Correction Research Article

INFLUENCE OF SILVOPASTORAL SYSTEM ON ANATOMICAL ASPECTS, DRY MATTER QUALITY OF MOMBASA AND MARANDU GRASSES

ABSTRACT: The goal of this paper was to evaluate the influence of shading levels in Silvopastoral system on the association level of anatomical structures to the nutritional parameters and gas production of Poaceaes. Two forage species were evaluated:*Panicum maximum* cv. Mombasa and *Brachiaria brizantha* cv. Marandu, exposed tothree shading levels: Full Sun System (FSS), 25 and 50% of shading arranged in contiguous bands and allocated in a completely randomized design with four replications.It was measured in the leaf blade the thickness of the parenchyma tissue (mesophyll), vascular bundlecomponents(metaxylem and metaphloem), vascular bundle sheath, sclerenchymaticsheath, sclerenchymatic cap, sclerenchymatic extension and epidermis secondary growth. It was also performed an analysis of NDF and ADF content; gas production and dry matter degradability at 96 hours of incubation. The variables were subjected to t-test at 5% and it was checked the correlation coefficients of each genotype with shading. The proportion of tissues that are able to lignify such as the secondary thickening and sclerenchyma reduce under shading on average 66% and 60%, respectively. The proportion of metaphloem in Mombasa in Full Sun was 5.5% and 3.7% for Marandu. However, this response was reversed with shading, reducing the proportion of metaphloem in Mombasa. Gas production in shaded Marandu grass was higher than in Mombasa grass as a result of the higher proportion of metaphloem. Considering the anatomical traits and gas production, Marandu grass showntheh higher dry matter degradability compared to Mombasa when shaded.

Key words: *Brachiaria brizantha*, degradability, *Panicum maximum*, parenchyma.

Introduction

The plant tissues are composed largely of cellulose fibrils in cell wall (GLORIA AND GERREIRO, 2006), ergastics substances and minerals that comprise the cellular content, which are considered highly digestible, as well as phloem cells (LIMA et al., 2001; PACIULLO et al., 2001).

Other anatomical structural elements from the leaf internal part also have essential roles for the functioning of the plant. The sclerenchyma fibres are responsible for the mechanical support functions and structural conformation, assisting the leaves and stems to stay erect (BASSO et al., 2014).Due to the structural duty, the fibres are composed of rigid compounds with more apparent thickness in the cell walls. Therefore, cells of these tissues are largely susceptible to secondary growth and cell death. Cell lignification from sclerenchymaticand xylem tissues is a result of pairing between compounds derived from the secondary metabolism and its deposition in these tissues and can affect animal intake variables (LEMPP, 2007).

Lignin is the major chemical entity responsible for the reduction in fibre digestibility. To the extent that the plant progresses in its age subsidized by optimal threshold production factors, some compounds derived from the secondary metabolism initiated the interference on the structural aspects of the plant.Paciullo et al. (2001) correlated lignin and sclerenchyma cell wall thickening with dry matter digestibility and it was found that in thehigher proportion of these tissues the correlation was significantly negative (LAJÚS et al., 2014).

The use of thesewall and cell content components is a function of the constitution of compounds of the cell wall and its degradation potential, which is defined by the participation of low degradability constituents (AKIN, 1989; JUNG and CASLER, 2006).

The cell wall constituents primarily include insoluble compounds such as cellulose, hemicellulose and lignin. However, some compounds of the secondary metabolism as hydroxinamic acids may be associated with lignin (JUNG AND CASLER, 2006; FUKUSHIMA AND HATFIELD, 2003) varying in concentration.

The association profile of these kinds of compounds derives from the genetic capacity of each species on express genes that modulate the synthesis of secondary metabolismcompounds, thereby degrading insoluble cell wall compounds may vary depending on the concentration of phosphocoumarins and ferulic acids associated with lignin (JUNG AND CASLER, 2006; GRABBER et al., 1998).

Gas production has a strong relationship with fibre digestibility and carbohydrate concentration contained in the leaf and can be mitigated by changes in the participation of lignified components in fibre (BOTTEN et al., 2011). Therefore,fluctuations in environmental variables can result in major changes in the physiological mechanisms of the plant, specifically in secondary metabolism and activation of enzymes responsible for photosynthesis, resulting in increased synthesis of phenolic compounds (TAIZ and ZEIGER, 2009).

Taking into account climatic variations, shaded plants tend to synthesize and to incorporate lesser amount of lignin (TAIZ AND ZEIGER, 2009) that encrust the cellulose of the cell wall and may impregnate sclerenchymatic tissues and metaxylem fibres.On the other hand, greater light offers are directly proportional to the synthesis of lignin and lactones. In addition, higher proportion of sclerenchymatic tissue and metaxylem can reduce dry matter degradability and gas production, and also increase the retention time in the rumen affecting consumption (AKIN, 1989; WILSON AND HATTERSLEY, 1983).

The goal of this paper was to evaluate the responses of two *Poaceae*, the different shading percentages on anatomical components, dry matter degradation and to check the association level between the variables, aiming also to evaluate gas production.

Materials and methods

The experiment was conducted at the School of Veterinary Medicine and Animal Science (SVMAS) of the Federal University of Tocantins, in Silvopastoral system of native shrubbery forest in a flat terrain (2% slope). According to Köppen the climate is classified as Aw (hot and humid);raining from October to May with an average annual rainfall of 1800 mm and average temperature of 28°C. The soil was classified as Quartzipsamment soil, in which was performed chemical characterization analysis at 0-20 cm depth (Table 1).

The experiment was implanted under natural shading ranges neighbouring vegetation in Cerrado-Amazon transition and enriched with two forage species. The design was completely randomized with four replications for each of the six treatments, which were arranged in a 2x3 factorial - two forage species (*Urochloa brizantha*cv. Marandu (syn.*Brachiaria brizantha*) and *Panicum maximum* cv. Mombasa) and three shading levels (0, 25 and 50%), totalling 24 experimental units.

The forest area was managed in October 2009, adopting as criteria for thinning shading levels of 0, 25 and 50% of light transmission in the environment and that was reviewed once morein June 2012 with the aid of a Luximeter.

Table 1. Chemical soil characterization at the beginning of the experiment, preceding the first cycle.

	pH	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	K^+	P	MO	CTC	V
	CaCl2	----------cmol$_c$dm^{-3}----------					---mgdm^{-3}-------		cmol$_c$dm^{-3}	%
BSP	4.78	0.67	0.59	0.66	2.26	0.012	2.92	5.62	3.54	36.18

B25	3.76	0.76	0.54	0.58	4.57	0.016	3.15	13.68	5.90	22.50
B50	3.84	0.61	0.59	1.17	5.61	0.016	2.79	14.05	6.83	17.83
MSP	4.57	0.86	0.57	0.42	4.64	0.009	2.80	8.04	6.08	23.70
M25	4.17	1.13	0.90	0.74	5.22	0.017	2.79	11.73	7.27	28.17
M50	3.97	0.78	0.78	1.10	4.76	0.013	3.13	12.51	6.35	24.95

Soil analysis at 0-20 cm depth in Quartzipsamment soil. BFS: Marandu Full Sun; B25: Marandu 25% shade; B50: Marandu 50% shade; MFS: Mombasa Full Sun; M25: Mombasa 25% shade; M50: Mombasa 50% shade.

Following the handling of trees, in October 2013 was performed the application of 800 kg lime/ha, 45 kg P_2O_5/ha and 50 kg K_2O/ha. Then, it was proceeded the foragesown, awaited the pasture establishment and at that time it was done one uniform cut at 20 cm above the ground with a brush cutter. After that,it was waited for the appearance of 3Mombasa grass leaves, 4.5 Marandu grass leaves and later the animals were put in paddocks, which one corresponded to 2,250 m². Given three days after removal of the animals from paddocks,40 kg N/hanitrogen fertilization was performed.

Anatomical Analysis

The harvest of forage samples in the seventh growth cycle prior to grazing was performed. The selected leaves, intended for histological sections, consisted of the last fully expanded leaf.During the harvesting process, the leaves intended to anatomical analysis were placed immediately in thermal box at 12º C as a conservation method of the fundamental systemcomponents of the vegetative organs.

Once collected, the material was conducted to the Biochemical and Morphology Laboratory of SVMAS where the leaves were sliced in the middle third in about 1 cm², dehydrated in absolute alcohol I and II at 70, 75, 80, 85, 90% and finally sent to absolute alcohol/xylene for sample clarification (each runsamples remained 30 minutes). Subsequently, the fractions were further clarified with xylene I and II (dimethyl benzene) and fixed with 5:5:90 solution(JOHANSEN, 1940) (40% formaldehyde, 50% ethanol and glacial acetic acid Pure Compound). The fragments were embedded in paraffin for subsequent cross-sections in5 µm in microtome. After that, the sections were attached to the blade for subsequent staining with toluidine blue.

In the leaf blade was measured the thickness of the parenchyma tissue (mesophyll), vascular bundlecomponents (metaxylem and metaphloem), vascular bundle sheath, sclerenchymaticsheath, sclerenchymatic cap, sclerenchymatic extension, and epidermis secondary growth (Fig. 1).The determination of the tissue thickness was done via light microscopy and using the Moticplus 2.0 software.

Gas production and dry matter degradability

In the seventh cycle, the samples taken as simulated grazing, considering 50% efficiency (sampled from3 expanded leaves for Mombasa and 4.5 for Marandu), were led to forced ventilation oven at 55° C for 72 hours, then were grinded in Willey mill and 2 mm sieve.

Approximately 0.2 grams of grinded samples were added in sealed Ankom bags and were sent to fermentation tubes receiving fluid collected from the rumen of a cannulated animal, kept grazing Marandu grass for 15 days. About 1 L of inoculum was placed in a preheated vacuum bottle at 39ºC. Immediately after harvesting, the material was sent to the laboratory where the inoculum was filtered.From the liquid fraction was added 10 mL to the sample+bag added to 20 mL of culture medium in the tube and sealed with petroleum jelly.

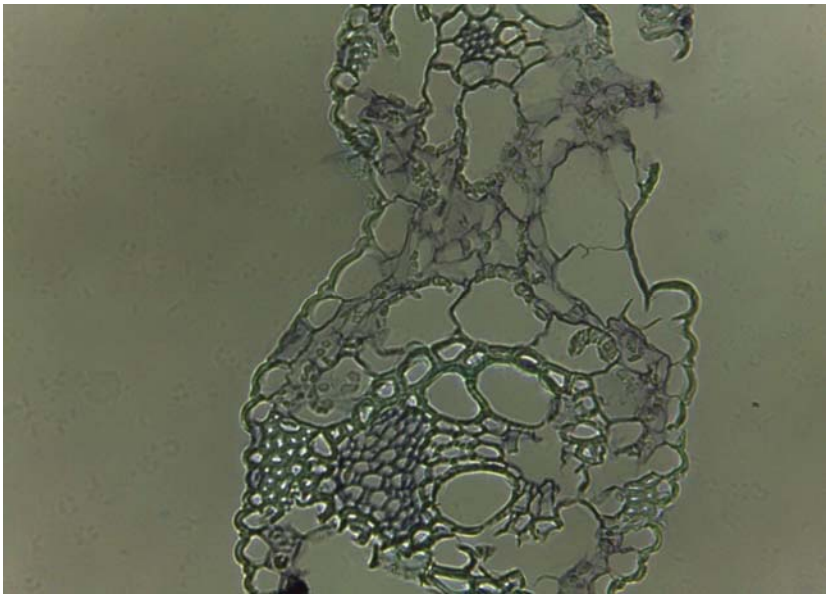

Figure 1. Anatomical structure of the leaf blade of *Urochloa brizantha* cv. Marandu (syn. *Brachiaria brizantha* cv. Marandu) grown in full sun.

Since the onset of incubation the measurement of gas production was carried out from time zero, three, six, nine, 12, 24, 48, 72 and 96 hours with the aid of graduated scale.The model of (FRANCE et al., 1993) was fitted to the data, as expressed below:

$$Y = A\{1 - \exp^{[-b(t-L) - c \times (\sqrt{t} - \sqrt{L})]}\}$$

The parameters regarding gas production kinetics are:
Cumulative Gas Production (mL), incubation time "t" (hours), total gas (mL) "A", lag time "T" (hours) and fractional degradation rate (h-1) "µ".The equations generated were compared usingparallel test and identity test according to Regazzi and Silva (2004).

The effective degradability at 96 hours incubation was obtained using the methodology of (FRANCE et al., 1993), given as:

$$EF = S_0\, e^{-kT}\, (1 - kI) / (S_0 + U_0)$$

In which:

EF = Effective degradability

k = passage rate; calculated for k=0.02; 0.03; 0.04 e 0.05.

S_0 e U_0= initially fermentable fractions and non-fermentable fractions, respectively, as follows: $I = \int\infty \exp -[(b + k)(t - T) + c(t - T)]dt$.

Statistical analysis

Analysis of variance and mean test were made after the residual normality test, data normality (SHAPIRO AND WILK, 1965) and homogeneity of variance. In the mean test was used t test at 5% significance level (for shading levels), the f test at 5% significance level was used to compare forage species and was tested the relationships between anatomical structures, shading and dry matter degradability by Pearson correlation test at 5%.

Results and discussion

Analogue proportions of sclerenchyma and parenchyma were obtained forthe respective shadings for Marandu and Mombasa grass (Table 2), with no difference regarding interaction (P<0.05).

However, the inference performed on shadings and species means shows a higher proportion of sclerenchyma (P<0.01) for Mombasa compared to Marandu. Shading caused a reduction in the proportion of sclerenchyma, occurring higher concentration of this type of tissue in Full Sun than shaded plants.These tissues for shade plants both in Mombasa and Marandu show no significant differences (P> 0.05) among the means for 25% and 50% shading.

Table 2. Sclerenchyma, parenchyma, metaphloem and epidermal thickening (µm) percentages (%) of cross-sections and degradability of Marandu and Mombasa grasses under different shade percentages.

	SSP	25%	50%	Means	CV(%)
		Sclerenchymaa(%)			
Marandu	3.4	2.0	2.0	2.5 b	
Mombasa	6.5	3.1	3.1	4.3 a	23
Means	4.9 A	2.6 B	3.3 B		
		Parenchyma(%)			
Marandu	56.9	57.3	60.0	58.1	
Mombasa	56.1	62.9	59.9	59.6	8
Means	56.5	60.1	59.9		
		Epidermal thickening (µm)			
Marandu	3.6 aA	2.5 aB	2.1aB	2.7	
Mombasa	2.5 bA	2.5 aA	1.9 aA	2.3	18
Means	3.8	2.5	2.0		
		Metaphloem(%)			
Marandu	3.77 bB	5.04aA	4.4aAB	4.5	
Mombasa	5.5 aA	2.55bB	3.2bB	3.7	16
Means	4.6	3.98	3.8		
		Associating parenchymal and sclerenchyma			
Marandu	18 aB	21 aB	35.2aA	24.7	
Mombasa	16 aB	22.5 aA	20.2bAB	19.1	17
Means	16.5	21.6	27.7		
		Degradability of dry matter			
Marandu	48.5 aC	53.1 aB	57.8 aA	53.1	
Mombasa	51.7 aA	51.5 aA	51.7 bA	51.6	5
Means	50.1	52.3	54.8		

Means followed by different lowercase letters in the same column (f test) and distinct uppercase letters in the same row (t test) differ at 5% significance (P <0.05). Degradability of dry matter in 96 hours of incubation. FS: Full Sun, 25%: Silvopastoral 25% shade and 50%: 50% Silvopastoral 50% shade. DM: Dry Matter.

Fluctuations in sclerenchyma proportions reflect oscillations in environmental conditions. Even Mombasa having less proportional reduction of this tissue, this forage also shows higher proportion of tissues that are able to lignify such as sclerenchyma and presents reduction in parenchyma, similar in Marandu. Greater participation of sclerenchyma in leaves may indicate forage with low degradability and lessnutrient availability (JUNG AND CASLER, 2006).

Associating parenchymal and sclerenchyma levels to the relationship analysis between the two variables (Table 2) indicates higher proportion of parenchyma tissue for Marandu grass compared to Mombasa grass (P <0.05) in that the shading is increased, particularly in 50% shade. In Mombasa grass the changes are more subtle. Marandu grass presents higher proportion of parenchyma, which indicates reduced synthesis of secondary metabolites and sclerenchymatic tissues, undergoing drastic changes on nutritional aspects, as indicated by dry matterdegradability (Table 2) and gas production (Figure 1).

Dry matter degradability values indicate similarity in responses between shadings for Mombasa grass butdifferences (P <0.01) for Marandu grass, in which this variable increased to the extent that the shading is increased.

Secondary epidermal thickening values were significant (P <0.01) for the split, with gradual reduction among the shading respectively from Full Sun to 50% shade in Marandu grass. However, the same response was not observed in Mombasa grass, which remained unchanged between shading levels but showed lower epidermal thickness under Full Sun compared to Marandu (Table 2).

Metaphloem (Table 2) was significant forits participation increasing in Marandu grass leaf and reduction in Mombasa grass, resulting in a higher proportion of metaphloemin the first grass specie.Aligning Table 2 values togas production in Figure 2 (A and B) it is seen that the stability of tissues that are able to lignifyand parenchyma tissues in Mombasa grass and the sudden reduction inmetaphloem, which is digested as a high degradability tissue (AKIN AND AMONS,1975), resulted in lower gas production (Figure 2B) and the shading equate each other. On the other hand, Marandu grass has increased gas production (Figure 2A) reflecting the higher proportion of metaphloem, which was crucial to affect gas production due to the higher degradability (GOMES et al., 2011;AKIN AND AMONS,1975). Researching two *Axonopus*species Lima et al. (2001) reported higher degradability in phloem and parenchyma compared to lignified tissues. The phloem hasa cell wall composed mostly of cellulose, giving it greater susceptibility to degradation in the rumen (ENGELS AND SCHUURMANS et al., 1992).

Cellulose as well as hemicellulose are part of the cell wall chemical entities which havesignificant degradability and also they constitute NDF fractions.

Table 3 presents the values for NDF andADF content. No significant interference between species and shading levels for NDF and ADF was found.

Table 3. NDF and ADF dry matter content of Marandu and Mombasa grasses under different shade percentages.

	SP	25%	50%	Means	CV
		Neutral detergent fibre (NDF), %			
Marandu	59.76	64.85	59.95	62.7 b	
Mombasa	62.68	66.48	63.73	63.9 a	9
Means	61.22A	65.68 A	61.84 A		
		Acid detergent fibre (FDA), %			
Marandu	23.18	26.72	24.38	25.36a	
Mombasa	24.96	27.88	25.65	25.57a	12
Means	24.07A	27.30A	25.01A		

Means followed by different lowercase letters in the same column (f test) and distinct uppercase letters in the same row (t test) differ at 5% significance (P <0.05). Neutral detergent fibre (NDF); Acid detergent fibre (FDA). FS: Full Sun, 25%: Silvopastoral 25% shade and 50%: 50% Silvopastoral 50% shade.

Considering that the neutral detergent fibre (NDF) is constituted by cellulose, hemicellulose and lignin whereas acid detergent fibre (ADF) is constituted by cellulose and lignin, and that they have a relationship with the ingestive behaviour, in which the higher is the increase in dry matter, the lower digestibility and consequently lower intake. However, the cellulose and hemicellulose proportions may vary due to climate changes and todo the increase of lignin in NDF reflecting different results in degradability variables (VAN SOEST, 1994). Lignins are rigid compounds present in vascular plants that have the purpose of preserving the physical integrity of the plant and its herbivory resistance capacity depends on the association with hydroxinamic compounds (p-ferulic acid, p-coumaric and sinapic acid) (HATIFEILD et al., 1994).

Even if there is no significant difference between treatments, greater phloem proportion in Marandu, which is basically composed of cellulose, allowed this forage to present greater dry matter degradability (GOMES et al., 2011).

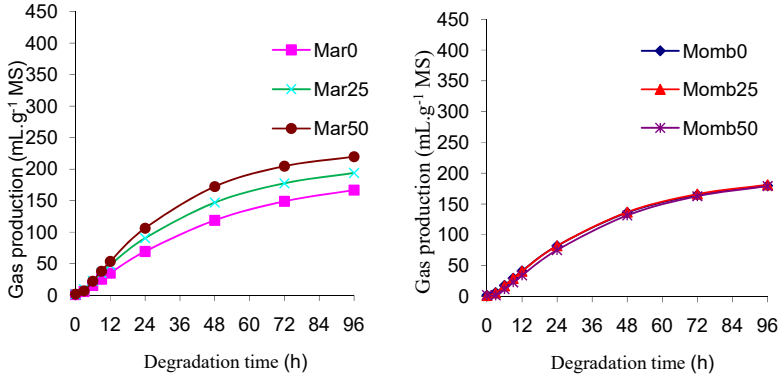

Figure 2. Cumulative gas production within 96 hours of *Panicum maximum* cv. Mombasa and *Brachiaria brizantha* cv. Marandu leavesgrownonFull Sun and 25 and 50% shading. Marandu Full Sun (Mar0) Marandu 25% shade (Mar25), Marandu 50% (Mar50), Mombasa Full Sun (Momb0), Mombasa 25% shade (Momb25), Mombasa 50% (Momb50).

The highest increase of gas production in MombasaFull Sun compared to MaranduFull Sun is given in detriment of a greater proportion of metaphloemand lower secondary epidermal thickening. The largest proportion of the sclerenchyma in Mombasa suggests that cell wall components can be encrusted by lignin, reducing the degradation time span of the cell wall (CASTRO et al., 2007). The soluble and structural carbohydrates profile also influence grass fermentability (CASTRO et al., 2007). It is possible that the shading has changed the proportion of polymers, reducing the quantity of lignin in the cell wall of Marandu shaded plants (HOBSON AND STEWART, 1997).

The high number of cells present in Mombasa grass parenchymal tissue was not sufficient to express differences (P> 0.05) within the same species between the shading levels, the result was found at the expense of proportional oscillation of sclerenchyma and the xylem (PACIULLOet al ., 2001). Environmental fluctuations have an influence on the anatomical changes that may still be associated with the peculiarities inherentto the species. AccordingQueiroz et al. (2000) it can be found anatomical differences within the same species or physiological group and further accentuated by climate fluctuations.

Table 4 presentsthe correlation coefficients of the anatomical structures, shading and dry matter degradability. The relationship level between the variables analysed evidence mismatch between the two species when the responses of somevariables were compared, such as metaphloem, sclerenchyma, dry matter degradability and secondary thickening. The mean test valuesshowed that the different results for each species (LEMPP, 2007) in the shade was affected by shading, reflecting on the relationship level between the variables.

In Table 4 are expressed the correlation coefficients between variables and also the shading percentages. Marandu metaphloem (0.28) had no significant association with shading as occurred in Mombasa (-0.67) because the structure of its metaphloemremained stable to changes in light spectrum.

Table 4. Linear correlations between leaf anatomical variables, shading and dry matter degradation.

Marandu	Metafl	Escl	Parênq	Par:Es	DMS	Espess	Somb
Metafl	1						
Escl	-0.43	1					
Parênq	-0.39	-0.17	1				
Par:Esc	-0.17	-0.44	0.61*	1			
DMS	0.033	-0.63*	0.43	0.74*	1		
Espessam	-0.23	0.75*	-0.35	-0.58*	-0.66*	1	
Somb	0.28	-0.78*	0.46	0.68*	0.79*	-0.83*	1
Mombasa	**Metafl**	**Escl**	**Par**	**Par:Es**	**DE 96**	**Espess**	**Somb**
Metafl	1						
Escl	0.69*	1					
Parênq	-0.50	-0.60*	1				
Par:Esc	-0.68*	-0.75*	0.50	1			
DMS	-0.01	0.08	-0.28	-0.51	1		
Espess	0.19	0.12	-0.25	-0.03	0.06	1	
Somb	-0.67*	-0.45	0.23	0.36	-0.009	-0.45	1

Metaph: metaphloem, Scl: sclerenchyma, Paren: parenchyma, Par:Scl: parenchymal:sclerenchyma relationship, DMD: dry matter degradation within 96 hours, Thick: Epidermal thickening, Shad: shading. * Significant at 5% (P <0.05).

The relationshipbetween sclerenchyma and secondary epidermal thickening to the shading in Marandu were also significant and exhibited an reverse relationship, that is, an increase in shading level resulted in a decrease in the proportion of these tissues, resulting in an increase in dry matter degradation (P<0.05), which had positive correlation (Figure 2). This result was not observed in Mombasabecause the stability of tissues, with the exception of metaphloem in response to environmental fluctuations, did not allow the associative response to be significant. Paciullo et al. (2001)correlated the anatomical components to chemical blades and stems from Marandu and Molasses grasses and found similar results to this study, in which the same variables showed no significant correlation in the two species.

In Table 4 it is clear that the significant negative correlation in Mombasa grass between parenchyma and sclerenchyma is explained by the reduction in sclerenchyma tissue. The same result was not found for Marandu, since reducing the mesophyll relative to sclerenchyma was not significant.

The number of significant correlations was lower in Mombasa and most wasmismatched to Marandu results (Table 4 and Figure 2A). In Figure 2, for example, it is clear that the behaviour in tissue proportions is an intrinsic result of the genetic resilience factor, whichdid not affect dry matter degradability of Mombasa.

Dry matter degradation after 96 hours of incubation for both forages shows that the results were different (Figure 3). Due to the higher proportion of metaphloem and reduction of tissues that are able to lignify under shading, Marandu grass showed an increase in degradation compared to Mombasa grass, which in turn remained unchanged between the shading levels. Tissues that are able to lignify: xylem, epidermis and sclerenchyma limit grass degradation, increasing the length of the digestive processes (LIMA et al., 2001; LEMPP et al., 2009). The cell wall lignification of xylem and sclerenchymatic tissues increase tissue resistance to degradation of bacteria and fungi (BOTTEN et al., 2011).

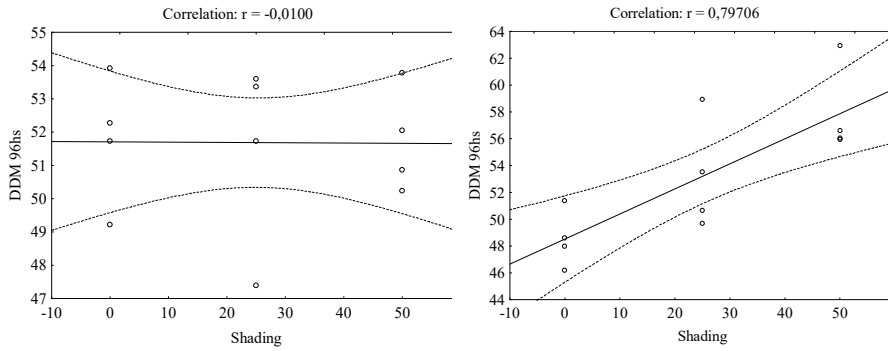

Figure 3. Quantitative association between shading and dry matter degradability in 96 hours of incubation for Mombasa (A) and Marandu (B). DDS: degradation of dry matter.

Conclusions

Considering anatomical traits and gas production in Silvopastoral system, Marandu grass shownnutritionally the best performance when shaded.Mombasa grass is less sensitive to environmental changes which culminated in greater preservation of low degradability tissues.

References

AKIN, D. E. Histological and physical factors affecting digestibility of forages. **Agronomy of Journal**, v. 81, n. 1, p. 17-25, 1989.

AKIN, D. E.; AMOS, H. E. Rumen bacterial degradation in of forage cell wallsinvestigated by electron microscopy. **Applied Microbiology**, v. 29, n. 5, p. 692-701, 1975.

BASSO, K. C.; CECATO U.; BARBEIRO, L. M.; LEMPP, B.; GOMES, J. A. N.; LUGÃO, S. M. B. Influence of nitrogen levels on leaf anatomy and nutritive value of millenium grass. **Bioscience Journal**, v. 30, n. 3, p. 792-802, 2014.

BOTTEN, T. J.; JOBLIN, K. N.; MCARDLES, B. H.; HARRIS, P. J. Degradation of lignified secondary cell walls of Lucerne (*Medicago sativa* L.) by rumen fungi growing in methanogenic co-culture. **Journal of Applied Microbiology**, v. 111 , n. 5, p. 1086-1096, Agosto, 2011.

CASTRO, G. H. F.; GRAÇA, D. S.; GONÇALVES, L. C.; MAURÍCIO, N. M.; BOGES, I.; TOMICH, T. R. Cinética da degradação e fermentação ruminal da *Brachiaria brizantha* cv. Marandu colhida em diferentes idades ao corte. **Arquivos Brasileiro de Medicina Veterinária e Zootecnia**, v. 59, n. 6, p. 1538-1544, 2007.

ENGELS, F. M.; SCHUURMANS, J. L.L. Relationship between structural development of cell walls and degradation of tissues in maize stems. **Journal of Science and Food Agriculture**, v. 59, p. 45-51, 1992.

FRANCE, J.; DHANOA, M. S.; THEODOROU, M. K.; LISTER, S. J.; DAVIES, D. R.; ISAC, D. A model to interpret gás accumulation profiles with in vitro degradation of ruminal feeds. **Journal of Theoretical Biology,** v. 163, n. 1, p. 99-111, 1993.

FUKUSHIMA, R.S.; HATFIELD, R.D. Composição fenólica de ligninas dioxano determinadas pela reação oxidativa com o nitrobenzeno. **Pesquisa Agropecuária Brasileira**, v. 38, n. 3, p. 373-378, 2003.

GOMES, R. A.; LEMPP, B.; JANK, L.; CARPEJANI, G. C.; MORAIS, M. G. Características anatômicas e morfológicas de lâminas foliares de genótipos de *Panicum maximum*. **Pesquisa Agropecuária Brasileira**, v. 46, n. 2, p. 205-211, 2011.

GLÓRIA, B. A.; GUERREIRO, S. M. C. **Anatomia Vegetal,** 2ª edição Viçosa, Editora UFV, 2006, 436p.

GRABBER, J H.; RALPH, J.; HATFIELD, R. D. Ferulate cross-linked limit the enzymatic degradation of synthetically lignified primary walls of mayze. **Journal Agriculture Food Chemical**, v. 46, n. 7, p. 2609-2614, 1998.

HATFIELD, R.D., JUNG, H.G., RALPH, J. A., BUXTON, D.R., WEIMER,, P.J. comparison of the insoluble residues produced by the klason lignin and acid detergent lignin procedures. **Journal Science Food Agriculture**. v. 65, p. 51 -58, 1994.

HOBSON, P. N.; STEWART, C. S. **The rumen microbial ecosystem**. London: Brackie Academic & Professional, 1997. 719 p.

JOHANSEN, D. A. **Plant microtechnique**. Mc Graw Hill, New York. 1940.

JUNG, H. G.; CASLER, M. D. Maise stem tissues: Impacto of development on cell wall degradability. **Crop Science**, v. 46, n. 4, p. 1801-1809, 2006.

LAJÚS C. R.; MIRANDA, M.; SCHEFFER-BASSO, S. M.; CARNEIRO, C. M.; ESCOSTEGUY, P. A. V. Leaf tissues proportion and chemical composition of *Axonopus jesuiticus x A. scoparius* as a function of pig slurry application. **Ciência Rural**, v. 44, n. 2, p. 276-282, 2014.

LEMPP, B. Avanços metodológicos da microscopia na avaliação de alimentos. **Revista Brasileira de Zootecnia**, v.36, suplemento especial, p. 315-329, 2007.

LEMPP, B.; GOMES, R. A.; MORAIS, M. da G. Importância da anatomia vegetal na qualidade da forragem. In: Simpósio, 7.; Congresso de Forragicultura e pastagens, 3., 2009, Lavras, **anais**. Lavras: UFLA, 2009, p. 1-16.

LIMA, L. M. S.; AQUINO, Y.; BRITO, C. J. F. A.; DESCHAMPS, F. C. Degradação ruminal dos tecidos vegetais e composição bromatológica de cultivares de Axonopus fissifolius (RADDI) KUHLM. **Ciência Rural**, v.31, n. 3, p. 509-515, 2001.

PACIULLO, D. S. C.; GOMIDE, J. A.; QUEIROZ, D. S.; SILVA, E. A. M. Correlações entre componentes anatômicos, químicos e digestibilidade in vitro da matéria seca de gramíneas forrageiras. **Revista Brasileira de Zootecnia**, v. 30, n. 3, p. 955-963, 2001.

QUEIROZ, D. S.; GOMIDE, J. A.; MARIA, J. Avaliação da folha e do colmo de topo e base de perfilhos de três gramíneas forrageiras. **Revista Brasileira de Zootecnia**, v. 29, n. 1, p. 61-68, 2000.

REGAZZI, A. J.; SILVA, C. H. O. Teste para verificar a igualdade de parâmetros e a identidade de modelos de regressão não-linear. **Revista de Matemática e Estatística,** v. 22, n. 2, p. 33-45, 2004.

SAHPIRO, S. S. and WILK, M. B. An analysis of variance for normality 9 complete samples). **Biometria**, v. 52, n. 3-4, p. 591-611, 1965.

WILSON, J.R.; HATTERSLEY, P.W. *In vitro* digestion of bundle sheath cell in rumen fluid and its relation to the suberized lamella and C4 photosynthetic type in *Panicum* species. **Grass and Forage Science**, v.38, nº 3, p.219-223, 1983.

TAIZ, L.; ZEIGER, E. **Fisiologia Vegetal**.4 ed. Porto Alegre, Artmed, 2009, 848 p.

VAN SOEST, P.J. **Nutritional ecology of the ruminant**. 2. ed. Ithaca: CornelI University Press. 1994.