


## Article

# How Are Warm-Season Pastures' Nutritive Value and Fermentation Characteristics Affected by Open Pasture, Silvopasture, and Sward Herbage Maturity?

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**Abstract:** This study aimed to evaluate the forage mass and nutritional value (NV) of Guinea Massai grass (*Megathyrsus maximus* × *Megathyrsus infestum*) in an open pasture (OP) or the silvopastoral system (SPS) at different stages of development (SDs). The experimental design was completely randomized, with five replications. The treatments were distributed in a factorial scheme 2 × 4, corresponding to types of systems (OP and SPS) and four SD (vegetative stage—S1, pre-flowering—S2, full flowering—S3 and maturity after flowering—S4). There was no interaction of the system × stage of plant development in any of the variables evaluated. The production of forage mass and Guinea Massai grass morphological components did not differ ( $p > 0.05$ ) between SPS and OP. However, they differed between the SDs ( $p \leq 0.05$ ), with an increasing linear effect for forage mass, percentage of the stem, and dead material, and decreasing for leaf percentage and leaf: stem ratio. Only protein content differed ( $p < 0.05$ ) among the chemical composition variables between culture systems. The shading caused by the SPS did not decrease the forage mass, NV, and in vitro dry matter digestibility of Guinea Massai grass compared to the system in OP. The advance of the SD of Guinea Massai grass increases the forage mass, with higher proportions of morphological components of low NV, such as stem and dead material. Silvopastoral system of Guinea Massai grass and eucalyptus is effective to prolongate the grazing season, with a greater amount of green leaves in the sward and better NV, without compromising herbage mass production in the forage growing season.

**Keywords:** Guinea Massai grass; forage management; integrated systems; nutritive value



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## 1. Introduction

Integrated systems are an option for efficient land use and intensification in ruminant production. The silvopastoral system (SPS), or forest livestock integration, is characterized by associating, in the same area, the forest component, the forage species, and the animal under grazing [1]. The combination of these components sustainably benefits the soil–plant–animal ecosystem.

The correct choice of the forestry and forage component plays a fundamental role in ensuring the success of the SPS implementation. Thus, the forest species should allow the luminous incidence to reach the forage canopy, must have low invasive potential, and provide marketable products [2]. In Brazil, the genus *Eucalyptus* has been highlighted for use in SPS because it presents desirable characteristics, besides being one of the most studied species for this purpose. On the other hand, the forage species must be adapted to the edaphoclimatic conditions of the region and have a good production capacity, be tolerant to the conditions generated by the system, especially shading [3], and meet the nutritional requirements of the animals. Thus, Guinea Massai grass (*Megathyrsus maximus*

× *Megathyrus infestus*) becomes an option to make up the SPSs, especially because it tolerates 30–50% shading [4–6].

The shade created by the tree component alters the microclimate of the understory by reducing temperature and luminosity [7]. The reduction in temperature, in turn, slows the advance of the phenological stages of the plant, keeping it in more initial stages and of better nutritional value (NV) [8]. As the plant matures, especially in the reproductive phase, there is an increase in the thickness of cell walls, increasing fiber content, which reduces cell content and digestibility [9,10]. Thus, management conditions that keep the forage longer in the vegetative stage can benefit the production system.

Although it is known that there is a difference in forage NV in systems of open pasture (OP) and shaded [6,11–13], there is still little information about this variation in the different stages of development (SD) in integrated systems. Thus, the development of research evaluating tropical grasses in different SD plays a fundamental role in defining the ideal management in different systems.

We hypothesized that the shadow caused by the SPS would reduce the forage mass and improve the nutritive value of Guinea Massai grass compared to an OP. Additionally, that the more advanced the stage of development, the lower the nutritional value. Given the above, the objective was to evaluate the forage mass and NV of Guinea Massai grass during the different SDs maintained in the OP and SPS.

## 2. Materials and Methods

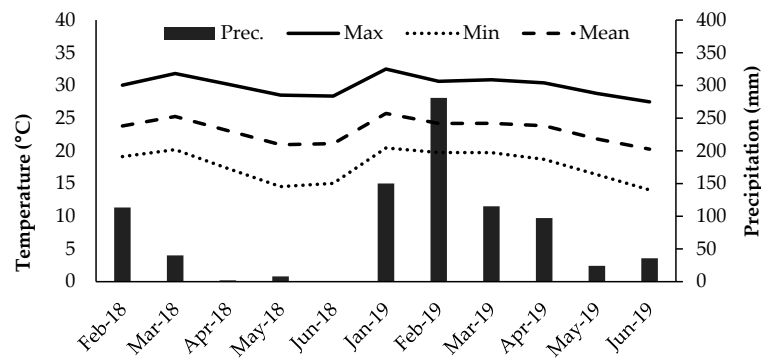
### 2.1. Experimental Area

The experiment was conducted in the Forage and Pasture sector at the São Paulo State University, Jaboticabal Campus, São Paulo, Brazil (21°14' S and 48°17' O, 598 masl), from February to June 2018, and from January to June 2019. The soil was classified as dystrophic yellow-red latosol [14]. In November 2017, soil analysis was performed, and there was no need for maintenance fertilization [15] (Table 1).

**Table 1.** Soil properties (depth 0–20) in the silvopastoral system (SPS) and open pasture.

| Properties   | SPS  | OP   |
|--|------|------|
| pH (CaCl <sub>2</sub> )                            | 5.3  | 5.4  |
| Organic matter (g dm <sup>-3</sup> )               | 26   | 25   |
| P (g dm <sup>-3</sup> )                            | 38   | 36   |
| S (g dm <sup>-3</sup> )                            | 5    | 5    |
| Ca (mmol <sub>c</sub> dm <sup>-3</sup> )           | 24   | 26   |
| Mg (mmol <sub>c</sub> dm <sup>-3</sup> )           | 13   | 13   |
| K (mmol <sub>c</sub> dm <sup>-3</sup> )            | 2.8  | 3.0  |
| Al (mmol <sub>c</sub> dm <sup>-3</sup> )           | 0    | 0    |
| H + Al (mmol <sub>c</sub> dm <sup>-3</sup> )       | 31   | 28   |
| Sum of bases (mmol <sub>c</sub> dm <sup>-3</sup> ) | 38.5 | 42.4 |
| Cation exchange capability                         | 69   | 70   |
| Base saturation (V%)                               | 56   | 60   |

According to the Köppen classification, the climate is of the type of Aw, described as tropical with a dry season from April to September and a concentration of rains in the summer months (October to March). The average annual precipitation is 1369 mm, and the average air temperature is 22.7 °C. Precipitation and temperature data were extracted from the data set of the area collection and Agrometeorology Department of Exact Sciences (Figure 1).



**Figure 1.** Accumulated precipitation (Prec.), mean, maximum (Max), and minimum (Min) temperatures recorded during the collection period, of the two experimental years (2018/2019) at Jaboticabal, São Paulo, Brazil.

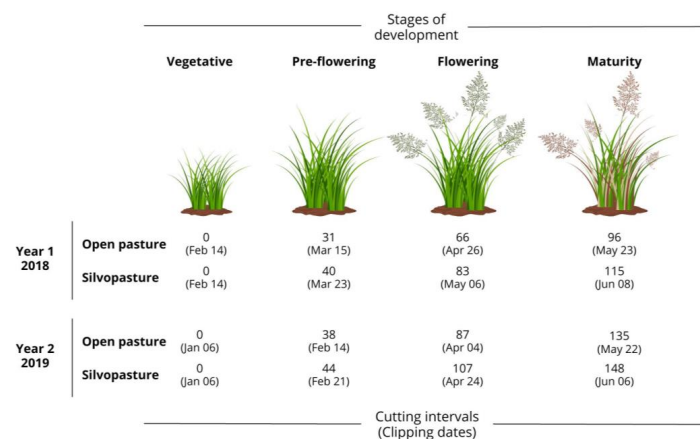
### 2.2. Implementation of the Pasture Systems

The experimental area of 8000 m<sup>2</sup> comprised OP and SPS established in 2012 (800 m<sup>2</sup> per plot). Initially, in March 2012, the entire experimental area was established with Guinea Massai grass (*Megathyrsus maximus* × *Megathyrsus infestus*) integrated with maize (*Zea mays* L.). After grain harvesting, an irrigation system was used to aid in pasture formation (June to August, dry season in the region). In September 2012, the SPS plots were implanted, and seedlings of 1.2 m high of *Eucalyptus grandis* × *Eucalyptus urophylla* (*Eucalyptus urograndis*) were introduced to the system in single rows, in an East–West direction in the sets, one with spacing of 9 m × 1.5 m and the other 18 m × 1.5 m. Five years after implantation (2017), the tree component was thinning, and only the spacing of 18 m × 1.5 m was kept. This study was conducted in 2018/2019, six and seven years after the system implantation, respectively.

### 2.3. Treatments and Experimental Design

The experimental design was completely randomized, with five replications. The treatments were distributed in a factorial scheme 2 × 4, corresponding to two types of systems (SPS and OP), during four stages of development (SD) (vegetative stage—S1, pre-flowering—S2, full flowering—S3, and maturity after flowering—S4).

In January 2018, homogenization grazing was performed with dairy heifers (average weight of 350 kg), to achieve the sward residue height of 25 cm [6]. After grazing, five grazing exclusion cages of 1 m<sup>2</sup> were randomly distributed in each system to prevent the forage inside from being grazed. The cuts in each SD of Guinea Massai grass were performed according to the methodology described by Neel et al. [16]. In addition, Guinea Massai grass samples were collected for two consecutive years on the dates and cut-off intervals shown in Figure 2.



**Figure 2.** Dates and intervals between cuts in the different stages of development of Guinea Massai grass in open pasture and silvopastoral system at Jaboticabal, São Paulo, Brazil.

#### 2.4. Evaluation of Forage Mass and Chemical Composition

A frame of 0.25 m<sup>2</sup> was used to delimit the sampling area within the cages. The forage samples in the four stages of grass development were collected at 5 cm of the soil. After collection, the material was weighed to obtain the green mass. The morphological components were separated into leaf blade (leaf), stem + sheath + reproductive structure (stem), and dead material obtained from a subsample. This subsample was dried in forced air circulation oven at 55 °C until constant weight, then the total dry mass of forage (kg DM ha<sup>-1</sup>) and the proportion of its morphological components (% of DM) was calculated on a dry mass base at 55 °C.

Subsamples containing the whole plant were used to determine the chemical composition [17]. The chemical composition was expressed on a dry matter base of 105 °C. The dry matter content at 105 °C was quantified in an oven regulated at 105 °C, and the mineral matter (MM) by combustion at 600 °C [17]. Organic matter (OM) was calculated by the equation  $OM = 100 - MM$ . Neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations were determined using procedures described by ANKOM Technology [18], and lignin (LIG) by acid hydrolysis. Hemicellulose was obtained by the difference between NDF content and ADF content, and cellulose by the difference between ADF and lignin content. Crude protein (CP) content was estimated using a LECO FP 528 nitrogen<sup>®</sup> io analyzer (Leco Corporation, St. Joseph, MI, USA).

#### 2.5. Gas Production and In Vitro Degradability

In vitro grass production kinetics were evaluated in two consecutive trials, using 500 mg of sample and incubation vials of an internal volume of 125 mL [19,20], totaling eight treatments (four stages vegetative of forage × two systems) and five replications.

In each trial, rumen liquids used as inoculum (a pool of the rumen content of three animals) were collected from three fistulated lambs in the rumen-fed Guinea Massai grass hay, adapted for 15 days. The rumen liquids were collected before the bulky supply to the animals. Rumen fluids were collected proportionally for pool formation. The buffered inoculum was prepared by adding the ruminal liquid pool to the incubation medium (buffer), in a proportion of one part of the rumen fluid for every four parts of buffer solution [21]. Then, 75 mL of this solution was added to 125 mL vials filled with CO<sub>2</sub>. The vials were sealed, measured for the holding of gases, and stored in a water bath at 39 °C for 48 h.

The gas pressure in the vials was measured at 3, 6, 9, 12, 24, and 48 h of fermentation, using a pressure transducer connected to a datalogger model GN200 (GN equipment, São Paulo, Brazil). The value recorded in the readings was converted to gas volume using the specific equation for laboratory conditions.

$$V = (5.4766 \times P) + 0.0934 \quad (1)$$

where  $V$  is the volume of gas in mL and  $P$  is the pressure measured in psi. The gas production was corrected by the average gas produced by the buffered inoculum and obtained from the Tifton 85 grass hay standard sample. In each assay, two weak ones containing standard samples and two vials with buffered inoculum were used. The production of gas accumulated during the 48 h was expressed in mL g<sup>-1</sup> of OM.

The lag time ( $L$ ) was estimated using the uncompartimentalized logistic model of Schofield et al. (1994) using the Equation (2):

$$Vt = Vf \times (1 + \exp(2 - 4 \times S \times (t - L))) - 1 \quad (2)$$

where  $Vf$  = final volume of accumulated gas (mL);  $S$  = degradability rate (h);  $t$  = time (h); and  $L$  = lag time (h).

The in vitro degradability of DM (DIVDM) was measured within 24 and 48 h of incubation. At the respective times, the containers were immediately immersed in ice, and, once open, the contents of the bottles were filtered on filter paper and the pH measured.

Then, the incubation residues were dried in a forced ventilation oven at 55 °C for 24 h and weighed on a precision scale. The difference between the forage weight calculated DIVDM and the residue after incubation was corrected for DM.

### 2.6. Statistical Analyses

Data from forage mass, morphological components, chemical composition, and DIVDM were submitted to variance analyses (ANOVA) according to the following model:

$$Y_{ijk} = \mu + S_i + E_j + (SE)_{ij} + \varepsilon_{ijk} \quad (3)$$

where:  $\mu$  = general mean;  $S_i$  = system effect  $i$ ;  $E_j$  = development stage effect  $j$ ;  $SE_{ij}$  = interaction effect between stage  $j$  and system  $i$ ;  $\varepsilon_{ijk}$  = random error associated with each observation.

The Shapiro–Wilk and Bartlett tests tested data for normality and homogeneity of variance, respectively. When significant, the systems were compared by the  $t$ -test, considering  $p < 0.05$ . When differences were observed between the SDs, orthogonal polynomial contrasts were used. All analyses were performed using the statistical program R (Version 4.0.2). The Tukey test compared the means, and differences were considered significant when  $p < 0.05$ .

## 3. Results

The adoption of the SPS compared to OP did not affect the forage mass and morphological components of Guinea Massai grass (Table 2). The production of forage mass and morphological components differed between SDs (Table 2). We observed a significant linear increase in the forage mass and the proportions of stem and dead material with the advance of plant maturity. On the contrary, the advance in the SD resulted in a linear reduction in the percentage of leaves and the leaf: stem ratio. Forage mass increased by 189% in S4 in relation to S1. The increase in forage mass in S4 comprised an increase of 261% in the proportion of stem and 233% in the proportion of dead material in plants compared to S1. However, the SD advance reduced the proportion of leaves in S4 by 48% in relation to S1.

**Table 2.** Forage mass and morphological components of Guinea Massai grass in open pasture (OP) and silvopastoral (SPS) systems (S) at different stages of development (SD) during 2018 and 2019 at Jaboticabal, São Paulo, Brazil. Values are means of five replicates of the S × DS interaction ( $n = 5$ ).

| Variable                 | Systems |       | SEM   | SD    |       |       |       | Effect | p-Value |        |        |
|--------------------------|---------|-------|-------|-------|-------|-------|-------|--------|---------|--------|--------|
|                          | OP      | SPS   |       | 1     | 2     | 3     | 4     |        | S       | SD     | S × SD |
| Forage mass <sup>a</sup> | 5740    | 5704  | 2811  | 2931  | 4322  | 7006  | 8475  | LIN    | 0.959   | <0.001 | 0.736  |
| Leaf (%)                 | 58.74   | 58.14 | 11.33 | 83.41 | 65.96 | 42.80 | 42.99 | LIN    | 0.826   | <0.001 | 0.523  |
| Stem (%)                 | 20.56   | 21.32 | 10.23 | 8.25  | 15.65 | 29.31 | 29.71 | LIN    | 0.762   | <0.001 | 0.769  |
| Dead (%) <sup>b</sup>    | 20.68   | 20.53 | 12.23 | 8.34  | 18.39 | 27.36 | 27.57 | LIN    | 0.959   | 0.004  | 0.953  |
| LSR <sup>c</sup>         | 5.10    | 5.19  | 3.09  | 11.98 | 5.23  | 1.76  | 1.99  | LIN    | 0.909   | <0.001 | 0.417  |

<sup>a</sup> in kg DM ha<sup>-1</sup>; <sup>b</sup> dead material; <sup>c</sup> leaf ratio: stem; linear (LIN). Values of  $p < 0.05$  differ, as per the  $t$ -test.

The interaction system × stage of plant development did not affect the variables of the chemical composition of Guinea Massai grass (Table 3). Except for the CP content, the pasture system did not significantly affect most of the chemically stable variables. The CP content in the pasture cultivated under the SPS was higher by 10% compared to the concentration of CP in the Guinea Massai grass in the OP. The plant development stage affected the forage chemical composition, except for the hemicellulose and cellulose content. These fractions comprised, on average, 34% of the DM of the forage, regardless of the SD evaluated. The forage NDF content showed a quadratic effect, with a higher content in S2. The contents of ADF and LIG showed a linear increase, with increments of 6% and 29% of S3 in relation to S1, respectively. The CP and ash contents showed a decreasing linear effect, with a reduction of 59% and 8% of S1 for S4, respectively.

**Table 3.** Chemical composition ( $\text{g kg}^{-1}$  DM) of Guinea Massai grass in open pasture (OP) and silvopastoral (SPS) systems (S) in different stages of development (SD) during 2018 and 2019 at Jaboticabal, São Paulo, Brazil. Values are means of five replicates of the  $S \times DS$  interaction ( $n = 5$ ).

| Variable         | System (S) |       | SEM  | Stage of Development (SD) |       |       |       | Effect | p-Value |        |               |
|------------------|------------|-------|------|---------------------------|-------|-------|-------|--------|---------|--------|---------------|
|                  | OP         | SPS   |      | 1                         | 2     | 3     | 4     |        | S       | DS     | S $\times$ DS |
| CP <sup>a</sup>  | 76.5       | 84.4  | 14.5 | 118.7                     | 81.3  | 65.8  | 48.0  | LIN    | 0.023   | <0.001 | 0.994         |
| NDF <sup>b</sup> | 742.4      | 736.6 | 22.3 | 736.1                     | 753.8 | 736.1 | 732.0 | QUA    | 0.243   | 0.020  | 0.250         |
| ADF <sup>c</sup> | 391.9      | 390.7 | 22.2 | 376.6                     | 385.5 | 397.8 | 404.4 | LIN    | 0.818   | 0.002  | 0.879         |
| HEM <sup>d</sup> | 353.9      | 345.7 | 31.9 | 353.8                     | 357.5 | 342.0 | 345.5 | NS     | 0.292   | 0.488  | 0.787         |
| CEL <sup>e</sup> | 347.4      | 345.2 | 20.0 | 347.9                     | 344.6 | 350.4 | 342.5 | NS     | 0.665   | 0.673  | 0.817         |
| LIG <sup>f</sup> | 29.4       | 34.0  | 10.8 | 28.9                      | 25.3  | 34.8  | 37.4  | LIN    | 0.074   | 0.004  | 0.406         |
| Ash              | 101.2      | 102.6 | 11.9 | 10.58                     | 110.1 | 94.6  | 97.3  | LIN    | 0.649   | <0.001 | 0.859         |

<sup>a</sup> Crude protein; <sup>b</sup> neutral detergent fiber; <sup>c</sup> acid detergent fiber; <sup>d</sup> hemicellulose; and <sup>e</sup> cellulose; <sup>f</sup> lignin, linear (LIN); quadratic (QUA); not significant (NS).

While there were a few significant effects for the main effect of S, and many for the main effect of DS, the  $S \times SD$  interaction did not affect Vf and L (Table 4). The Vf of the forage cultivated in SPS was 6% higher than Vf provided by the OP. The lag time presented a similar response. The SPS presented an L of 84% higher than the forage cultivated in an OP (Table 4).

**Table 4.** Parameters of ruminal kinetics in vitro of Guinea Massai grass in open pasture and silvopastoral (SPS) systems during 2018 and 2019 at Jaboticabal, São Paulo, Brazil. Values are means of five replicates of the  $S \times DS$  interaction ( $n = 5$ ).

| Variable                                 | System (S)   |      | SEM  | Stage of Development (DS) |       |       |       | SEM  | p-Value |         |               |
|--|--------------|------|------|---------------------------|-------|-------|-------|------|---------|---------|---------------|
|  | Open Pasture | SPS  |      | 1                         | 2     | 3     | 4     |      | S       | DS      | S $\times$ DS |
| Vf ( $\text{mL g}^{-1}$ OM) <sup>a</sup> | 158          | 168  | 3.89 | 196a                      | 164b  | 140c  | 150bc | 14   | 0.0389  | <0.0001 |               |
| L (h) <sup>b</sup>                       | 2.90         | 5.36 | 0.75 | 6.37a                     | 6.79a | 1.51b | 1.85b | 2.39 | 0.0213  | 0.0098  |               |

<sup>a</sup> The final volume of gas produced in  $\text{mL g}^{-1}$  OM of organic matter, <sup>b</sup> lag time in hours. Means followed by the same letter on the line did not differ from each other by the Tukey test ( $p \geq 0.05$ ).

As the SD of the forage plant increased, with the progress of the development stage, there was a reduction in Vf production. The maximum volume of gas from the fermentation of organic matter was observed during S1, which, when purchased with S4, was reduced by 23%. There was no difference in the colonization period between stages S1 and S2. In these stages, the lag time was higher by 74% compared to the other stages that did not differ from each other (Table 4).

The average pH was 7.0 and 6.9 in 24 h and 48 h, respectively. There was no effect of the interaction system  $\times$  stage of development on DIVDM (Table 5). The pasture systems also did not affect DIVDM in incubation times of 24 h and 48 h. DIVDM in both evaluated times presented decreasing linear effects with the development of SD, with a reduction of approximately 34% from DIVDM in S1 to S4 in both incubation periods.

**Table 5.** In vitro degradability of dry matter (DIVDM;  $\text{g kg}^{-1}$  DM) of Guinea Massai grass in open pasture (OP) and silvopastoral (SPS) systems (S) at different stages of development (SD) after 24 h and 48 h of incubation during 2018 and 2019 at Jaboticabal, São Paulo, Brazil. Values are means of five replicates of the  $S \times DS$  interaction ( $n = 5$ ).

| Variable   | System (S)   |       | SEM | Stage of Development (SD) |       |       |       | Effect | p-Value |        |          |
|------------|--------------|-------|-----|---------------------------|-------|-------|-------|--------|---------|--------|----------|
|            | Open Pasture | SPS   |     | 1                         | 2     | 3     | 4     |        | S       | DS     | S vs. DS |
| DIVDM 24 h | 284.5        | 297.6 | 7.8 | 344.0                     | 319.8 | 274.9 | 225.4 | LIN    | 0.235   | <0.001 | 0.127    |
| DIVDM 48 h | 447.9        | 453.8 | 7.1 | 552.4                     | 502.0 | 394.0 | 354.8 | LIN    | 0.562   | <0.001 | 0.147    |

Linear (LIN). Values of  $p \leq 0.05$  differ by the *t*-test.

#### 4. Discussion

The similarity in the forage mass observed between OP and SPS (Table 2) suggests that the edaphoclimatic conditions imposed by the trees on the pasture in the understory were not limiting. This is possible due to the forage plant's ability to adapt to the reduction in the quantity and quality of sunlight [4–6]. Thus, it is inferable that, in a scenario similar to this research, eucalyptus can be used as a tree component in a spacing of 18 m between rows without the forage mass of Guinea Massai grass being affected. In a previous study in the same area of this research, Van Cleef et al. [22] observed that denser eucalyptus spacings (9 m between rows) reduce the forage mass of Guinea Massai grass. Lima et al. [13] found that tree age can also affect the development of grasses in SPS due to increased treetops and shading in the lower extract. The authors reported that between 6 and 7 years after planting, there was no reduction in the forage mass of *Urochloa decumbens*. The experimental period of this study occurred between the sixth and seventh year after planting eucalyptus seedlings, which may explain the similarity between the evaluated systems.

The absence of systems effect on forage mass (Table 2) ensures that the herbage allowance will not be a limiting factor for animal performance. However, while the forage mass was not different between systems, the level of shading may influence animal behavior regarding intake and rumination in the shade or full sunlight, especially if the paddocks were not fully shaded, which could cause animals to graze more briefly in the sun and lay down longer in the shade for thermal comfort. The difference between systems in CP and, potentially, lignin also would likely be a factor in animal performance. Moreover, the silvopastoral systems are known to provide thermal comfort to the animal [1], land use efficiency, and diversification of income sources within the property [23,24]. In this study, we did not measure the quality and quantity of sunlight. Further studies should measure the levels/extent of shading to better interpret the effect of the silvopastoral system on forage mass and nutritive value.

The chemical composition of Guinea Massai grass showed greater nutritive value in SPS due to the CP content in forage (Table 3). These results corroborate the responses of grasses grown in integrated production systems under conditions similar to this study's [6,11–13]. Kephart and Buxton [25] observed the highest protein content in plants submitted to shading, and the amount of nitrogen practically constant per cell results in a higher concentration of this nutrient. Moreover, according to these authors, the stress caused by shading can reduce the amount of photoassimilation for the development of the secondary cell wall, maintaining more cellular content, which contains higher protein content. Wilson [26] attributes the increase of protein in shaded environments to a higher soil moisture, which contributes to accelerating the degradation of organic matter and the cycling of nutrients in the soil and, consequently, greater utilization by plants. Neel et al. [16] relate this effect to the development of plants which, when submitted to shading, delay the time to reach physiological maturity. It is known that young plants have a higher protein content compared to those that are fully developed [13,22].

With the advance of the SD of Guinea Massai grass, there was a decrease in the percentage of leaves and, conversely, growth in the percentage of stem and dead material (Table 2). These results are in line with those observed by Caldeira [27] in Guinea Massai grass, Khral et al. [28] in different cultivars of giant missionary grass, and Peralta et al. [29] for cultivars of *Urochloa*. As the forage develops physiologically, moving from the vegetative to the reproductive stage, the stem elongates and prevents the generation of new leaves [30]. Advancing age resulted in a higher proportion of stems for two reasons. The first may be related to increased mass and self-shading level of the canopy. In these cases, the primary response of the plant is to lengthen the stem to expose the younger leaves to light. The second is related to flowering itself, which in Guinea Massai grass is induced by the reduction in daylength. Therefore, with the stimulation of flowering, the grass invested more energy in producing stems, reducing the leaf to stem ratio.

The advance of SD is responsible for the thickening and lignification of the cell wall, increasing the proportion of fiber constituents [31]. Young plant cells have only one outer

layer, called the primary wall. However, as they mature, the secondary wall is formed, consisting mainly of structural carbohydrates, which attribute larger amounts of fiber to the forage in a more advanced SD [32]. A fact observed in this study is that the levels of ADF and LIG increased linearly with the advance of SD (Table 3), which corroborates the former studies [33,34]. The change in the wall and cell content proportion is also related to the decrease in CP content [26], due to the reduction in nitrogen availability due to the greater complexation of nitrogen compounds at the ADF fraction [33]. Another factor linked to the reduction in protein content is the decrease in hemicellulose, since it is in the leaves with higher concentrations of CP [35]. Low protein contents limit the animal's voluntary consumption, because nitrogen's low availability as a substrate for microbial synthesis reduces the activity of microorganisms in the rumen [35].

The *in vitro* gas production technique assumes the conversion of all major sources of carbohydrates into carbon dioxide, methane gas, and the reaction of volatile fatty acids [36]. It is known that the volume of gas produced during the *in vitro* fermentation process is related to the degradability of the substrate in the medium [13]. In this study, although a higher accumulated volume of gases ( $\text{mL g}^{-1} \text{DM}$ ) was observed in Guinea Massai grass cultivated in SPS, no difference was observed in DIVDM compared to the OP (Table 4). This response suggests that the increase in gas production was not due solely to DIVDM, and that the lignin content in forage may have limited substrate degradation.

As for the stages of grass development, it was found that, with the advance of forage maturity, there was an increase in fiber content and a reduction in protein intake. This fact may have resulted in decreases in the maximum volume of gas produced during 48 h, and in the reduction in DIVDM (Table 5). It is known that the synchronism between energy and protein strongly influences the final volume of gas *in vitro* fermentation. In the same way, the lignification of structural tissue hinders the performance of digestive enzymes produced by ruminal microorganisms, reducing degradability [27,33]. Similar results were observed in Guinea Massai grass [31], Marandu palisade grass (*Urochloa brizantha*) [37], and different *Cynodon* cultivars [28], in which there was a reduction in degradability as the forage age progressed.

Different factors can affect microorganisms' colonization time and forage DIVDM. For example, the chemical composition, the physical characteristics of the cell wall, and the presence of readily-available carbohydrates are some determinant parameters in the lag time and degradability [35,38]. In this research, the forage cultivated in the SPS had a higher lag time (Table 4). This result may be due to the longer colonization time required by cellulolytic bacteria, since there was a tendency to increase the lignin content in the Guinea Massai grass submitted to SPS (Table 4), and cellulolytic bacteria populations have slower growth with a tendency to reduce; therefore, they require greater lag time.

Generally, plants in shaded environments tend to present a lower development speed and remain in a vegetative stage for longer [11,16]. In contrast, plants grown in the OP have a higher photosynthetic rate, providing accelerated growth [13]. Guinea Massai grass in SPS showed delayed physiological maturity compared to OP (Figure 2), prolonging the vegetative phase, and keeping the forage physiologically immature for a longer period [39]. This is a desirable feature in animal production systems, as the nutritional quality of forage generally declines with advancing age or during the reproductive phase [40].

## 5. Conclusions

The shading in the SPS did not decrease the forage mass, nutritive value, and value or DIVDM of Guinea Massai grass compared to the open pasture system, but it did increase the CP content. Forage mass averaged  $5700 \text{ kg ha}^{-1}$ , CP 8% of DM, and DIVDM 29% at 24h and 45% at 48h. The shading also induced Guinea Massai grass to remain vegetative for longer. The advance of the stage of development of forage results in a decrease in the proportion of leaves from 83 to 43%, crude protein from 11 to 4% content, and an increase in fiber fractions.



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