78 $^{b}$ |
| **30** | 15.54 $^{a}$ | 6.87 $^{a}$ | 15.85 $^{a}$ | 28.23 $^{a}$ | 5.35 $^{ab}$ | 27.15 $^{c}$ |
| **40** | 16.86 $^{a}$ | 7.56 $^{a}$ | 18.36 $^{a}$ | 26.11 $^{ab}$ | 4.74 $^{b}$ | 26.36 $^{c}$ |
| **CV (%)** | 7.0 | 8.8 | 11.08 | 4.87 | 7.98 | 4.38 |
| **Canopy height (cm)** | **Tissues (%) on dry** | | | | | |
| | EpAd | EpAb | Sc | VS | VT | Mes |
| **10** | 14.07 $^{b}$ | 6.96 $^{b}$ | 10.33 $^{b}$ | 24.2 $^{b}$ | 4.81 $^{b}$ | 39.64 $^{a}$ |
| **20** | 14.04 $^{ab}$ | 8.58 $^{a}$ | 11.21 $^{b}$ | 23.76 $^{b}$ | 5.18 $^{ab}$ | 36.32 $^{a}$ |
| **30** | 18.11 $^{a}$ | 8.31 $^{ab}$ | 14.26 $^{a}$ | 25.98 $^{ab}$ | 4.51 $^{b}$ | 28.84 $^{b}$ |
| **40** | 18.31 $^{a}$ | 9.60 $^{a}$ | 14.96 $^{a}$ | 27.60 $^{a}$ | 6.50 $^{a}$ | 23.04 $^{c}$ |
| **CV (%)** | 11.30 | 8.30 | 6.98 | 5.70 | 12.63 | 8.03 |

Means followed by the same letter in the column do not differ ($p>0.05$) by the Tukey test. EpAd – Epidermis on the adaxial surface of the leaf; EpAb – Epidermis on the abaxial surface of the leaf; Sc – Sclerenchyma; VS – Vascular Sheath; VT – Vascular Tissue; Mes – Mesophyll; CV – Coefficient of variation. Source: Authors.

The leaves of Mulato II showed higher crude protein contents when managed at 10 cm height, both in the rainy and dry seasons. Regarding NDF and ADF, no differences were observed between heights in the rainy season, and in the dry season, the contents were lower in 10 cm pastures. The coefficient of *in vitro* dry matter digestibility (IVDMD) in the rainy and

dry season was higher in 10 cm pastures, 83.74% and 81.28%, respectively. At the other heights, no significant differences were observed, with an average of 79.4% in the rainy and 79.05% in the dry seasons (Table 3).

**Table 3 -** Crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and *in vitro* digestibility (IVDMD) in leaves of *Urochloa* hybrid cv. Mulato II (syn. *Brachiaria*) in the rainy and dry seasons, kept under different canopy heights and simulating grazing with continuous stocking.

| Height (cm) | CP (%) | | NDF (%) | | ADF (%) | | IVDMD (%) | |
|---|---|---|---|---|---|---|---|---|
| | Rainy | Dry | Rainy | Dry | Rainy | Dry | Rainy | Dry |
| **10** | 13.45[a*] | 12.28 [a] | 64.41 [a] | 63.02 [b] | 31.08 [a] | 29.50 [b] | 83.74[a] | 81.28[a] |
| **20** | 12.75 [b] | 12.02 [b] | 63.71 [a] | 64.52 [a] | 31.08 [a] | 30.76 [a] | 80.28[b] | 79.41[b] |
| **30** | 12.70 [b] | 11.90 [b] | 63.48 [a] | 64.72 [a] | 30.36 [a] | 30.86 [a] | 78.86[b] | 79.30[b] |
| **40** | 12.45 [b] | 11.52 [b] | 63.72 [a] | 65.50 [a] | 31.55 [a] | 31.68 [a] | 79.06[b] | 78.43[b] |
| **CV (%)** | 3.09 | 2.14 | 0.80 | 1.03 | 2.73 | 1.53 | 1.73 | 0.83 |

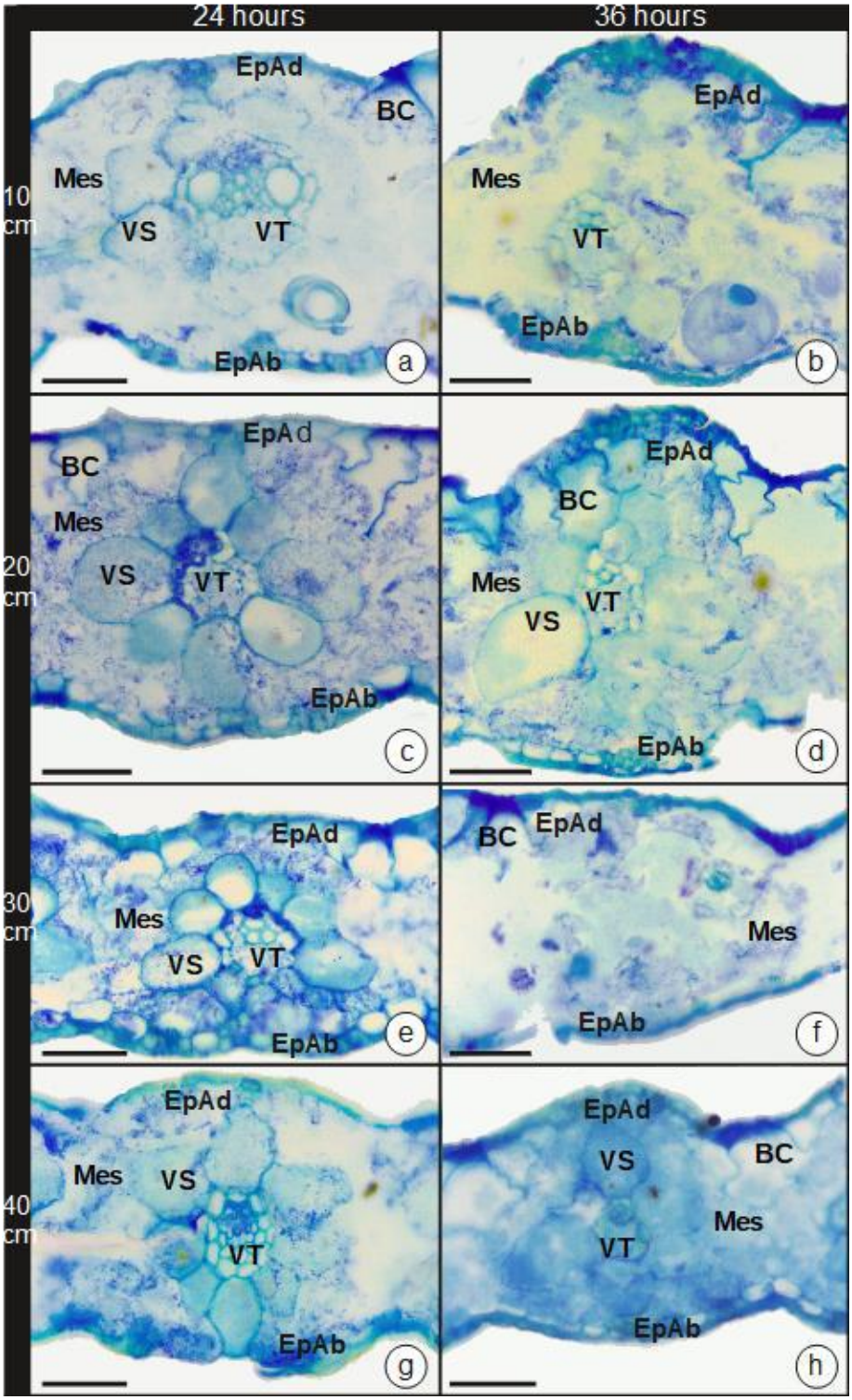
*Means followed by the same letter in the column do not differ (p>0.05) by the Tukey test. CV = Coefficient of variation. Source: Authors.

Crude protein values remained above 11%, and above 13% in plants kept at 10 cm height. The frequency of defoliation performed to simulate the different levels of continuous grazing provided constant renewal of tillers and maintained a supply of younger tissues. However, it was verified that during the dry season, the Mulato II grass plants maintained at 10 cm had lower levels of fibrous components when compared to the other heights (Table 3), with a higher percentage of mesophyll, lower sclerenchyma and vascular sheath being also observed (Table 2). These were probably composed of a thinner cell wall, providing better results in terms of digestibility.

In association with IVDMD, the evaluation of leaf fragments digested *in situ* for 24 and 36 hours of Mulatto II grass reinforce the higher digestibility in leaves maintained at 10 cm height (Figure 2). Anatomical analyzes showed greater degradation of the mesophyll and vascular tissues at the height of 10 cm, in both digestion times, being more intense after 36 hours of incubation. At other handling heights, tissues were more preserved after 24 hours of degradation, and naturally, after 36 hours in the rumen environment, the tissues were less preserved.

Both after 24 and 36 hours of incubation digestion of Mulato II grass leaves occurred first in the mesophyll and later in the epidermis (Figure 2), possibly due to the impregnation of lipid compounds in the epidermis that confer resistance. Furthermore, the digestion pattern was similar between the rainy and dry seasons, so we only present images from the rainy season.

**Figure 2 -** Cross sections of leaves of *U*. hybrid cv. Mulato II (syn. *Brachiaria*) collected in the rainy season, kept under different canopy heights simulating grazing with continuous stocking (10, 20, 30 and 40 cm), and submitted to *in situ* digestion by 24 (a, c, e, g) and 36 (b, d, f, h) hours. a, b - Height of 10 cm; c, d - Height of 20 cm; e, f - Height of 30 cm; g, h - Height of 40 cm.




Abbreviations: BC = Bulliform cells, EpAd = Epidermis on the adaxial surface, EpAb = Epidermis on the abaxial surface, VT = Vascular tissues, VS = Vascular sheath. Bars = 50 µm. Source: Authors.

## 4. Discussion

According to Wilson (1993), the outer walls of the epidermis cells become thick, lignified and covered with cuticle and wax as they develop, which is more expressive in EpAb than in EpAd. The cuticle can make difficult the digestion of the epidermis (Wilson et al., 1983) and even the passage of rumen fluid to the mesophyll tissues. Furthermore, according to Wilson and Mertens (1995), the EpAb in tropical grasses is tightly bound to the vascular tissues by the sclerenchyma, while the epidermis on the adaxial surface of the leaf is not always attached to the tissues. The connection between the epidermis, the sclerenchyma and the sheath cells are called the girder T structure and when this connection occurs between the epidermis on both leaf faces it is called the girder I structure. The girder structure prevents or make it dificultt to remove the epidermis by digestion and reduces the access of ruminal microorganisms to the mesophyll and parenchyma (Basso et al., 2023) and 93% of the vascular tissues of *Brachiaria brizantha* have a type I girder structure, and that these are the ones that most affect leaf degradability (Lempp et al., 2002).

In the cultivar Mulato II, the cuticle is thin, which can facilitate and speed up the process of foliar digestion. The smaller area of epidermis on the abaxial surface of the leaf presented by the plants in the canopy managed at 10 cm height may favor its own digestibility and, as well as the type of cuticle, facilitate the entry of rumen fluid reaching the internal tissues.

The sclerenchyma is located subepidermal, close to the vascular tissues (Figure 1c) and is composed of fibers, these cells being elongated and with a secondary thick and lignified cell wall (Carvalho & Pires, 2008; Sfiligoj et al., 2019) which makes them indigestible (Wilson et al., 1983). The lower sclerenchyma investment in rainy and dry observed in 10 and 20 cm pastures (Table 2) indicate that younger and shorter leaves have less wall thickness in sclerenchyma cells, probably because sclerenchyma is a tissue with structural function (Batistoti et al., 2012; Gomes et al., 2011).

Regarding the vascular sheath, also known as endoderm, its cells have two cell walls and constitute tissues that are partially degraded. Its digestion is slow or incomplete, making it difficult for ruminal microorganisms to access vascular tissues and may contain, in some cases, more than 50% of the carbohydrate and protein reserves of the leaf (Wilson, 1994), even representing 20 to 35% of the cross section (Lempp, 2007). The smallest volume of vascular sheath cells at 10 and 20 cm (Table 2) height can facilitate the access of microorganisms to the tissues, increasing the acquisition of reserves. In the leaves collected in these pastures, a greater amount of month was also observed, characterized by the presence of a thin primary wall and without lignin (Cheng et al., 1980), which allows its rapid digestibility and characterizes it as an efficient source of carbohydrates.

In Mulato II grass, a fiber ring surrounds the digestible elements of the xylem and phloem, making these regions difficult for rumen microorganisms to act, regardless of height management. Although the percentage of vascular tissue occupies only a small proportion compared to the other tissues (Table 2), the differences observed may be related to the differentiation and growth of phloem cells in the rainy of 10, 20 and 30 cm pastures, and to the thickening of the cell wall of vascular tissues, mainly the xylem in 40 cm pastures in the dry season.

According to Almeida (2013), levels below 65% of NDF, as observed here in the leaves of Mulato II grass, guarantee ruminal microorganisms a better use of nutrients from the diet consumed by cattle and, consequently, provide better animal performance. In this sense, the leaves of plants kept at 10 cm in height showed better performance during the dry period, when the lowest NDF values were observed (Table 3).

According to Eastridge (1997), ADF levels have a greater relationship with the digestibility of a food when compared to NDF levels, since the fraction of indigestible fiber (lignin) represents a greater portion of the ADF. For the present study, the ADF values are close to those recommended by Mertens (1992), who reported that forages with ADF value around 30% or less, unlike those with levels above 40%, are more consumed by ruminants due to the lower fiber composition in the diet.

The use of optical microscopy added to data on digestion residue and nutritional value can provide a better understanding of the results obtained in grazing. Pastures that are managed with greater frequency and defoliation intensity have higher percentages of more digestible tissues in relation to those grazed less intensively and, consequently, may undergo faster ruminal digestion, in addition to having a greater amount of protein, digestibility and lower levels of NDF and ADF.

## 5. Conclusion

Mulato II grass pastures managed with greater frequency and intensity of defoliation have higher percentages of more digestible tissues compared to those grazed less intensely and, consequently, can have faster ruminal digestion, in addition to having a greater amount of protein, digestibility and lower levels of NDF and ADF.

Researchs that evaluate the percentage of tissues in grass leaves can improve the understanding of the nutritional value characteristics of the pasture and can be important tools for new studies related to canopy structures of the new cultivars.

## References

Akin, D. E. & Rigsby, L. L. (1985). Influence of phenolic acids on rumen fungi. *Agronomy Journal*, 77, 180-182. https://doi.org/10.2134/agronj1985.00021962007700010043xa

Akin, D. E. (1982). Section to slide technique for study of forage anatomy and digestion. *Crop Science*, 22(1), 444-446. https://doi.org/10.2135/cropsci1982.0011183X002200020059x

Almeida, C. M. (2013). Características estruturais e composição bromatológica do capim marandu submetido a duas alturas de manejo. Dissertation in Tropical Agriculture. Faculty of Agronomy and Veterinary Medicine, Federal University of Mato Grosso, Cuiabá (MS), Brazil.

Basso, K. C., Galzerano, L., Silva, W. L., Ruggieri, A. C, & Reis, R. A. (2023). Anatomical, morphogenic and structural characteristics of Xaraés palisade grass under grazing. *Bioscience Journal*, 39, e39067. https://doi.org/10.14393/BJ-v39n0a2023-60937

Basso, K. C. & Barbero, L. M. (2015). Anatomia foliar de forrageiras e a sua relação com o valor nutritivo. *Veterinária Notícias*. 21, 1-10. https://doi.org/10.14393/VTv21n1a2015.24423

Batistoti, C., Lempp, B., Jank, L., Morais, M. G., Cubas, A. C., Gomes, R. A. & Ferreira, M. V. B. (2012). Correlations among anatomical, morphological, chemical and agronomic characteristics of leaf blades in *Panicum maximum* genotypes. *Animal Feed Science and Technology*. 171(2-4), 173–180. https://doi.org/10.1016/j.anifeedsci.2011.11.008

Bukatsch, F. (1972). Bemerkungen zur doppelfärbung: astrablau-safranin. *Mikrokosmos*. 8, 255.

Carvalho, G. G. P. & Pires, A. J. V. (2008). Organização dos tecidos de plantas forrageiras e suas implicações para os ruminantes. *Archivos de Zootecnia*. Córdoba. 57(R), 13-28.

Cantarutti, R. B., Martins, C. E., Carvalho, M. M., Fonseca, D. M., Arruda, M. L., Vilela, H. & Oliveira, F. T. T. (1999). Pastagens. In: Ribeiro, A. C., Guimarães, P. T. G., Alvarez, V. V. H. Comissão de Fertilidade do Solo do Estado de Minas Gerais. Recomendação para o uso de corretivos e fertilizantes em Minas Gerais. Viçosa, 5ª Aproximação.

Cheng, K. J., Fay, J. P. & Howarth, R. E. (1980). Sequence of events in the digestion of fresh legume leaves by rumen bacteria. *Applied Environment Microbiology*. 40, 613-625.

Daykin, M. E. & Hussey, H. S. (1985). Staining and histopathological techiques in nematology. In: Barker, K. R., Carter, C. C., Sasser, J. N. (Eds.). An advanced treatise on *Meloidogyne*: methodology. Raleigh. North Carolina State University Graphics. 39-48.

Detmann, E., Souza, M. A., Valadares Filho, S. C., Queiroz, A. C., Berchielli, T. T., Saliba, E. O. E., Cabral, L. S., Pina, D. S., Ladeira, M. M. & Azevedo, J. A. G. (2012). Métodos para análise de alimentos. (INCT - Ciência animal). Viçosa, Universidade Federal de Viçosa. p.350.

Eastridge, M. L. (1997). Fibra para vacas leiteiras. Proceedings of Simpósio sobre Produção Animal. Fundação de Estudos Agrários "Luiz de Queiroz", Piracicaba, Brazil, p.33-50.

Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA (2009). Manual de análises químicas de solos, plantas e fertilizantes. Brasília, Brazil. p.627.

Evert, R. F. (2006). Esau's Plant Anatomy. Wiley-Interscience, 63.

Gomes, R. A., Lempp, B., Jank, L., Carpejani, G. C., & Morais, M. G. (2011) Características anatômicas e morfofisiológicas de lâminas foliares de genótipos de *Panicum maximum*. *Pesquisa Agropecuária Brasileira*, Brasília, Brazil, 46 (2), 205-211. https://doi.org/10.1590/S0100-204X2011000200013

Habermann, E., San Martin, J.A.B., Contin, D. R., Bossan, V. P., Barboza, A., Braga, M. R., Groppo, M. & Martinez, C. A. (2019). Increasing atmospheric CO2 and canopy temperature induces anatomical and physiological changes in leaves of the C4 forage species *Panicum maximum*. *Plos one*. 15 (8), e0212506. https://doi.org/10.1371/journal.pone.0212506

Johansen, D. A. (1940). Plant microtechnique. Mc Graw.

Kraus, J. E., & Arduin, M. (1997). Manual básico de métodos em Morfologia Vegetal. EDUR.

Laca, E. A. & Lemaire, G. (2000). Measuring sward structure. In: Mannetje, L., & Jones, R. M. (Ed.). Field and laboratory methods for grassland and animal production research. Cabi, 103-122.

Lempp, B. (2007). Avanços metodológicos da microscopia na avaliação de alimentos. *Revista Brasileira de Zootecnia*, 36, 315-329. https://doi.org/10.1590/S1516-35982007001000029

Lempp, B., Valle, C. B., Torres, F. E., Alves, R., Victor, D. M. & Morais, M. G. (2002). Proporção e arranjo de tecidos de nove acessos de *Brachiaria brizantha*. Proceedings of Reunião Anual da Sociedade Brasileira de Zootecnia, 39, Recife, Brazil.

Maranhão, S. R., Pompeu, R. C. F. F., Araújo, R. A. de, Lopes, M. N., Souza, H. A. de, Cavalcante, A. C. R., Fontinele, R. G., Rogerio, M. C. P. (2021). Morphophysiology of tropical grasses under different water supply in two growing seasons: II. BRS Massai and BRS Tamani grasses. *Semina: Ciências Agrárias*. 42, 301-318. http://dx.doi.org/10.5433/1679-0359.2021v42n1p301

Mcdougall, E. I. (1948). Studies on ruminant saliva. 1. The composition and output of sheep's saliva. *Biochemistry Journal*, 43(1), 99-109.

Mertens, D. R. (1992). Análise da fibra e sua utilização na avaliação e formulação de rações. Proceedings of Simpósio Internacional de Ruminantes. Lavras, Brazil, 188- 219.

Mott, G.O. (1970). Evaluacion de la produccion de forrajes. In: Hughes, H. D., Heath, M. E., Metcalfe, D. S. (Eds.) Forrajes - la ciencia de la agricultura basada en la producción de pastos. México. 131-141.

Nunes, S. G., Boock, A. & Penteado, M. I. de O. (1985). *Brachiaria brizantha* cv. Marandu. Embrapa/CNPGC, Campo Grande, Brazil.

O'Brien, T. P., Feder, N. & Mccully, M. E. (1964). Polychromatic staining o plant cell walls by toluidine blue O. *Protoplasma*, 59, 368-373.

Paciullo, D. S. C. (2002). Características anatômicas relacionadas ao valor nutritivo de gramíneas forrageiras. *Ciência Rural*, 32(2), 357-364. https://doi.org/10.1590/S0103-84782002000200029

Paciullo, D. S. C., Gomide, J. A., Queiroz, D. S. & Silva, E. A. M. (2001). Composição química e digestibilidade *in vitro* de lâminas foliares e colmos de gramíneas forrageiras, em função do nível de inserção no perfilho, da idade e da estação de crescimento. *Revista Brasileira de Zootecnia*, 30 (3), 964-974. https://doi.org/10.1590/S1516-35982001000400009

Sfiligoj S. M., Hribernik, S., Kurečič, M., Urbanek K. A., Kreže, T. & Stana K. K. (2019). Anatomy of Plant Fibres. Surface Properties of Non-Conventional Cellulose Fibres, 7–15.

Silva, D. J., & Queiroz, A. C. (2002). *Análise de alimentos: Métodos químicos e biológicos*. (3a ed.), UFV, Brazil.

Wilkins, R. J. (1969). The potencial digestibility of cellulose in forages and faces. *Journal of Agricultural Science*, 73, 57-64.

Wilson, J. R., & Mertens, D.R. (1995). Cell wall accessibility and cell structure limitations to microbial digestion of forage. *Crop Science*. 35, 251-259. https://doi.org/10.2135/cropsci1995.0011183X003500010046x

Wilson, J. R. (1994). Cell wall characteristics in relation to forage digestion by ruminants. *Journal of Agricultural Science*. 122, 173-182. https://doi.org/10.1017/S0021859600087347

Wilson, J.R., Brown, R. H. & Windham, W. R. (1983). Influence of leaf anatomy on the dry matter digestibility of C3, C4, and C3/C4 intermediate types of *Panicum* species. *Crop Science*. 23(1), 141-146. https://doi.org/10.2135/cropsci1983.0011183X002300010041x

Wilson, J. R. (1993). Organization of forage plant tissues. In: Jung, H. G., Buxton, D. R., Hatfield, R. D. et al. (Eds.) Forage cell wall structure and digestibility. *Madison: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America*, 1-32.

Wilson, J. R., Deinum, B. & Engels, F. M. (1991) Temperature effects on anatomy and digestibility of leaf and stem of tropical and temperate forage species. *Netherlands Journal of Agriculture Science*. 39(1), 31-48. https://library.wur.nl/ojs/index.php/njas/article/view/16551/15965